

Medical Radiology

Radiation Oncology

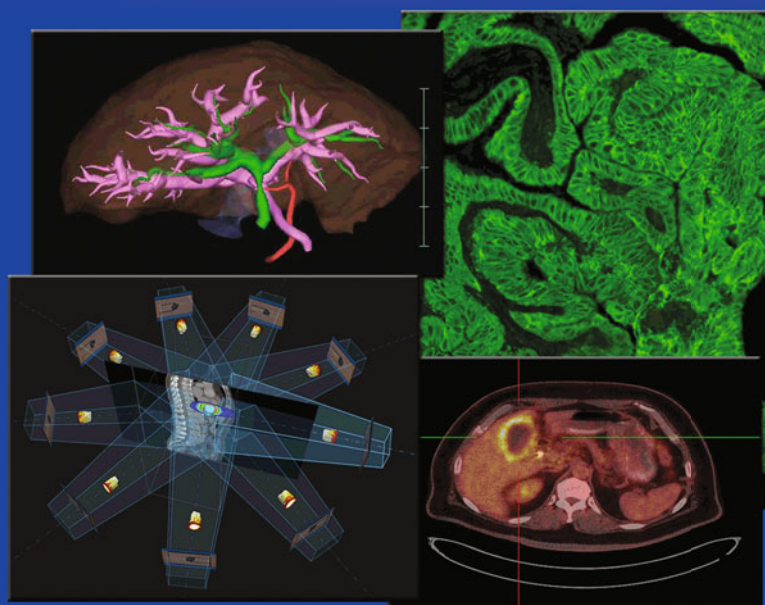
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Editors

Biliary Tract and Gallbladder Cancer

A Multidisciplinary Approach

Second Edition



Medical Radiology

Radiation Oncology

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Biliary Tract and Gallbladder Cancer

A Multidisciplinary Approach

Second Edition

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First Edition Demos Medical Publishing, 2008

ISSN 0942-5373 ISSN 2197-4187 (electronic)
ISBN 978-3-642-40557-0 ISBN 978-3-642-40558-7 (eBook)
DOI 10.1007/978-3-642-40558-7
Springer Heidelberg New York Dordrecht London

Library of Congress Control Number: 2014932826

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Foreword 1

The second edition of this book represents an update on primary biliary tract and gallbladder cancer. This is an increasing problem worldwide. It represents a difficult tumor for which treatment is frustrating.

The emphasis is on the current knowledge base for multidisciplinary management and covers aspects not only to do with novel serum biomarkers and pathologic staging but also molecular profiling and the entire range of current and emerging imaging strategies. Further chapters are devoted to epidemiology, the role of growth factor pathways and signal transduction, histopathology, and molecular pathogenesis. Significant emphasis is placed on surgical, radiation therapy, and chemotherapy approaches.

With the increasing incidence of the disease, there has been a greater interest in developing new cutting-edge technical knowledge for treatment including not only interventional radiologic techniques such as radiofrequency ablation and chemoembolization, but novel surgical approaches and image-guided technologies.

Biliary Tract and Gallbladder Cancer: A Multidisciplinary Approach edited by Drs. Herman, Pawlik, and Thomas represents the experience of a group of dedicated, well-informed practitioners and will serve as an invaluable source of information on the newest diagnostic and treatment technologies for biliary tract and gallbladder cancer. This book should be available to all oncologists as a concise but significant introduction to a tumor of growing incidence for not only students, but for residents and practitioners as well.

Philadelphia
Hamburg
München
Bodø

Luther W. Brady
Hans-Peter Heilmann
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Carsten Nieder

Foreword 2

Biliary tract adenocarcinoma comprises a rare, diverse, and, in many ways, enigmatic group of malignancies. Despite sharing a common origin with biliary epithelial cells, from the intrahepatic radicles that line the canals of Hering down to the distal common bile duct just above the ampulla of Vater, they beget a wide spectrum of clinical disease. Until recently, the factors leading to neoplastic change within the biliary tree were largely unknown, and it was unclear if this clinical variability was dictated solely by the anatomic site of origin or the result of fundamental differences in molecular pathogenesis. Indeed, in many clinical studies, it was common to group biliary cancers together, as if one diagnosis. This practice, although partly stemming from the rarity of these tumors and the need to ensure an adequate sample size, reflected a high degree of naiveté fueled by a lack of understanding of biliary cancer biology.

Advances in deciphering the molecular events that led to the development and progression of biliary tract cancer has lagged behind that of other, more common diseases. As such, it comes as no surprise that significant evolution in treatment approaches, as seen, for example, in hepatic colorectal cancer, have not been realized. However, on the heels of progress in other areas, several recent studies have shed some light on the pathogenesis of biliary tract cancer and have provided compelling evidence that they are different diseases, with unique molecular biological underpinnings that likely account for the clinical differences that have long been recognized. This deeper understanding of biliary tract cancer on a more basic level, while still rudimentary, holds promise for therapeutic advances.

In parallel with advances in our understanding of the molecular biology of biliary tract cancer, have come improvements in other areas that have enhanced our ability to manage patients with these diseases. Examples include greater survival in patients with advanced cancer treated with systemic chemotherapy and improved perioperative morbidity and mortality after resection. Despite these positive signs, however, there is still much to be accomplished, as evidenced by the persistent, very high operative mortality rate associated with resection of hilar cholangiocarcinoma, confusion regarding optimal treatment of gallbladder cancer, and the limited treatment options and poor survival in patients with unresectable tumors.

The publication of *Biliary Tract and Gallbladder Cancer: A Multidisciplinary Approach* is particularly timely. With a critical mass of investigators and clinicians with sufficient experience and interest to make a difference, there is a renewed sense of optimism that important, paradigm-changing advances are at hand. The editors, Drs. Herman, Pawlik, and Thomas have captured this sentiment by including a comprehensive list of topics authored by experts from around the world. Providing the latest insights into epidemiology, molecular biology, imaging and disease staging, therapy, and outcome, this textbook represents a valuable contribution to the field and will serve as an important resource for investigators and clinicians at all levels of expertise.

New York

William R. Jarnagin

Foreword 3

Until relatively recently, there existed little acknowledgement of the importance of cancers of the biliary tract. Management of these diseases typically consisted of surgical resection, often unsuccessful. There has been a substantial increase in the incidence of these diseases, at least partially attributable to the fact that many tumors initially classified as liver metastases from an unknown primary tumor now represent intrahepatic cholangiocarcinomas. Advances in imaging, especially with improved magnetic resonance imaging (MRI) techniques, have allowed more accurate determination of disease extent. There are also vastly enhanced techniques available to the pathologist to define the true origin of biliary tract tumors. As investigators are now able to better anatomically define these tumors, biologists are capable of identifying relatively unique characteristics that provide opportunity for newer management approaches.

Treatment of biliary tract tumors is complicated due to underlying biological and anatomical reasons. Although the tumors are often aggressive, most patients are asymptomatic until the cancer has reached an advanced stage. Local recurrence is common with surgical resection alone, and the vast majority of patients die from disseminated disease as chemotherapy has not been very effective. Radiation therapy has had a relatively limited role because delivery techniques are inadequate to deliver high doses to the tumor while preserving sufficient hepatic function.

Technology has largely improved our ability to better define and manage tumors in many locations; this is especially true for biliary diseases. Despite the widespread advent of technological advances thus far, enormous opportunity for further progress in management technique persists. These include imaging approaches, not only with MRI, but also with fludeoxyglucose-positron emission tomography (FDG-PET), and newer positron emitting isotopes. Surgical and anesthetic techniques have evolved enormously, allowing operations to be performed successfully in situations where this was not possible 10 or 15 years ago.

New radiation therapy equipment now allows precise millimeter dose localization to small, defined regions in and around the liver, with delivery of high doses to volumes that are tracked as they are moving due to respiratory motion. Advancing technology has allowed newer interventional approaches that enhance both diagnostic and therapeutic interventions that are utilized both by the interventional radiologist as well as the gastroenterologist. Changes in delivery techniques of transarterial chemoembolization and radioembolization have made a meaningful impact on tumor control.

Technology has also allowed a markedly improved understanding of these diseases in addition to opening new avenues for therapy with biologically targeted agents and possible immunotherapeutic approaches.

Thus, there is great need for a compilation of the known clinical and translational information on this group of diseases. The second edition of this book, *Biliary Tract and Gallbladder Cancer: A Multidisciplinary Approach* edited by Herman, Pawlik, and Thomas, is a needed addition to the literature that consolidates the knowledge generated by disparate medical and biological specialties that are essential for improved management of these tumors. The breadth of the information provided should be valuable to all physicians who treat this challenging group of tumors.

Chapel Hill

Joel E. Tepper

Preface

While there has been some progress in the field of biliary and gallbladder cancers, effective strategies to prevent, diagnose, and treat these malignancies remain a challenge for investigators and clinicians. Compared with other gastrointestinal malignancies, biliary and gallbladder cancers are somewhat rare. However, the incidence of biliary and gallbladder cancers appears to be on the rise worldwide. In addition, while uncommon, advanced biliary and gallbladder cancers are often associated with a high case-mortality. As such, there is a need for greater investigation into the causes and optimal therapeutic interventions of biliary and gallbladder cancer. Investigation into the mechanisms underlying the biology, as well as implementation of the optimal treatment, of biliary and gallbladder cancer, requires a multidisciplinary approach. Given the relative rarity of the disease and the multifaceted information necessary to understand its pathogenesis, much of the data on biliary and gallbladder cancer are scattered across the scientific literature. The purpose of the current book is to provide a comprehensive, unified, and definitive overview of biliary and gallbladder cancers.

Much like the first edition, we sought to recruit a broad representation of world experts to provide a global perspective on biliary and gallbladder cancers. In addition, authors from a wide range of disciplines were included to provide state-of-the-art updates on the epidemiology, pathogenesis, as well as diverse treatment of biliary and gallbladder cancers that include radiation, medical, and surgical oncology. The multidisciplinary approach to discussing the topics of diagnosis, treatment, and intervention is one of the main strengths of the book. As importantly, the reader is provided a comprehensive overview of the treatment of biliary and gallbladder cancer from a modern twenty-first-century perspective.

It is our sincere hope that the book helps to cultivate collaboration among scientists and clinicians who will continue to seek new knowledge to improve patient's care for patients with these malignancies. The book is dedicated to the researchers, clinicians, and support staff challenged with understanding and treating these difficult diseases. Most of all, the book is dedicated to all patients we treat with biliary and gallbladder cancer. It is our hope that in sharing the collective wisdom contained in this text we can help stimulate and encourage future collaborative efforts to find new ways to improve the quantity and quality of life of our patients with biliary and gallbladder cancers.

Joseph M. Herman
Timothy M. Pawlik
Charles R. Thomas, Jr.

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Epidemiology of Cholangiocarcinoma and Gallbladder Carcinoma

Ijeoma A. Azodo, Rowan W. Parks, and O. James Garden

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Abstract

Cholangiocarcinoma (CCA) and gallbladder carcinoma (GBCA) are rare but lethal cancers of the liver and biliary tract. While surgical resection offers the best chance for cure, many cancers present late in the disease course when surgery does not alter patient survival or quality of life and is often unable to achieve a margin-negative (R0) resection. Despite advances in imaging and diagnostic modalities, appropriate screening protocols are yet to be developed due to the lack of known and modifiable risk factors. This chapter describes the epidemiology of cholangiocarcinoma and gallbladder carcinoma across world populations. Special attention is paid to describing the incidence, prevalence, and mortality of cholangiocarcinoma in the high-risk populations from Thailand, Japan, Korea, and China where infection with the liver flukes, *O. viverrini* and *C. sinensis*, and positive Hepatitis B and C infection are strongly implicated in CCA development. Intrahepatic stones, primary sclerosing cholangitis (PSC), biliary tree infection, and altered bilio-pancreatic anatomy may contribute to a chronic inflammatory state that promotes biliary epithelial metaplasia and CCA. In the last 10 – 15 years, there has been a trend of increased intrahepatic cholangiocarcinoma (ICC) and concurrent decreased extrahepatic cholangiocarcinoma (ECC) incidence, especially in low-risk populations of the United States, Europe, and Scandinavia. Gallbladder carcinoma incidence and mortality continues to be high in populations from northern India, Pakistan, and Eastern Europe, and is disproportionally higher in women. While the overall recent trend of GBGA incidence and mortality is on the decline, it remains high in women of high Amerindian ethnicity in South America and the United States. The differential decreased mortality in populations from the United States, Europe, and Scandinavia compared to South America and Asia is attributed to differential access and utilization of cholecystectomy. Specific risk factors for GBGA include longstanding cholelithiasis,

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S. typhi infection / chronic carrier state, and polypoid lesions of the gallbladder. However, these are not sensitive or specific modifiable risk factors are limited, thereby restricting the ability to design a screening protocol or mandate prophylactic surgery to protect against GBCA. Ongoing research efforts are focusing on the multifactorial contributions of environmental toxins, diet, obesity, and molecular mechanisms of CCA and GBCA development to improve early diagnosis and develop targeted therapies to complement surgical resection.

1 Cholangiocarcinoma

Cholangiocarcinoma is a rare cancer of the biliary tract that develops from the epithelial cholangiocyte cells [1]. It is the second most common form of primary liver cancer, representing 10–25 % of cases, and the third most common gastrointestinal malignancy [2–4]. Worldwide, the highest incidence of cholangiocarcinoma is found in males and in Asians in Thailand, Korea, China and Japan, and individuals of Asian descent in the United States [5–7].

Cholangiocarcinomas are described as intrahepatic (ICC) or extrahepatic (ECC) based on their location along the biliary tree. The World Health Organization (WHO) and International Agency for Research on Cancer (IARC) assign intrahepatic cholangiocarcinoma the International Classification of Diseases-Oncology (ICD-O) morphology code ICD-O 8160/3 which aligns topographically with other primary tumors of the liver (C22.0, C22.1), such as hepatocellular carcinoma (HCC) [8–10]. Furthermore, molecular research also supports this topographic grouping based on a common hepatic stem/pluripotent cell origin for ICC and HCC [11, 12]. Intrahepatic cholangiocarcinomas typically are mass-forming and contiguous with the ductal system. Intrahepatic tumor metastases in advanced stages are common, as is infiltrative spread along the portal tracts. Extrahepatic cholangiocarcinomas are assigned the morphology code ICD-O 8140/3 and are topographically described with gallbladder carcinomas (C23) and other tumors of the biliary tree (C24) [8, 9, 13].

ECCs can be polypoid, nodular, scirrhous, or diffusely infiltrating. Hilar cholangiocarcinomas, or Klatskin tumors, (ICD-O 8162/3) have largely been studied, treated, and reported as a separate entity from both ICC and ECC, but originate from the same cell type [14]. The assignment of a unique ICD-O code to Klatskin tumors in its second edition introduced additional difficulty with classifying and reporting, as hilar cholangiocarcinomas are now cross-referenced topographically to both intra- and extrahepatic

locations in edition 3 in comparison with being aligned with the intrahepatic location in edition 2 [15–18].

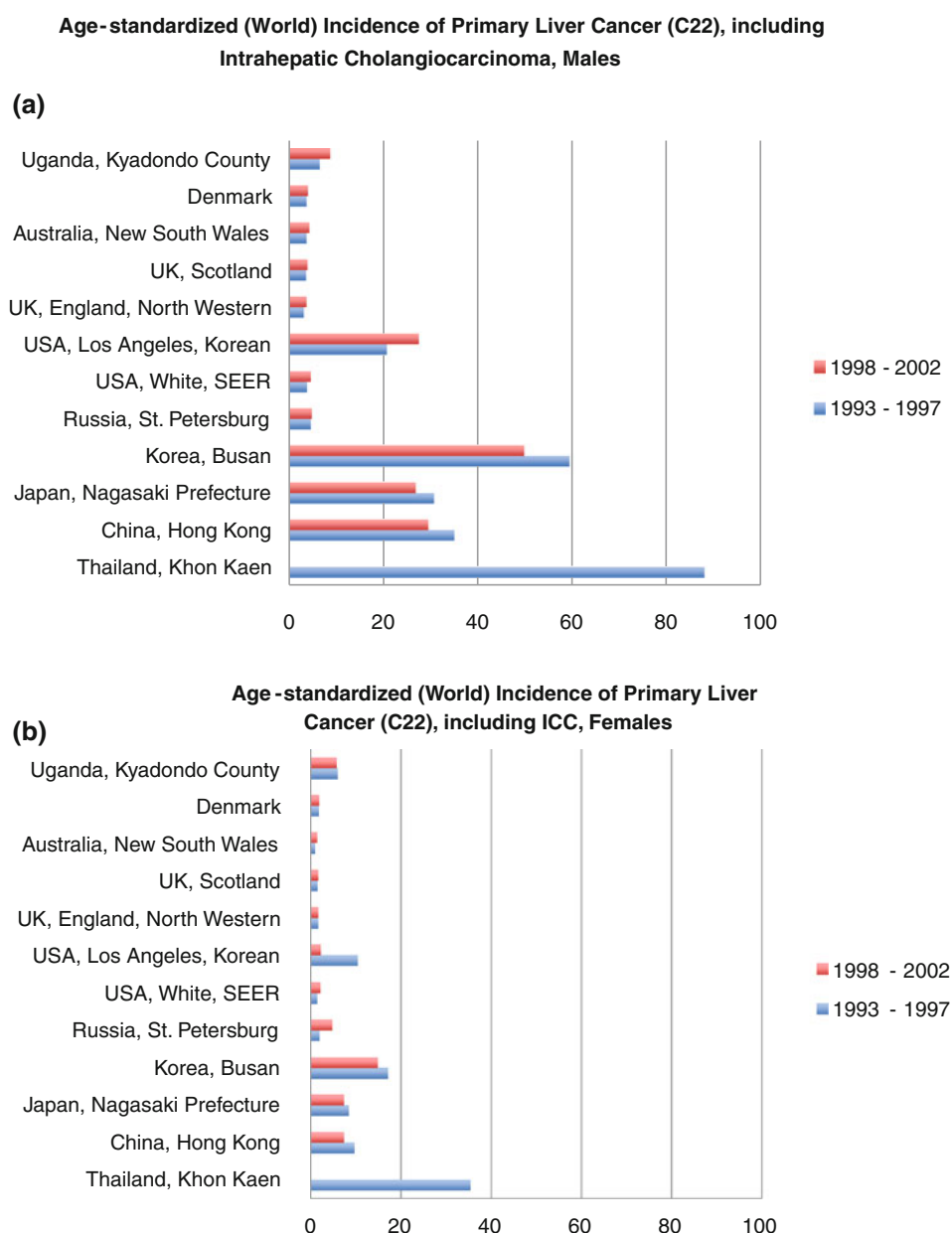
In its recent usage, cholangiocarcinoma (CCA) frequently refers to both carcinomas of the intrahepatic and extrahepatic (hilar, mid, and distal) biliary tree [1, 19]. The division of tumors in this manner dictates the approach to surgical resection. Historically, distribution of cholangiocarcinoma has been reported as perihilar in 50–70 %, intrahepatic in 6–10 %, and distal in 25–27 % [1, 19]. This distribution, however, has recently been called into question given the concern for underestimation of ECC and overclassification of ICC that occurred with adding a specific ICD-O-2 code for Klatskin tumors [15–18]. Re-analysis of 3,350 CCA cases in the United States (US) Surveillance, Epidemiology, and End Results (SEER) database from 1992 to 2000 suggests that the errant ICD classification overestimates the incidence of ICC by 13 % and underestimates that of ECC by 15 % [17].

Within the published literature, ICC and ECC may not be distinguished from each other or may be reported in conjunction with either HCC or gallbladder carcinomas, respectively. Without the accompanying histopathology of the tumors, documenting and reporting the true incidence, prevalence, and risk factors associated with cholangiocarcinoma are fraught with uncertainty. Known risk factors for CCA include liver fluke infection, primary sclerosing cholangitis, and hepatolithiasis [20–24]. Chronic inflammatory biliary conditions causing a repetitive cholangiocyte injury and repair cycle, such as hepatitis B or C virus infection, cirrhosis, alcohol use, obesity, non-alcoholic fatty liver disease (NAFLD), and exposure to certain carcinogens are also implicated in CCA pathogenesis [2, 25–27].

Publications from large, population-based registries provide the best national and regional epidemiologic data on cholangiocarcinoma. There continues to be increasing support for differential risk factors in developing ICC versus ECC [7]. Certainly, there is wide demographic and geographic variability in the development of cholangiocarcinoma as reflected in the regularly updated WHO/IARC publication, *Cancer Incidence in Five Continents* (Fig. 1a, b). Differential outcomes following surgical resection for ICC versus ECC have also been reported [28]. The incidence of ICC continues to rise while that of ECC is relatively static or minimally decreasing [29–31].

Although significant progress has been made in the diagnosis and treatment of CCA in the last two decades, mortality continues to rise in low-prevalence areas of the United States, Europe, United Kingdom, and the non-high-risk areas of Asia and the Middle East [29, 30]. This chapter describes the incidence, risk factors, and mortality associated with cholangiocarcinoma.

Fig. 1 Age-standardized (World) incidence (per 100,000) of primary liver cancer (C22) including ICC (C22.1) in high-risk, low-risk and endemic population areas, **a** males, **b** females. Rates shown on *x*-axis are per 100,000 person-years. Source WHO IARC [5, 6]



1.1 Incidence and Mortality Trends: United States of America

In the US for the year 2012, there were 2,580 cases of ICC and 7,410 ‘other biliary,’ mostly ECC, cancers reported, amounting to approximately 3 % of total gastrointestinal malignancies using the US SEER program. SEER cancer statistics predict national incidence rates for cancer based on aggregate information from the North American Association of Cancer Registries (NAACR), which represents approximately 95 % of the US population. In 2013, it is estimated that there will be a total of 30,640 cases of ICC and primary liver cancer [3, 4]. The SEER Cancer Statistics Review (1975–2009) covers 18 designated areas and approximately

26 % of the US population, and reported a total age-adjusted incidence for ICC as 0.7 per 100,000 males and 0.6 per 100,000 females for 2005–2009. These latest incidence figures reflect a 1.5 % annual percentage change (APC) decline in males and a 0.6 % APC decrease in females between 2000 and 2009 [3, 4]. The overall trend for all US race and ethnicities (white, black, Hispanics, Native Americans, and Asian-Pacific Islanders) shows an increase in ICC from initial data collected in 1973–1975 (Table 1a and b). The age-adjusted incidence rates are highest in Hispanic Americans (1.22 per 100,000) and lowest in African-American males and females (0.3 per 100,000) [32].

Mortality rates have increased across all racial and ethnic groups by at least 3.5 % annual percentage per year, except

Table 1 SEER incidence, US mortality and survival percent for all and selected races in (a) ICC, and (b) ECC

	Incidence	Mortality	Survival (%)
(a) SEER incidence, US mortality and survival for ICC, 2005–2009			
All races, total	0.6	1.2	6.6
All races, male	0.7	1.4	6.0
All race, female	0.6	1.1	7.1
Whites, total	0.6	1.2	6.4
Whites, male	0.7	1.4	6.4
Whites, female	0.5	1.1	6.3
Blacks, total	0.5	1.1	7.9
Blacks, male	0.5	1.3	0.0
Blacks, female	0.5	1.0	9.7
(b) SEER incidence, US mortality and survival for ECC, 2005–2009			
All races, total	1.8	0.4	15.7
All races, male	2.2	0.5	16.9
All race, female	1.5	0.4	14.4
Whites, total	1.8	0.5	15.6
Whites, male	2.2	0.5	17.1
Whites, female	1.5	0.4	14.4
Blacks, total	1.7	0.4	13.3
Blacks, male	2.1	0.4	14.1
Blacks, female	1.5	0.3	12.7

Incidence and death rates are per 100,000 and are age-adjusted to the 2000 US Standard population. Mortality data are derived from US Mortality Files, National Center for Health Statistics, Centers for Disease Control and Prevention (CDC). Surveillance, Epidemiology, and End Results (SEER) data is from 18 US areas. Survival data (2002–2008) is based on follow-up of patients into 2009. *Source* SEER Cancer Statistics Review, 1975–2009 [3]

in Asian/Pacific Islander women in whom mortality rates have decreased at a rate of 0.2 % per year [32]. The age-specific distribution of ICC and ECC peaks between the ages of 65–84 years and is relatively uncommon below age 45 and above age 85 [3, 4, 32]. The SEER-reported incidence of ECC, reported as ‘other biliary cancers,’ incorporates other non-epithelial tumors, though a similar age distribution and male preponderance for ICC is seen. Using cumulative SEER data from 13 sites registered from 1992 to 2009, there has been a 3.5 % APC increase in incidence of primary liver cancer and ICC. Recent United States data corroborate a continued trend of an increasing incidence of ICC from 0.13 per 100,000 in 1973 to 0.67 per 100,000 in 1997 [31].

In a review of the 30-year trend in SEER mortality since 1973, Nathan et al. demonstrated improved survival for both ICC and ECC. Five-year survival for resected ICC in the period 1973–1992 when compared to 1993–2002 demonstrated a significant improvement to 22.9 % from 16.5 % [29]. Their model reported yearly increased survival from 1992 onwards, resulting in a 34.4 % improvement through

Table 2 Incidence rates (per 100,000) of extrahepatic bile duct cancers, including ECC in the United States, 1997–2002 age-standardized to the US 2000 standard population

Age-standardized incidence rates of extrahepatic bile duct cancers, United States, 1997–2002		
	Male	Female
All races/ethnicities	0.93	0.61
American Indian/Alaska Native	0.90	0.76
White	0.91	0.60
Black	0.82	0.55
Asian-Pacific Islander	1.50	0.92
Hispanic	1.14	0.87
Non-hispanic	0.92	0.60

Source Goodman, 2007 [7]

all three decades. Improved survival was more marked in ECC where multivariate modeling demonstrated a 23.3 % increase in adjusted survival per decade and a cumulative improved survival of 53.7 % from 1973 to 2002 [29].

Mortality from ECC is also steadily increasing from 0.07 per 100,000 in 1973 to 0.69 per 100,000 in 1997. The incidence of ECC has been otherwise static to slightly decreased in the last few decades. This may be a result of discrepant coding, or the impact of a decreasing incidence of gall-bladder cancer [17]. The trend of increasing incidence of ICC and decreasing to static incidence of ECC is preserved even when making allowances for the differential coding that place Klatskin tumors with ICC with ICD-O-2 (1995–2001) and to either ICC or ECC with ICD-O-3 [15, 17, 18, 33].

Within a descriptive study of biliary tract cancers (gall-bladder, extrahepatic, ampulla of Vater) from 1997 to 2002, cancers from 11,261 men and 15,722 women were identified from network or US population-based registries and normalized to the US census population in 2000 [7]. Populations of Native Alaskan/Native American and Asian-Pacific Islanders showed significantly higher rates of ECC compared with whites or blacks. Incidence rates for ECC were higher overall in men than women. Incidence rates were comparatively lower in all women with the highest rates in Native Alaskan/Native American and Asian-Pacific Islanders (Table 2).

Age-specific incidence rates in men showed a steady increase in incidence beginning in the early 20s during which the incidence curves for Asian-Pacific Islanders diverged and increased at a dramatically different rate than white or black males after age 50 [7]. Similar divergence was seen with Native Alaskan/Native Americans with the significant divergence occurring after age 75. A recent longitudinal view (1973–2007) of biliary tract cancers (ICC, ECC, and ampulla of Vater) in Native Alaskans established a rate of 2.6 per 100,000 compared to 1.2 per 100,000 and 1.0 per 100,000 in the US white and black populations, respectively, using the age-adjusted rates to the world standard million

population. Remarkably, an increase in the rate of biliary tract cancer in Native Alaskan women from 0.9 per 100,000 (1973–1992) to 2.6 per 100,000 (1993–2007), without a similar increase in the males for the same interval periods (3.5 per 100,000 and 3.4 per 100,000), was noted [34].

Various regional US populations comprised of non-specific risk groups, such as Olmsted County, Minnesota, reported trend results that are consistent with US national incidence and mortality data for biliary tract cancers and CCA. Over the study period of 1976–2008, the age-sex-adjusted incidence of ICC in 116 patients increased from 0.3 to 2.1 per 100,000 person-years with the increased incidence in men accounting for the majority of the change [35]. During this time period, no overall increase in biliary tract cancer was seen, which was ascribed to concomitant decreases in gallbladder cancers, in women predominantly. Other variations within the US population may reflect socioeconomic status, underlying ethnicity, and other environmental factors. In general, Hispanics have a higher prevalence of hepatobiliary cancers and ICC than whites in the US, with a contrastingly higher predominance in females than males [32]. The prevalence of ICC in Asian-Pacific Islanders is not significantly different from other US population groups when reported for cancer cases 1990–2000 [32].

1.2 Incidence and Mortality Trends: United Kingdom, Europe, and Australia

In the United Kingdom, the Office of National Statistics (ONS) capture cancer data for England and Wales. The ONS noted an increase in the age-adjusted incidence and mortality from primary liver cancer, attributable primarily to a rise in HCC. The age-adjusted incidence for ICC (not histologically verified) was lowest in the period 1971–1973 at 0.11 per 100,000 males and 0.09 per 100,000 females, but steadily increased by 12-fold over the next three decades, to 1.33 per 100,000 and 1.06 per 100,000, respectively [36]. The age-adjusted incidence of ECC (separate from gallbladder cancers) declined between 1971–1973 and 1999–2001 in both males and females, although the rates rose in males in the decade between 1971–1973 and 1981–1983, before and then subsequently declining [36]. The mortality rate from primary liver tumors (including ICC) doubled between 1976 and 1994 [37]. Specific morphologic analysis attributed the rise to the increase in ICC rather than HCC [36, 38].

Age-adjusted mortality rates in England and Wales standardized to the European population increased from <0.02 per 100,000 in 1976 to just over 1 per 100,000 in 1994, accounting for differences in coding and uncertainties in death registration. Deaths from ICC reliably

exceeded HCC as the most common cause related to a primary liver tumor in 1993 [38]. Gallbladder tumors and ECC were reported together and showed an overall decrease in age-adjusted mortality from 1974 to 1994. In absolute numbers, the total ECC mortality fell from 413 deaths in 1974 to 176 deaths in 1996. Data from Scotland (1968–1997) are consistent with the rest of the United Kingdom data, with a documented dramatic increase in incidence in ICC of 817 and 851 % in males and females, respectively [39]. The incidence of ICC increased to 1.53 per 100,000 in males and 1.24 per 100,000 in females from 1993 to 1997 compared with 0.12 in males and 0.17 in females (1968–1972). These data reflect small sample size with 9 diagnoses in 1968 and 68 diagnoses in 1997.

In a recent analysis of 12,638 biliary tract cancers, including ECC, diagnosed in England and Wales from 1998 to 2007, the median age at diagnosis was 75 years with an incidence of 2 per 100,000 adjusted to the European standard population [40]. The incidence was relatively static over time and between males and females. A further in-depth analysis of the incidence of ICC and ECC in England and Wales for the period of 1990–2008 reported an increase in age-adjusted incidence of ICC for males from 0.43 to 1.84 per 100,000 and 0.27 to 1.51 per 100,000 in females [33]. The trend in ECC declined to 0.51 from 0.78 per 100,000 in males and to 0.39 per 100,000 in females in the same time period. The noted trends in incidence were maintained in the England and Wales populations after addressing possible discrepancies in the coding of ‘Klat-skin’ or ‘hilar’ tumors, by analyzing the data with Klatskin tumors in both the ICC and ECC groups [33] (Fig. 2).

Comparative incidence reporting in a variety of populations worldwide, using the official WHO-validated mortality database, showed steady age-adjusted increases related to ICC from 1979 to 1997 in the United States, Japan, Australia, England and Wales, and Scotland [41]. The opposite trend was seen for ECC and gallbladder cancers in the same group of nations. Of the countries with complete WHO mortality data that were studied, France displayed no change in age-adjusted mortality for ICC and ECC. Japan and Italy both reported increased mortality for ECC and gallbladder cancers [41] (Fig. 3).

Reports of cancer incidence and survival data from Norway, Sweden, Denmark, Iceland, and Finland is made possible through the NORDCAN collaboration network. Primary liver (including ICC) and gallbladder (including ECC) each represent 1–2 % of the cancer burden within these countries [42, 43]. Specific data extracted from the Danish Cancer Registry shows a decrease in age-adjusted incidence of ICC (1.5 to 0.62 per 100,000) and ECC (1.05 to 0.65 per 100,000) from 1978 to 2002 in both men and women. The highest incidence of ICC and ECC was found in diagnosis groups aged 60–79 and aged 80 and older. The

Fig. 2 Age-standardized incidence rates (ASIR) per 100,000 of ICC and ECC in England and Wales, 1990–2008, Males and Females combined. ICC (C22.1), excludes Klatskin tumors (M8162/3). ECC (C24.0), includes Klatskin tumors (M8162.3). Modified and reprinted with permission from Khan et al. 2012 [33]

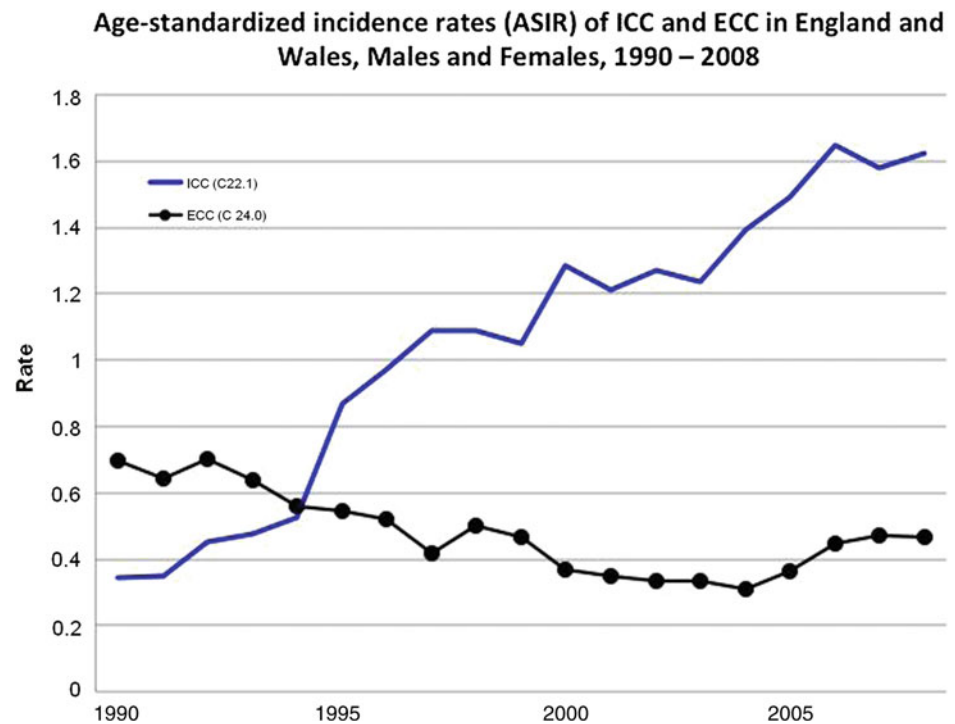


Fig. 3 Age-adjusted percentage change in incidence rate (per 100,000) of ICC and ECC in Select Countries, 1979–1997 [41]. Percent change is shown on y-axis and select countries on the x-axis. Numerical values for absolute percentage change are shown with the corresponding column

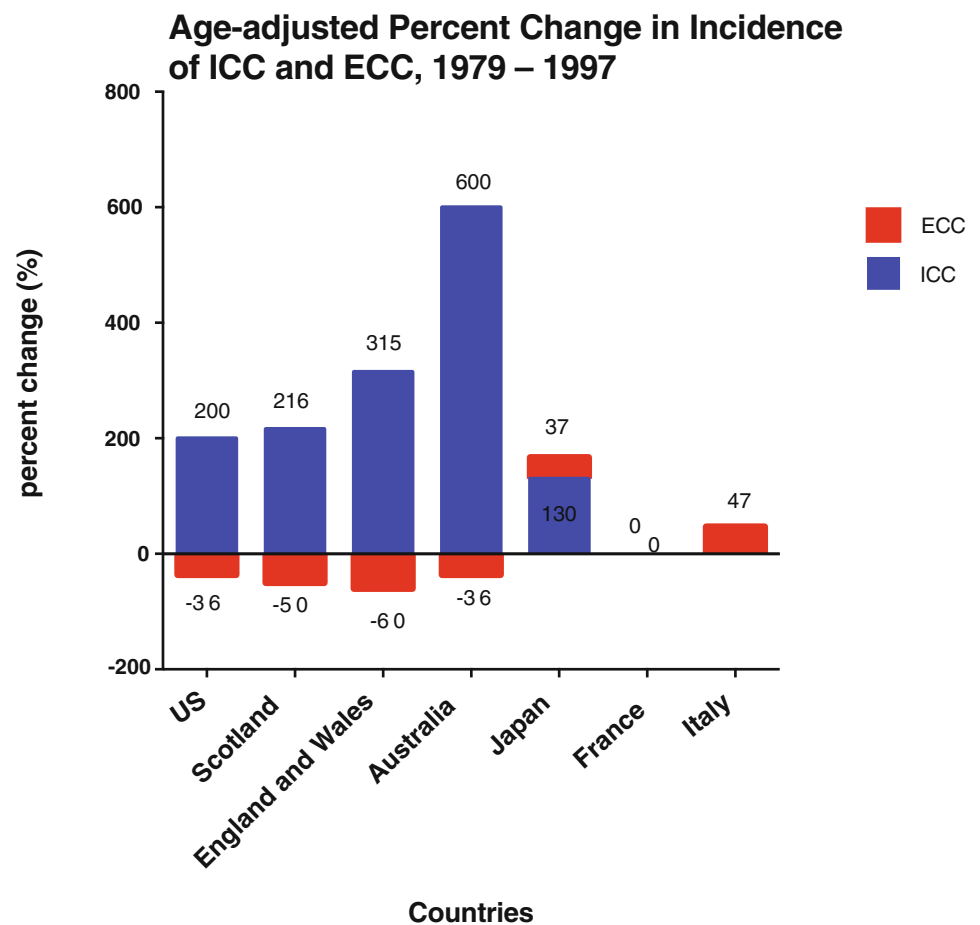
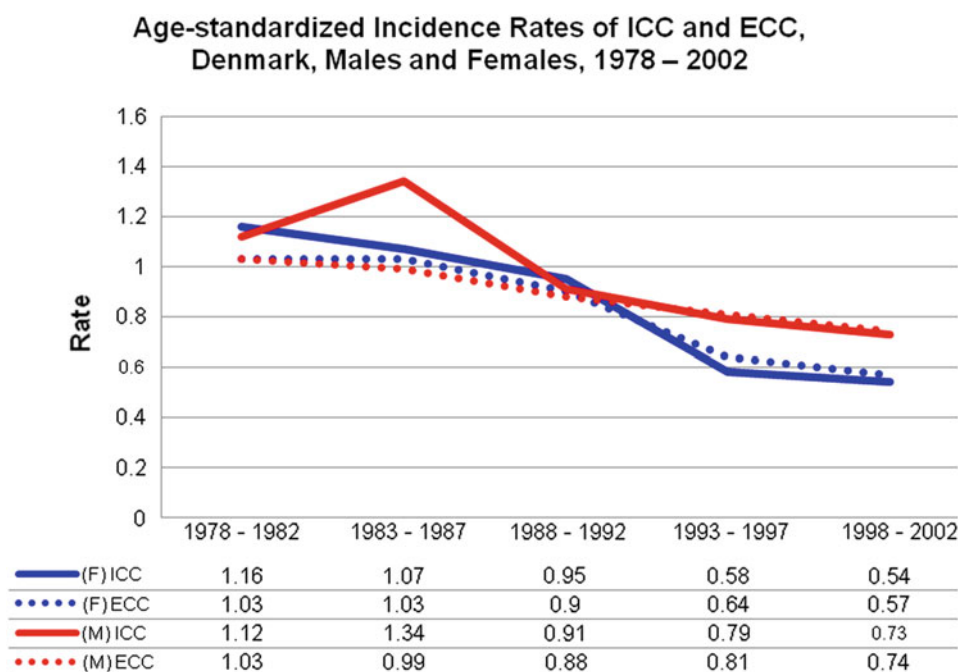


Fig. 4 Age-standardized (US 2000) incidence rates (per 100,000) of intrahepatic cholangiocarcinoma (ICC) and extrahepatic cholangiocarcinoma (ECC) for Denmark, Males (M) and Females (F), 1978–2002 [44]. Incidence rate is shown on the y-axis and grouped time intervals in years are shown on the x-axis with the graphed incidence data shown below in tabular format



overall temporal incidence of ECC was 3.67 per 100,000 and 5.32 per 100,000 in the age division 60–79 and 80 and older, respectively [44] (Fig. 4).

In the Netherlands, a different trend was seen than that in Scandinavia. ICC diagnoses corresponding to ICD-O codes C22.1 from 1989 to 2009 were analyzed from the Netherlands Cancer Registry (NCR) data [45]. For both males and females, the three-year moving averages showed a 5 % annual percentage decline in age-adjusted incidence from 1989 to 1998 and a subsequent 9.4 % annual percentage increase from 1998 to 2009. Most marked was a 3 % annual percentage increase in ICC incidence within the group aged 45–49 years, where the increases in age-specific incidence are seen in later years in other low-endemic area studies [3, 34, 46]. Epidemiologic data from Italy reflect a trend most consistent with the UK data for 55,000 patients diagnosed in the period 1998–2005. Similarly, a French population-based study from Burgundy reported stable rates of age-adjusted ICC incidence. The adjusted incidence rates for ICC in 1976–1980 and 2001–2005 were 0.3 per 100,000 and 0.2 per 100,000, respectively, and 1.1 per 100,000 in 1976–1980 and 2001–2005 for ECC [47].

Regional-specific cancer incidence and mortality data across Europe are available in the WHO/IARC publications, *Cancer Incidence in Five Continents, Vol. IX* and Scientific Publication No. 159 [6, 48]. Determining the specific incidence and trend data for ECC and ICC in these aggregated publication is difficult due to the grouping of liver and intrahepatic bile duct tumors, and gallbladder cancers with other biliary tumors. Significant efforts have been made to understand the coding of tumors between ICD-8, 9, and 10

and ICD-O and O-2, in reporting data for ICC and ECC as biliary tract cancers. Notwithstanding this, the data bear out the worldwide trend of increased incidence of ICC and stable to declining incidence of ECC in the low- to normal-risk populations, and select high-risk groups within low-risk populations in North America, Europe, and the United Kingdom, with the exception of Denmark [17, 30, 31, 33, 36, 39, 47, 49].

1.3 Cholangiocarcinoma in Asia and Liver Fluke Infestation

The IARC Working Group on the evaluation of carcinogenic risks to humans formally addressed the association of cholangiocarcinoma to infections with the trematodes, *Opisthorchis viverrini*, and *Clonorchis sinensis* [20, 23, 50–52]. The liver fluke *Opisthorchis viverrini* is endemic to north and northeastern Thailand, western Malaysia, Cambodia, Vietnam, and Laos. Transmission to humans is via consumption of raw fish and water contaminated with sewage and agricultural pollutants [20, 50, 53, 54]. Infection with the trematode, *Clonorchis sinensis*, is the more common infectious agent related to cholangiocarcinoma in China, Korea, Taiwan, and Japan [50]. Cholangiocarcinoma in the Eastern European nations of Kazakhstan, Russia, Siberia, and Ukraine is likely related to transmission of *O. felinus* in freshwater fish and polluted water [50, 52]. The evidence supporting the association of *Schistosomiasis japonicum* with liver cancer and cholangiocarcinoma has been less convincing [20, 55].

The highest national prevalence data for *O. viverrini* infections are found in Laos (37 %) and Thailand (9.4 %) and for *C. sinensis* infections in Hong Kong (5.6 %) by most recent estimation [56]. Of the 35 million people infected with *C. sinensis* worldwide, an estimated 10–15 million live in China [57]. While public health efforts aimed at prevention and treatment are most advanced in these endemic trematode infection areas, the population-at-risk for infection is in excess of 67 million for *O. viverrini* alone [50]. Indeed, despite educational efforts, biliary tract complications related to liver fluke infection include cholestatic damage, inflammatory lesions, cirrhosis, and cholangiocarcinoma. Carcinogenesis is thought to be related to the chronic inflammation and attempts at cholangiocyte repair incited by trematode infection [58–60]. Cholangiocarcinoma related to food-borne trematodiasis is the most common cause of death. The calculated odds ratio (OR) for developing CCA following *C. sinensis* infection is 6.1 and with *O. viverrini* infection is 4.4 [56]. A recent meta-analysis of CCA risk factors in Asian countries calculated an OR of liver fluke infestation (both *C. sinensis* and *O. viverrini*) of 4.8 [61].

The trends in primary liver cell cancer, including ICC, are detailed in Table 3 [5, 6]. While these data do not specifically detail the trends in ICC alone in areas of high-risk and low-risk populations and in endemic areas of cholangiocarcinoma, the data reflect the demonstrated trends in ICC seen in population-based studies given a rather static incidence in HCC [5, 6].

The region of Busan (Pusan) in South Korea continues to have one of the highest areas of primary liver cell cancer worldwide with 1990 incidence rates as high as 74.8 per 100,000 and 15.6 per 100,000 in males and females, respectively, for the age group of 35–64 years [62]. Intrahepatic cholangiocarcinoma is estimated to account for 20 % of total cases. High relative risk (RR) values for *C. sinensis* infection and alcohol consumption were associated with CCA in this population, whereas hepatitis B and C infections were more strongly associated with HCC. Additionally, case-matched studies from a tertiary referral practice demonstrated a high OR of ICC (OR 16.0) and ECC (OR 7.0) with associated *C. sinensis* exposure in cases with at least one of six parameters—history of ingesting raw freshwater fish or evidence of *C. sinensis* infection on stool microscopy, serology, pathology examination, or skin testing [63]. Again, an increased risk of CCA was not seen with concurrent hepatitis B or C infections [63].

The major risk factors for CCA in Asia are male gender, excess alcohol consumption, *C. sinensis* infection, and consumption of raw fresh water fish based on the report published in 2010 from the Korean Multicenter Cancer Cohort [64]. The study, based on 2000–2004 data, demonstrated that incidence and mortality rates closely aligned with the varying incidence rates of *C. sinensis* infection in

Table 3 Age-standardized world (ASRW) incidence rates (per 100,000) showing trends in incidence of primary liver cancer (C22) including intrahepatic cholangiocarcinoma for high-risk, low-risk and endemic population areas

Age-standardized incidence rates in primary liver ICC in high-risk, low-risk and endemic population areas				
1993–1997		1998–2002		Location
Male	Female	Male	Female	
88.0	35.4	–	–	Thailand (Khon Kaen)
35.0	9.7	29.5	7.3	China (Hong/Kong)
30.7	8.4	26.8	7.3	Japan (Nagasaki Prefecture)
59.4	17.1	49.8	14.9	Korea (Busan)
4.6	2.0	4.8	2.1	Russia (St. Petersburg)
3.8	1.4	6.2	2.2	USA (White SEER)
20.7	10.4	27.5	10.2	USA (Los Angeles, Korean)
3.1	1.7	3.7	1.7	UK, England (North Western)
3.6	1.5	3.9	1.7	UK, Scotland
3.7	1.0	4.3	1.4	Australia (New South Wales)
3.7	1.8	4.0	1.9	Denmark
6.5	6.0	8.7	5.8	Uganda (Kyadondo County)

Source Parkin et al. [5] (1993–1997) and Curado et al. [6] (1998–2002)

the studied areas. The low and moderate endemic *C. sinensis* infections areas of Chungju (7.8 %) and Haman (31.3 %) showed age-adjusted incidence rates of 1.8 per 100,000 and 5.5 per 100,000 with corresponding age-adjusted mortality of 1.1 and 2.6 per 100,000, respectively. A further study using the Korean National Cancer Incidence Database (KNCID) from 1995 to 2004 attributes 9.5 % of CCA to liver fluke infection in low- to moderate-risk areas and in up to 22.6 % of CCA cases in high-endemic areas of Korea [65]. Incidence rates in Korean males were more than twice those of females [65]. Incidence rates of 4.1 per 100,000 in males and 1.8 per 100,000 in females were roughly twice those in the United States (1996–2000) and four times those in England and Wales (1991–2001). As outlined in Table 4, the rates of primary liver cancer (HCC and CCA) are decreasing and have been since 1985 [65, 66].

Age-adjusted rates of ICC and ECC in east and south-eastern Asia, using data collected from 18 cancer registries in Asia together with data from US and other Western registries, corroborate the rise in CCA worldwide [66]. Among populations of China, Korea, Japan, and Thailand, regional variations in the incidence of CCA are seen, with the highest rates of ECC noted in Korea. The areas of highest prevalence are concentrated around the Nakdong River and its lower extents [61, 62]. Though the majority of CCA incidence variation is related to varying prevalence of liver fluke infection, there are additional, as yet unknown, environmental, and ethnic factors that may explain the differential presentation of ICC and ECC within a population.

Table 4 Age-standardized incidence rates of intrahepatic cholangiocarcinoma (ICC) and extrahepatic cholangiocarcinoma (ECC) for selected regions in Asia, males and females, 1998–2002

Age-standardized incidence rates of intrahepatic cholangiocarcinoma (ICC) and extrahepatic cholangiocarcinoma (ECC) for selected regions in Asia, males and females, 1998–2002				
	ICC (C22.1)		ECC (C24)	
	Male	Female	Male	Female
China, Qidong	10.3	4.6	0.0	–
China, Guangzhou	0.3	0.1	1.1	0.8
China, Hong Kong	2.3	1.7	0.3	0.2
China, Shanghai	7.4	4.9	1.4	1.4
Japan, Hiroshima (1996–2000)	1.7	0.8	2.4	1.2
Japan, Osaka	1.7	0.9	2.7	1.5
Korea KCCR (1999–2002)	5.4	2.5	3.3	1.5
Korea, Busan	5.8	3.4	4.2	2.3
Korea, Daegu	5.7	2.6	4.1	2.2
Korea, Daejeon	5.0	2.1	2.8	1.3
Philippines, Manila	1.3	0.9	0.1	0.1
Singapore, Chinese	1.1	1.1	0.4	0.3
Taiwan	4.3	3.9	0.7	0.5
Thailand, Khon Kaen	71.3	31.6	0.4	0.1
Thailand, Chiang Mai	8.2	4.0	0.4	0.2
Thailand, Bangkok	2.5	1.4	0.3	0.1
Thailand, Songkhla	1.6	0.5	0.1	0.2
Viet Nam, Hanoi	0.1	0.1	0.0	0.0

Source Shin et al. [66]

The Khon Kaen region of Thailand accounts for 70–90 % of primary liver tumors, as compared to high-risk areas, like Busan, Korea (20 %), Japan (5 %), and worldwide (10–25 %) [54, 65–67]. The highest prevalence of liver fluke infections occurs in the north (19.3 %), north-east (15.8 %), and central (3.8 %) regions of Thailand [54, 61]. *O. viverrini* infection is the strongest risk factor for developing CCA, and all areas have shown appreciable decreases over the last three decades [61, 68]. The prevalence of *O. viverrini* declined to an overall 9.6 % (6 million people) in 2001 from 14 % (7 million people) in the early 1980s [54, 61]. Average rates of CCA in the Khon Kaen Province area were 118 per 100,000 with an average prevalence of 24.5 % using data from 1990 to 2001 [54].

For the rest of Thailand, data in 2002 demonstrated an incidence of 71.4 per 100,000 for ICC in males and 31.6 per 100,000 in females. Corresponding rates of ECC in Korea are dramatically less at 0.4 per 100,000 in males and 0.1 per 100,000 in females [66] (Fig. 5a, b). Recent results documenting the outcomes of resected ECC in a cohort of 58 patients in Khon Kaen, Thailand, reported a 10.8 % 5-year

survival rate that is somewhat reflective of the outcomes worldwide [69]. Survival remains poor despite public health education regarding the consumption of raw fish, more rigorous surveillance of at-risk populations, and increased efforts to treat the trematode infections that precede development of cholangiocarcinoma. Studies show similar poor long-term outcomes following surgery in patients resected for cholangiocarcinoma with and without an underlying trematode infection [53, 69, 70].

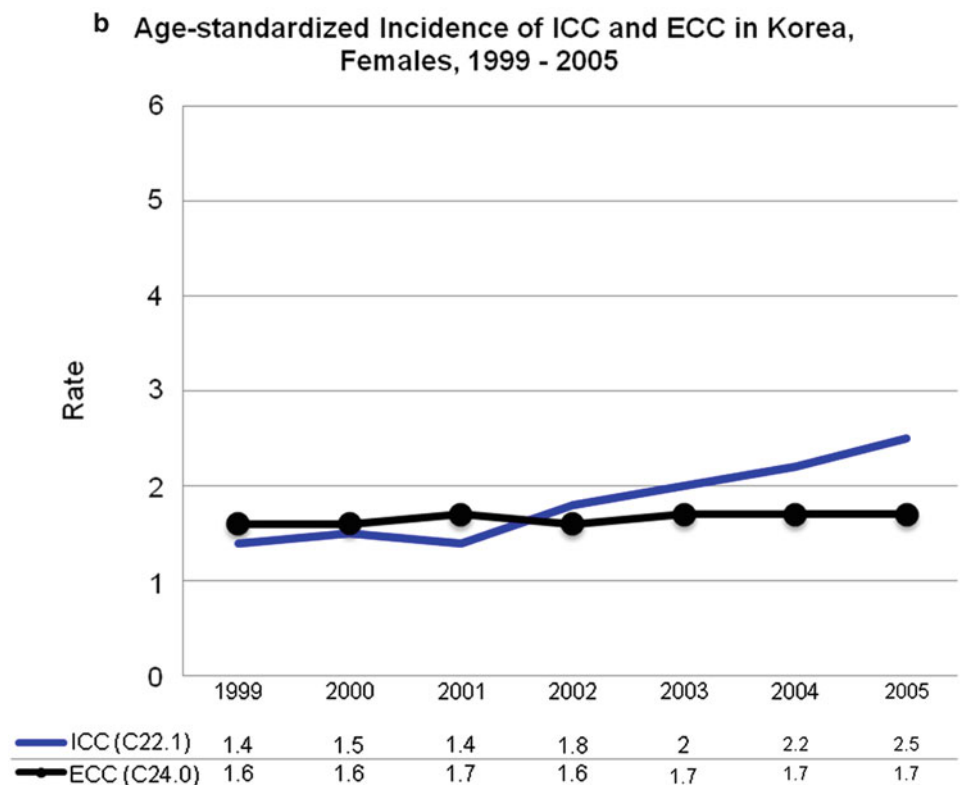
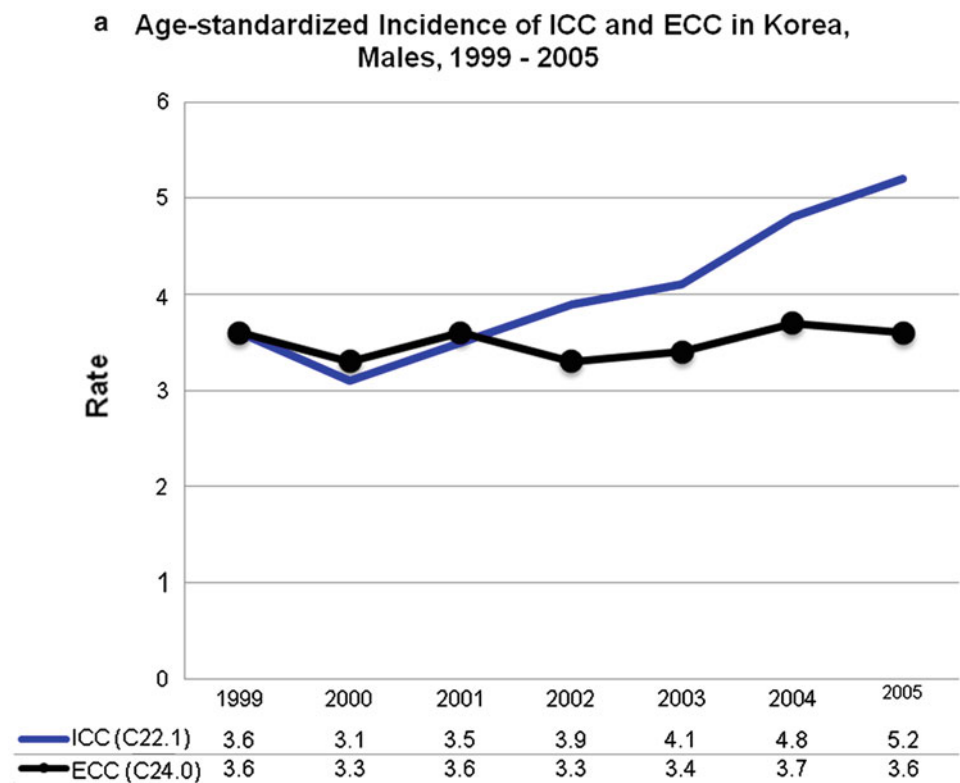
The island of Japan has a higher risk than the rest of world for CCA, but a lower risk than Korea, China, Japan, and Thailand (Table 1) [5, 6, 41]. Bile duct cancers account for over 45 % of biliary tract cancers studied through the Japanese Biliary Tract Cancer Statistics Registry [71, 72]. The Japan Public Health Center Prospective Study reported outcome data on patients with ECC collected between 1990 and 1994 and found an association of cholelithiasis with the development of ECC in a study limited to gallbladder cancer and ECC [73]. Improvements in diagnostic techniques, patient selection for operation, and safety of extensive resections have improved over the last decade. Even though rates of CCA have declined overall in Asian populations, the overall survival still remains poor for this cancer once it develops.

1.4 Choledochal Cysts, Caroli's Disease, and the Abnormal Pancreatobiliary Junction

Choledochal cysts (Types I–IV) involve the extrahepatic biliary tree, while Caroli's disease (Type V) is defined as cystic dilation of the intrahepatic biliary tree [74]. Choledochal cysts are most common in Asia, particularly in Japan, and account for one-half to two-thirds of reported cases [74–76]. Type 1 choledochal cysts are most common, and there is a 3–4:1 preponderance in females [77–80]. The frequency of choledochal cysts is estimated between 1:13,000 and 1:2 million live births [78, 81].

From very early reports, an association of cancer concurrent with choledochal cysts was noted [74, 75, 82]. In a large study of 1,433 Japanese patients across all ages, the incidence of cancer related to a choledochal cyst was 3.2 % and predominantly adenocarcinoma, though cases of squamous carcinoma and mucinous adenocarcinoma have been reported. Of all patients in the series, 45 % were 10 years of age or less and the choledochal cyst-related cancers occurred in patients with a mean age of 32 (range, 15 to 66 years) [75]. The prevalence of cancer is <1 % in the first decade of life, increasing to 6.8 % in the second decade of life, and rising to >14.3 % in those over the age of 20 [82, 83]. Adenocarcinoma in patients as young as 12 and 15 years of age has been described [74, 84].

Fig. 5 Age-standardized incidence rates (ASIR) of intrahepatic cholangiocarcinoma (C22.1) and extrahepatic cholangiocarcinoma (C24.0) in Korean, **a** males and **b** females. Rates shown on x-axis are per 100,000 person-years. Overall rate for ICC is 4.1 males, 1.8 females with a +7.9 % and +10.6 % annual percentage change (APC), respectively, for time period. Overall rate for ECC is 3.5 males, 1.7 females with a +0.3 % and +1.3 % APC, respectively, for time period. Source Shin et al. 2010 [65]



Cholangiocarcinoma is the most common cancer that occurs with choledochal cysts, and the patient risk is 20–30 times that of the normal population [79]. Gallbladder malignancy comprises 10 % of cancers associated with choledochal cysts, and CCA occurs in about 7 % of patients with Caroli's disease [85, 86]. Synchronous or metachronous identification of CCA with treatment of choledochal cysts is reportedly as high as 20–28 % in Western literature and 8–30 % in reports from Asia [78, 81–83, 87]. More often, the literature describes adult patients who develop cholangiocarcinoma on long-term follow-up after a surgical intervention in infancy or adolescence [85, 88]. In the most recent two decades, published literature has shown a rate of 0–33 % CCA development following excision of choledochal cysts [79].

The theories of CCA pathogenesis in choledochal cysts include biliary stasis, exposure to infected bile and its carcinogenic byproducts, exposure to pancreatic amylase because of an abnormal pancreato-biliary junction (APBJ), and chronic inflammation related to biliary track stones [58, 59, 89, 90]. An APBJ is found concurrently with choledochal cysts in 39–92 % of cases [89]. Manometric studies have demonstrated high pancreatic amylase secretion with the cyst substance and noted pressure changes within the biliary tract system whereby reflux of pancreatic enzymes occurs [91]. The Japanese literature suggests that choledochal cysts do not occur without an APBJ [89]. The biliary tract epithelium may of itself be abnormal and more susceptible to the malignant degeneration that normally occurs in an age-dependent fashion. Mucosal adenomatous hyperplasia of cholangiocytes in response to chronic inflammation initiates the hyperplasia-metaplasia-carcinoma sequence that culminates in carcinogenesis [92, 93].

Other conditions that cause chronic inflammation of the biliary tree predispose patients to cholangiocarcinoma. Prior manipulation of the biliary tree for benign disease is an example of an acquired risk factor for CCA. A 5.5 % increased risk has been reported for patients that have undergone biliary-enteric bypass procedures, usually for choledocholithiasis [94]. The risk of CCA is highest following choledochoduodenostomy (7.6 %) and lowest following Roux-en-Y hepaticojejunostomy (1.9 %) [94].

Transduodenal sphincteroplasty for treatment for sphincter of Oddi dysfunction is also implicated as causative in the development of CCA (7.4 %) [95–97]. Other reported cases indicate that the risk continues to be present as many as 1–40 years following the initial intervention [95, 96]. The proposed mechanism for carcinogenesis is stasis of bile infected with intestinal contents secondary to the new biliary-enteric configuration. Conflicting data suggest that manipulation of the biliary tract for otherwise benign, or non-bile duct cyst disease, is itself a risk factor for cholangiocarcinoma [94, 98], while other reports do not support an increased cancer susceptibility [99]. Secondary stone

formation caused by anastomotic stricturing and recurrent cholangitis after biliary-enteric anastomosis is also implicated in CCA metaplasia [100–102]. The constant irritation of the biliary tract by stones, which may be infected, also contribute to cholangiocyte metaplasia [100, 101].

Primary intrahepatic stones, or hepatolithiasis, are common in East Asia and a known risk factor for the development of CCA. Japan and Taiwan have the highest incidence of cholangiocarcinoma complicating long-standing hepatolithiasis at 1.5–9.4 % and 2.4–5.0 %, respectively [21, 103–106]. The overall incidence in the literature is estimated at 4–11 %, but has been reported to be as high as 20 % in a Taiwanese population [104]. Following hepatectomy for stone disease, one series of 29 patients from Japan documented a 17.1 % incidence of ICC. Stone disease predominantly affects the left lobe of the liver, though right-sided and bilateral stone disease is also described [105, 107, 108]. Tumors typically develop first within the intrahepatic ducts in close proximity to where stones are located though hilar tumors and ECC have been described [21, 104, 105, 108].

Survival following surgical resection in patients with hepatolithiasis and CCA is poor and is similar to patients with uncomplicated CCA. The cycle of biliary sepsis requiring drainage can delay and may prevent a patient from presenting for surgical resection. Diagnosing CCA is difficult in the face of hepatolithiasis; however, it should be suspected in patients with long-standing hepatolithiasis (>10 years), deranged liver function tests, age >40–50 years, and lobar atrophy, stricture, or obvious tumor mass on imaging [104, 108, 109]. As such, overall resectability rates may be lower due to advanced disease stage at time of presentation. Favorable long-term survival is associated with completeness of surgical resection rather than any specific impact of hepatolithiasis.

1.5 Primary Sclerosing Cholangitis and Cholangiocarcinoma

The development of CCA in patients with primary sclerosing cholangitis (PSC) is an important risk factor in Western populations. PSC is a chronic cholestatic disease with no optimal liver-directed therapy to appreciably treat disease. Use of ursodeoxycholic acid (UDCA) and immunosuppression with corticosteroids, tacrolimus, cyclosporine, and methotrexate may result in biochemical improvements of liver function tests in affected patients [110]. The natural history of the disease is an insidious onset of intra- and extrahepatic biliary strictures punctuated by bouts of treatment-refractory sepsis [110]. Stenting is used to alleviate biliary obstruction caused by dominant strictures. Cirrhosis, liver failure, CCA, and death are the

most significant complications. In the absence of tumors, the median survival for patients with PSC is between 10 and 12 years, with asymptomatic patient survival of 70 % at 16 years [111–113].

PSC is closely associated with inflammatory bowel disease (IBD) (60–80 %), both ulcerative colitis (UC) (26–68 %) and, to a lesser extent, Crohn's disease (13 %) [1, 22, 67, 114, 115]. The estimated prevalence of IBD with PSC ranges from 60 to 80 % in the United States, Nordic Countries, and Northern European populations with little variation over the last two decades [1, 22, 67, 112, 115]. The annual incidence risk of CCA with PSC ranges from 0.6 to 1.5 % [58, 116–118]. The prevalence of CCA complicating PSC is 7–18 % in the United States and has been reported as 8 and 14.3 % in Sweden and Germany, respectively [112, 114, 119, 120]. On average, patients develop CCA 30–63 months following the diagnosis of PSC [2, 22, 114] with up to 50 % diagnosed with CCA concurrent with their PSC diagnosis [114, 116, 121]. The primary tumor location in PSC-CCA is extrahepatic or hilar in 60–76 % of cases, 16–60 % intrahepatic, and indeterminate or both in 20–35 % [22, 111, 114, 117, 122].

The most complete datasets summarizing the known relationship of PSC with cholangiocarcinoma come from a large referral-based population in Minnesota (USA) and the Nordic Countries (Denmark, Finland, Sweden, Norway, Iceland) [22, 112, 122–125]. The cohort of 604 patients obtained from the Swedish Cancer and Deaths Registry (1970–1998) showed a 13.3 % incidence of patients with PSC developing a malignancy (any cholangiocarcinoma, gallbladder cancer, or HCC). The standardized incidence risk (SIR), adjusted for age and sex, compared to the general population of Sweden was 161 for developing any malignancy [118]. The updated data present information from a specific region of Sweden in patients with PSC who developed CCA between 1992 and 2005 [124]. The incidence of CCA in this cohort was 8.5 % with 5-year and 10-year cumulative incidences of 7 and 11 %, respectively. The SIR for all HPB malignancies was 177 and for CCA was 868 [124]. In the Swedish studies, no influence of duration of PSC to the development of CCA has been documented, in contrast to other Western studies [111, 118, 124]. The literature is relatively sparse on the interaction of PSC and CCA in Eastern populations. After documenting the pattern of PSC in the Japanese population, a further cross-sectional study reported a 3.4 % incidence of CCA in patients with PSC. Approximately 50 % of patients were diagnosed with CCA at the time of the PSC diagnosis. The time to CCA diagnosis was an average of 2.5 years later in the remainder of patients [126].

The gains in improved survival for patients with PSC and CCA have been seen with rapid treatment of sepsis, recognition of and stenting of dominant strictures, and liver transplantation. Using a neoadjuvant chemotherapeutic protocol followed by transplantation, actuarial 5-year

survival rates of 82 % with transplantation compared to 21 % with resection alone have been reported [127]. Other series have seen modest survival rates of 35 % at 5 years with liver transplant for PSC without a chemotherapy protocol [117]. Recurrences in the transplant group are also demonstrably lower (13 vs. 27 %). Up to 20 % of patients with PSC on the waiting list may harbor an undiagnosed cholangiocarcinoma, as found on waiting list surveillance testing or in the liver explant [117, 128].

1.6 Hepatitis B, Hepatitis C and the Effect of Chronic Inflammation, Hepatocyte Injury, and Cholangitis

Historically, no association between chronic Hepatitis B (HBV) and Hepatitis C virus (HCV) infections in the pathogenesis of CCA was reported [62, 129], but this has changed in recent years [27]. The IARC linked, with limited supporting evidence, HBV and HCV infections to CCA through a causal mechanism of inflammation, chronic liver disease, cirrhosis, and fibrosis [51]. Chronic HBV infection is responsible for cirrhosis and the progression to HCC in sub-Saharan Africa and in East Asia. Chronic HCV infection is the predominant risk factor for HCC in Japan and Western countries [5, 6]. The influence of HBV and HCV on CCA is multifactorial. In conjunction with other probably contributory risk factors such as alcohol consumption, smoking, and consumption of nitrosamine-preserved foods, it has emerged as a more likely direct influence, though the exact mechanisms are unknown.

Studies from the United States, Japan, and Italy demonstrated an increased risk of ICC with HCV [130–133]. In direct contrast, other studies from the East, in Taiwan, China, and Korea, show a closer association of HBV infection and ICC [134–137]. In a series that published tumor location, the relationship of HBV or HCV infection is more commonly associated with ICC than ECC [130, 133, 135, 136]. The differential findings do correspond with population and geographic areas in which HBV and HCV are endemic. A recent publication from Taiwan, where both HBV and HCV are endemic, reported a significant association of ICC with HBV and HCV [135]. Additionally, co-infection with hepatitis surface antigen (HBsAg) and anti-HCV core antigen (anti-HCV) confers a higher risk of ICC formation than with either HBsAg or anti-HCV alone [135]. The findings of two separate meta-analyses support the view that HCV and HBV infection plays a significant role in the development of CCA, more often ICC than ECC [138, 139].

Although a new risk factor related to CCA has been described, the relative importance of this contribution for CCA pathogenesis is unclear. The HBV and HCV

seropositivity rate in the patient populations from which results derive ranges from 1.6–49 to 1.2–36 %, respectively [131, 132, 137, 140]. In a 4:1 matched case-control study from Korea, an association of HBV (OR = 2.2) to ICC was found with more significant contributions from diabetes (OR = 3.2), hepatolithiasis (OR = 50), choledochal cysts (OR = 10.7), cirrhosis (OR = 13.6), and *C. sinensis* infection (OR = 13.6) [136]. The relationship to other risk factors is supported by other HCV studies with PSC, diabetes playing a stronger role in CCA pathogenesis than HBV or HCV [132, 141]. The RR of HBV pathogenesis in ICC is stronger in reports from Asian populations compared with other nationalities [138].

Molecular pathologic analysis of tissue samples has found HBV DNA and HCV RNA within the biliary epithelium and has been proposed this as a possible mechanistic theory for tumor growth [142]. The chronic inflammatory process incited by HCV core protein contributes to cellular proliferation of a damaged biliary epithelium [143]. The initiating events of cholangiocarcinoma involve cytokine activation of inflammatory cells and induce nitric oxide synthase (iNOS) within damaged biliary epithelia. DNA damage occurs in conjunction with nitrosylation of thiol and tyrosine residues to propagate further damage [2, 144]. iNOS-damaged DNA contributes to tumor suppressor *p53* upregulation and may also induce damage that renders the *p53*-mediated DNA repair mechanism inactive [92]. This inflammatory cycle is better described in patients with PSC and CCA, and liver fluke infestation and CCA [2, 59]. However, HBV and HCV infection, as an example of a chronic inflammatory process, is a suitable mechanism for induction of the nitric oxide synthase process and its pleiotropic effects on biliary tract carcinogenesis [10]. While HBV and HCV primarily affect hepatocytes, research evidence supports a common hepatocyte progenitor cell origin for hepatocytes and cholangiocytes [11, 12, 145, 146]. Carcinogenic processes within the liver may thus affect susceptible cholangiocytes in close proximity, perhaps also explaining the increased association of HBV and HCV infection with ICC rather than ECC.

1.7 Environmental Factors

Thorotrast, discontinued as a colloidal intravenous contrast agent after 1955, derived from thorium-232, is recognized as a Class I carcinogen by the IARC [67]. Preferential deposition was seen within the substance of the liver (60–70 %), but its affinity was also for the reticuloendothelial system, the spleen and bone marrow. Historically, tumors of the liver presented as HCC or hepatic

angiosarcoma, 10–12 years following the exposure [67, 147]. Dysplasia, or abnormal proliferation of bile ducts, was seen in Thorotrast-induced cases of liver cancer [67]. ECC was the more common of the biliary tract tumors. The IARC drew several conclusions regarding Thorotrast exposure and cholangiocarcinoma from initial data and follow-up of large cohort series from the United States, Germany, Denmark, Japan, and Sweden [148–153]. Thorotrast exposure caused an excess cancer (liver and bile duct) risk of 97 % persisting for up to 50 years, increasing risk and mortality from liver cancer with increasing amount of injected Thorotrast, and an increasing standardized mortality rate (SMR) and RR compared to controls as more time elapsed from first exposure to Thorotrast. One proposed mechanism of pathogenesis is alpha-ionizing radiation particles inducing instability in human mismatch repair genes [154].

1.8 Emerging and Other Risk Factors

Obesity and diabetes as elements of the metabolic syndrome are gaining increasing attention in the pathogenesis of CCA. Both are known risk factors for HCC in NAFLD and other gastrointestinal cancers (esophagus, pancreas, gallbladder, stomach) [155]. In one population case-control study from China, the risk of ECC and other biliary tract cancers increased with the increasing number of diagnostic components of the metabolic syndrome [156]. The increased risk profile persisted for patients with body mass index (BMI) <25 and non-diabetics with three other elements of the metabolic syndrome. Interestingly, high high-density lipoprotein (HDL) (OR = 8.17) and high triglyceride levels (OR = 5.28), additional elements of the metabolic syndrome, gave a higher OR for ECC than for metabolic syndrome as a single risk factor. In a low-risk population from Denmark (1978–1991), diabetes, but not obesity was significantly associated with ICC [157]. Data from the United Kingdom (1987–2002), another low-risk population, also showed a positive association with obesity (BMI ≥ 30) and conferred a 1.5-fold increased risk of developing CCA [158].

The relationship of obesity to CCA is not clearly defined and contradictory associations have emerged. Some reports postulate a carcinogenic mechanism based on the pro-inflammatory and carcinogenic components of bile when bile flow is impaired, as in obesity [73]. Still other less well-delineated risk factors for CCA may include heavy alcohol consumption, *H. pylori* infection, smoking, cholelithiasis, thyrotoxicosis, cirrhosis from all causes, and human immunodeficiency virus (HIV) [25–27, 132, 141, 157, 159].

1.9 Summary

Accurate morphologic and histologic tissue sub-typing will facilitate better classification of this disease and allow more precise calculations of incidence and risk factors. Strategies to attempt the cure of CCA include extensive surgical resection and or transplantation. However, the underlying liver disease, as in PSC, HBV, HCV, cirrhosis, and hepatolithiasis may complicate timely diagnosis of the tumor. Diagnostic tools and imaging have improved over the last few decades, though overall resectability remains low. Resection with negative margins remains the best predictor of long-term survival given the lack of suitable adjuvant therapeutic options. Major survival gains with CCA may be made by targeting prevention of the disease and modification of definite risk factors. Public health education to dissuade raw fish consumption and treatment of liver fluke infection in endemic areas are examples of what can be done to reduce the development of this tumor. Continued widespread HBV vaccination and HCV surveillance efforts should help address early CCA recognition in at risk population groups. Given the emerging risk factors for ICC and ECC, general lifestyle modification toward increased health and wellness may minimize the impact of obesity, diabetes and insulin resistance, smoking, and excess alcohol consumption. Further research into the molecular mechanisms of CCA development may be fruitful in developing tailored diagnostic, prognostic and treatment efforts [160].

2 Gallbladder Carcinoma

Gallbladder carcinoma (GBCA) is an epithelial tumor of the gallbladder, which is part of the extrahepatic biliary tract. The majority of tumors described in the literature are adenocarcinoma, but the category includes squamous, adenosquamous, neuroendocrine, and other mixed or undifferentiated malignant neoplasms of biliary, intestinal or foveolar features [8, 13]. The WHO and IARC assigns intrahepatic cholangiocarcinoma the ICD-O morphology code ICD-O 8140/3, 8144/3, 8310/3, 8480/3, 8490/3, 8560/3, 8503/3, 8470/3, 8070/3, 8020/3, which corresponds topographically to gallbladder cancer (C23.0). [8–10]. ECC share the same morphologic codes as gallbladder carcinomas (*see above*), and are topographically described with gallbladder carcinomas (C23) and other tumors of the biliary tree (C24) [8, 9, 13]. In the reporting of outcomes, GBCA outcomes are sometimes grouped with other tumors of the extrahepatic bile duct (EHBD), such as ECC. Data trends, and risk factors for GBCA will be discussed in this chapter and where there is overlap with EHBD tumors or ECC, this will be specifically mentioned.

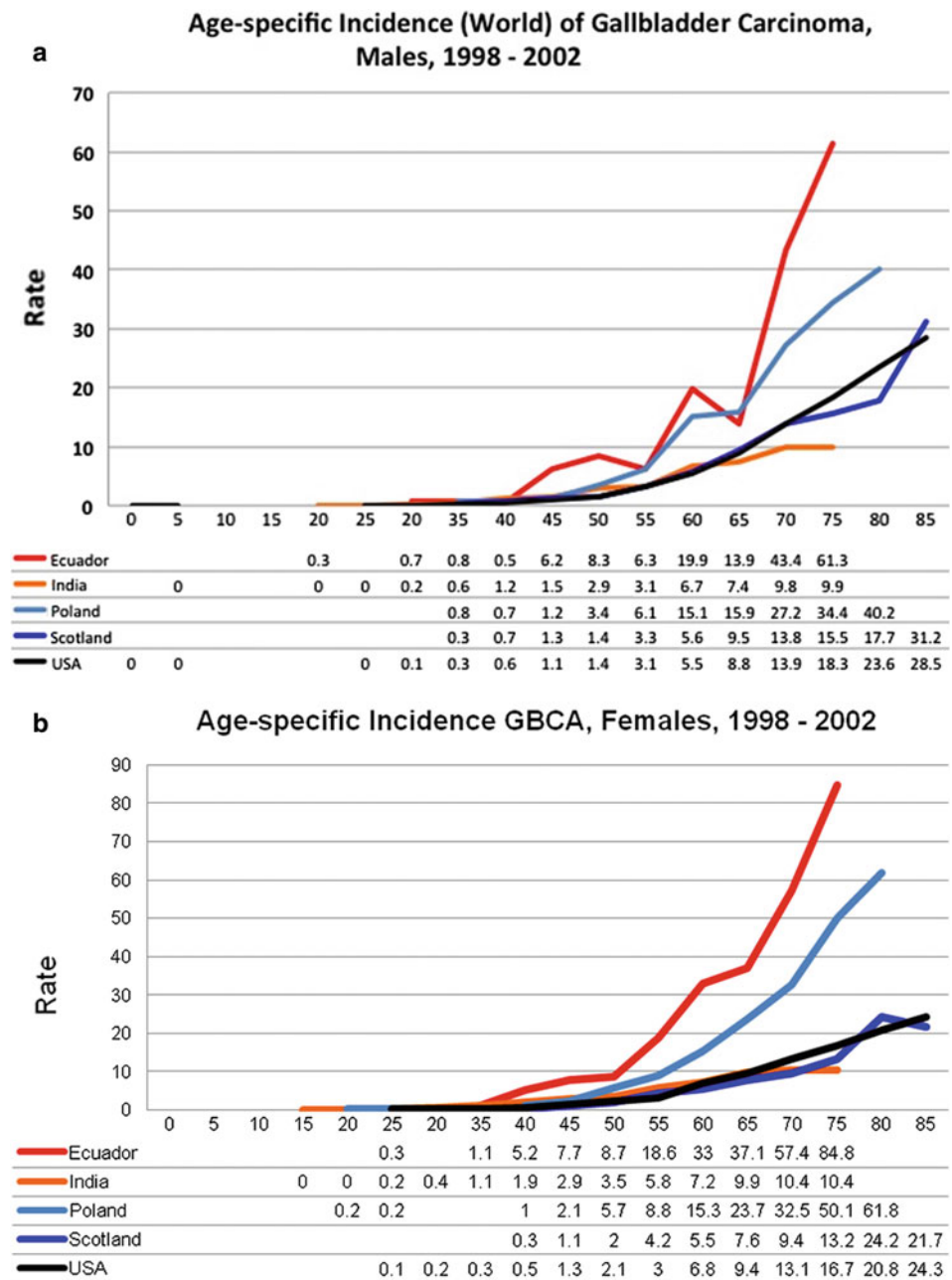
Table 5 Age-standardized world incidence rates (ASR-W) for GBCA, 1998–2002

	ASR-W	Cases
(a) Age-standardized incidence rates of GBCA, World, Males, 1998–2002		
Japan, Yamagata prefecture	5.7	335
US, California, Los Angeles: Korean	4.8	24
Ecuador, Quito	3.4	78
Czech Republic	3.2	1,072
Italy, Torino	2.8	114
Slovakia	2.7	389
Slovenia	2.6	175
USA, Connecticut: Black	2.5	15
Italy, Ragusa Province	2.4	29
Brazil, Goiana	2.4	37
China, Shanghai	2.3	493
Poland, Warsaw City	2.3	132
(b) Age-standardized incidence rates of GBCA, World, females, 1998–2002		
Ecuador, Quito	5.8	152
Slovakia	4.5	364
Colombia, Cali	4.4	164
Czech Republic	4.3	1,821
Spain, Granada	3.3	109
US, California, Los Angeles: Hispanic White	3.2	218
Japan, 3 registries	3.1	1,787
China, 2 registries	2.9	1,279
Poland, 2 registries	2.9	326
Italy, Torino	2.5	121
US, California, Los Angeles: Korean	2.4	16
Costa Rica	2.4	167
Slovenia	2.4	205

Shown are the highest rates reported to the IARC publication with associated cases for *a* males, *b* females. Representative data from both population registry data and individual regions within a country

The incidence, prevalence, and geographic distribution GBCA vary widely with differential susceptibility based on gender, infectious exposure, ethnicity and genetics or family history, but correlate with the incidence and prevalence of cholelithiasis. Worldwide incidence ratios are difficult to parse out from ECC and other EHBD tumors; however, given the poor survival with this tumor, incidence rates generally follow mortality rates. Consistently high ASR-W incidence areas for GBCA are in India and Chile with rates in women of 8.6 and 27.3, respectively, and rates in men of 3.9 and 12.3, respectively, for ages 0–74 years [6]. Table 5 details the highest and lowest incidence rates of GBCA worldwide based on the most recent last IARC *Cancer*

Fig. 6 Age-specific incidence world rates (ASRW) per 100,000 of GBCA in, **a** males and **b** females, 1992–2009 [195]. Rates shown are per 100,000 person-years on the y-axis. Patient age in years are shown on the x-axis



Incidence in Five Continents, Vol. IX publication covering data from 1998 to 2002 [6, 161]. Trends in GBCA show an overall declining mortality over the last 2 decades, although increases have been noted in Italy, Iceland, Korea and Costa Rica [5, 6, 41, 48, 162, 163]. Few reports regarding the prevalence and incidence of GBCA in Africa are published in the literature.

Individuals in their late fifth to seventh decades of life are more commonly afflicted with GBCA [5, 6, 164] (Fig. 6). The female to male ratio of GBCA is generally 3:1 in the United States, Australia, Europe, and Asia, and as high as 5:1 in India, Pakistan, Ecuador, and Israel [5, 6, 163–165]. Geographic areas of high prevalence include

populations in India, Ecuador, Chile, Pakistan, and Japan [5, 6, 166]. High prevalence rates occur in specific populations in low-risk nations, like the Pima Indians, Hispanics, and Alaska Natives in the United States [3, 4]. The Mapuche Indians in the high-risk nation of Chile, and women in India and Pakistan, have some of the highest incidence of GBCA worldwide [5, 6, 167].

Risk factors for the development of GBCA include longstanding gallstones and the inflammatory changes that may impact the progression to carcinoma [168, 169]. The overall incidence of GBCA in the presence of cholelithiasis is <0.2 and greater than 80 % of patients with GBCA have gallstones [8, 168]. GBCA may also progress through an

adenoma-carcinoma sequence or a dysplasia-carcinoma sequence [170]. In either case, the evidence supports an increased risk of GBCA with increasing number and size of gallstones, and other polypoid lesions of the gallbladder. Both theories of GBCA pathogenesis are plausible [170–173]. Additional risk factors for GBCA include multiparity, obesity, and biliary tract infections with *S. typhi* and *Helicobacter* spp. [174–180]. Many studies support an increased risk of GBCA in women with more childbirths (>3) and pregnancies [166, 178, 179]. The association of age at first birth, age at last birth, use of the oral contraceptive pill, menopausal status, and use of hormone-replacement therapy (HRT) with GBCA is less well-defined [164, 178, 179, 181].

Current data point to increasing cholecystectomy rates, especially since the introduction of laparoscopic cholecystectomy, are partially responsible for the improved incidence and survival trends in this rare and often deadly cancer [182–186]. Aggressive surgical resection remains the best option for cure [187–189]. Even with high-quality and advanced imaging and diagnostic techniques, patients with GBCA present late, given the lack of specific signs and symptoms early in the disease. Once GBCA achieves regional and/or nodal spread, there are few efficacious chemotherapeutic or radiotherapeutic options that improve prognosis, with 5-year survival rates <10 % in Stage II and <3 % in Stage IV disease [190–194].

2.1 Incidence and Mortality Trends: United States of America

In the United States, it is estimated that there will be about 10,310 cancers of the gallbladder and biliary tract per annum [4]. GBCA accounts for 3.5 % of all digestive system cancers and is currently the 7th most common cancer with 4,740 cancers in men and 5,570 cancers women (includes cancers of the biliary tree and EHBD). Deaths from this cancer remain high with an estimated 1,260 and 1,970 deaths expected for males and females in 2013, respectively. These data represent an increased total number of cases from 2012 ($n = 9,810$) spread evenly between men and women, with a similar mortality rate in males and females [3]. As of 2009, GBCA was not in the top 5 causes of cancer-related death for the United States population [4].

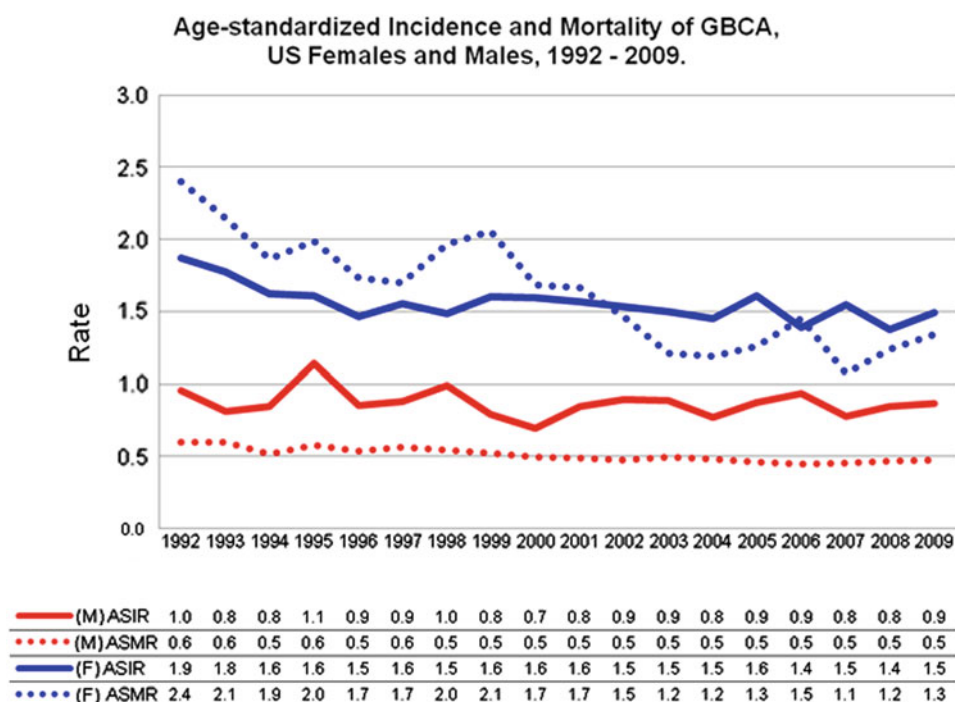
Using the SEER program population-based cancer registry results, age-adjusted GBCA incidence per 100,000 fell from 1.8 in 1993 to 1.5 in 2009 for White females. Corresponding GBCA incidence rates per 100,000 for Hispanic females ranged from 2.8 in 2006 and 2008 to a maximum of 5.8 in 1993, with the most recent incidence rate reported as 3.2 per 100,000 [3, 195]. GBCA incidence rates over the

same time period in males were comparatively lower than in females with 2009 incidence rates per 100,000 of 1.1 in Hispanic males and 0.8 in White males, representing a slight decrease over the 1992–2009 time period [3, 195]. The SEER program reports are a collaborative effort of the National Cancer Institute (NCI), the Center for Disease Control (CDC) and the North American Association of Central Cancer Registries (NAACR) to collect and publish incidence and mortality data for cancer in the United States. SEER 13 represents data from 26 % of the United States population while the current version SEER 18 collects between 87 and 93 % of the US population. The most robust longitudinal data use SEER incidence data from 1992 to 2009 [3] (Fig. 7).

In a study using United States SEER data to review the incidence and management of GBCA over 10,000 cases across 2 time periods from 1973 to 1992 and 1993 to 2002, the gender distribution of GBCA remained the same with women accounting for 73 and 72.1 % of cancers, respectively [196]. The study noted a gradual reduction in the incidence of GBCA during the second time period, and most notably in patients aged 50 years or older. More Black patients were diagnosed with GBCA during the second time period, which reflected an increase from 5.3 to 8 % of 5,918 and 4,179 total patients, respectively. Regional data from a large epidemiology project in Minnesota (USA) reporting from 1976 to 2008, support the decreasing trend in GBCA overall, and in women [35]. Within this cohort, 81 % reported a history of cholelithiasis. During the study time period, the overall age-sex-adjusted incidence per 100,000 declined from 4.0 to 2.2, with the decrease in female incidence rates from 5.0 (1976–1990) to 1.6 (2001–2008) per 100,000 accounting for the significant difference [35]. The mean/median age at diagnosis of 72 in this study is consistent with the age of diagnosis between 72 and 73 reported in other US data sources [193, 197].

The ratio of GBCA in females to males in the United States was roughly 2–2.5:1 from 1992 to 2009 and closer to 1.2–1.3:1 in recent years (Fig. 8) [3, 4, 193, 195, 197]. Approximately 70 % of cancers were found in White or White Hispanic women and this remains unchanged in published data over the last 30 years [194, 196, 197]. Ethnic and racial differences in GBCA exist within the US population. The age-adjusted incidence rates per 100,000 for Black American males and females (2009) are 1.4 and 1.5, respectively [195]. Since the 1990s, the incidence rates of GBCA in Black American males and females have surpassed that of White males and females though the prevalence of gallstone and gallbladder disease is lower in this group [161, 198]. The APC in incidence and mortality for American Whites has declined at a faster rate than in American Blacks (Table 6) [3, 161, 195, 198].

Fig. 7 Age-standardized (US 2000 Standard Population) incidence rates (per 100,000) of GBCA in the United States. Incidence rate is shown on the y-axis and grouped time intervals in years are shown on the x-axis with the graphed incidence data shown below in tabular format. *ASIR* age-standardized incidence rate. *ASMR* age-standardized mortality rate [3]



The highest US incidence rates for GBCA are found in those with Hispanic, American Indian and/or Native Alaskan heritage. Trend outcomes report that Hispanic females have the highest age-adjusted incidence of GBCA in the United States, followed next by American Indian and Native Alaskan females [7]. Hispanic males have a higher incidence of GBCA than White males, but it is still half that of Hispanic females. One recent comprehensive review of GBCA in 189 American Indian/Alaskan Native (AIAN) Americans (1999–2004) found wide geographic variation in the GBCA incidence rate of 0.5 (East) to 5.5 (Alaska) per 100,000 [199]. Incidence rates were consistently higher in AIAN and non-Hispanic White females than males. In comparison to non-Hispanic Whites, many more in the AIAN population were diagnosed with GBCA at an earlier age [199]. These results hold over a longer time period (1973–2007) in a study of 213 AN (only), 73 of whom developed GBCA [34]. AIAN, Hispanics and Chileans present with GBCA at 61–63 years of age, a stage that is 1–1.5 decades earlier than in low-prevalence populations [169, 200].

2.2 Incidence and Mortality Trends: United Kingdom and Europe

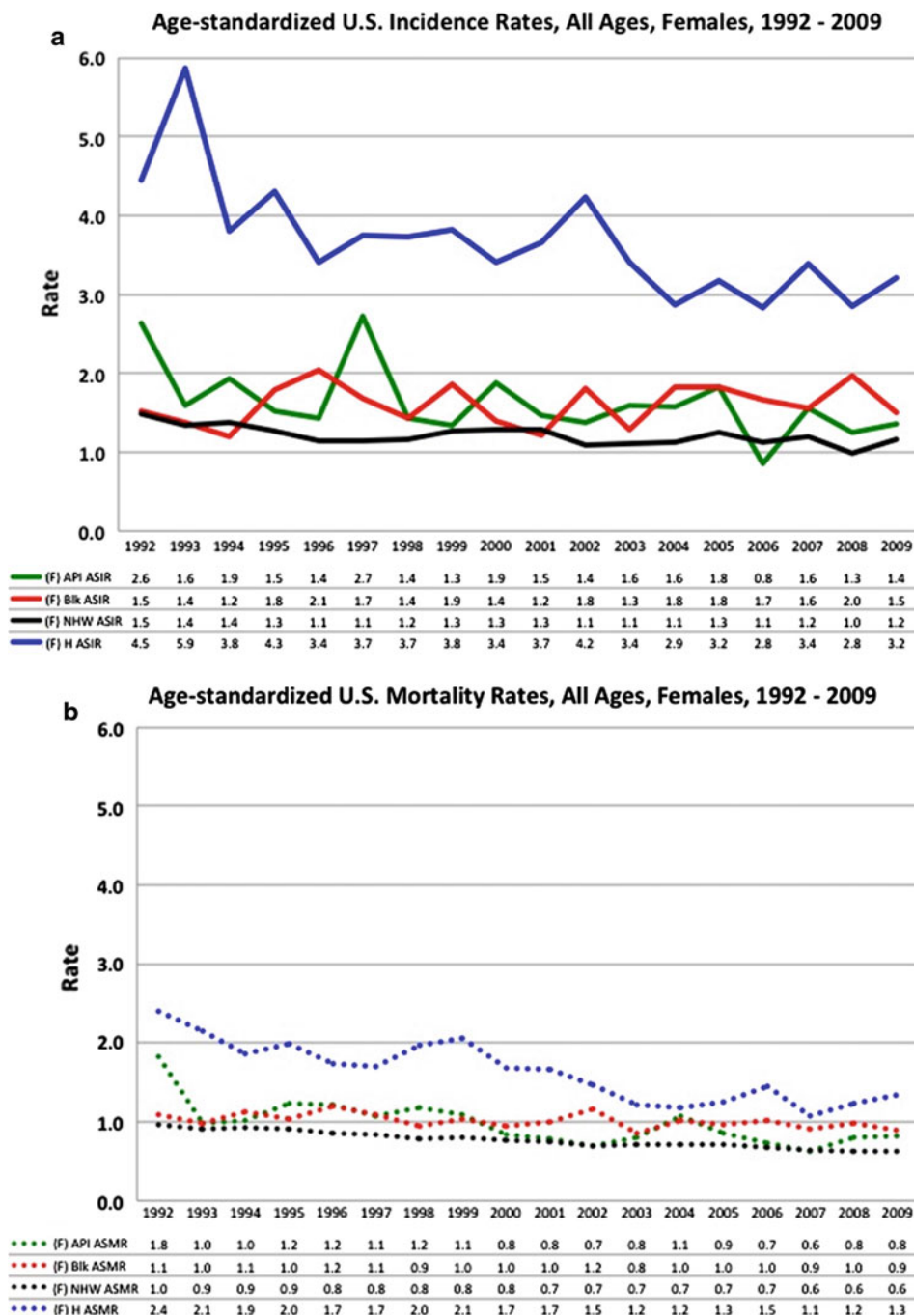
Incidence and mortality trends for GBCA in Europe are comparable to those of the US population [6, 161, 162, 201]. Decreased mortality for GBCA is seen through most northern European countries except for high incidence areas of Poland, Italy, Spain and France in Central and Eastern

Europe [5, 6, 162, 164, 165]. Mortality of GBCA across Europe declined by as much as 30 % in women and 10 % in men in the time period 1980–1999 [162]. For females, ASR-W incidence ratios per 100,000 in 2001–2002 for France (1), Denmark (1.1), the Netherlands (1.54), United Kingdom (0.74), and Scotland (1.1) were similar to figures from the United States [5, 6, 161]. Higher incidence rates for females were found in Poland (4.2), Spain (2.05) and Italy (2). The ASR-W for males in Europe were low in 2001–2002 in Denmark (0.9), France (1.25), Switzerland (1), the Netherlands (1.1), United Kingdom (0.6) and Scotland (1.23), with higher incidence rates in Italy, Poland and Slovakia, but not Spain [5, 6, 161].

The high incidence rates of GBCA seen in Central European countries, including Hungary, Poland, Czech Republic, and Slovakia is not associated with any specific known etiology such as a higher incidence of gallstones nor a decreased cholecystectomy rate [163, 202]. Though ASR-W rates of GBCA remained stable across Europe from 1992 to 2002, appreciable declining mortality trends were noted in Slovenia, Hungary, the Czech Republic and Slovakia [163, 202].

The Nordic countries, Finland, Sweden, Denmark, Iceland and Norway, produced a 30-year review of cancer, and reported declines in GBCA and ECC incidence and mortality from the late 1980s, and notably in Denmark since the 1970s [43]. Iceland did show a significant increased GBCA mortality rate in males during the period 1992–2002, but a similar trend was not noted for women [163]. The decreased mortality from GBCA was significant for both sexes in Denmark and Sweden, and in females only in Finland [163].

Fig. 8 Age-standardized (US Census 2000) **a** incidence and **b** mortality of gallbladder carcinoma in the United States, all females, 1992–2009. Rates shown are per 100,000 person-years. ASIR, age-standardized incidence ratio. ASMR age-standardized mortality ratio. *F* females. *API* Asian/Pacific Islander. *Blk* black. *NHW* non-Hispanic white. *H* Hispanic (inclusive of all races). Trends are significant for incidence in all groups. API, -2.7 . Blk, -0.6 . NHW -1.0 . H, -2.7 Trends are significant for mortality in all groups. API, -13.5 . Blk, $+0.9$. NHW -2.2 . H, -3.7 . Source US SEER Data [3, 195, 265, 266]



Trends in excess mortality in the month following initial diagnosis of GBCA showed a modest improvement in survival, however, 5-year survival in the Nordic countries is still 10–40 %, as seen worldwide [43]. A historic multicentent, case-controlled study conducted in Canada, the Netherlands, Australia, and Poland provided supportive evidence implicating a history of gallstones and previous cholecystectomy in the pathogenesis of gallbladder cancer [167]. In England, similar to the United States, the median age at diagnosis for

GBCA is 73 years, 70 % of the diagnoses are in women and 75 % of patients are diagnosed between the ages of 65–85 years [40]. The incidence rate of GBCA from 1998 to 2007 was stable in comparison to a declining rate seen from 1971 to 2001 [36, 40]. The mortality rate for GBCA in the United Kingdom declined significantly from 1992 to 2002 in both sexes [163]. Longitudinal incidence and mortality data in Scotland from 2001 to 2010 show ASIR and ASMR data for GBCA are shown in Fig. 9.

Table 6 SEER incidence, US mortality and survival percent for all and selected races in GBCA

	Incidence	Mortality	Survival (%)
SEER incidence, US mortality and survival for GBCA, 2005–2009			
All races, total	1.2	0.6	16.6
All races, male	0.8	0.5	14.8
All race, female	1.4	0.8	17.3
Whites, total	1.1	0.6	16.9
Whites, male	0.8	0.4	14.5
Whites, female	1.4	0.7	17.8
Blacks, total	1.5	0.8	12.8
Blacks, male	1.3	0.7	15.8
Blacks, female	1.7	1.0	13.0

Incidence and death rates are per 100,000 and are age-adjusted to the 2000 US standard population. Mortality data are derived from US Mortality Files, National Center for Health Statistics, Centers for Disease Control and Prevention (CDC). Surveillance, Epidemiology, and End Results (SEER) data is from 18 US areas. Survival data (2002–2008) is based on follow-up of patients into 2009. *Source* SEER Cancer Statistics Review, 1975–2009 [3]

2.3 Incidence and Mortality Trends: Asia

The populations in North India, including neighboring Pakistan, have high incidence rates of GBCA compared to other parts of the world and to Indians in the southern part of the continent [164, 203–205]. The ASIR in Delhi, India, a high prevalence area, was 8.6 per 100,000 in females and 3.9 per 100,000 for males from 1998 to 2002 and relatively unchanged (9.4 and 3.9, respectively) since the 1993–1997 evaluation, for ages 0–74 years. GBCA is the leading cause of death for women in North India [206]. In stark contrast, the GBCA incidence rate in females from South India ranges from 0 to 0.7 per 100,000, for ages 0–74 years [5, 6, 164].

The Japanese Public Health Center-based prospective study (JPHC) launched from 1990 to 1994 examined over 100,000 Japanese people and the impact of known and likely risk factors on the biliary tract cancers, GBCA and ECC [73]. The major advantage of this study was that it separately analyzed GBCA and ECC with associated risk factors. The cohort analysis of the Japanese population followed through 2004 found a strong association (hazard ratio, HR 3.10) of cholelithiasis with GBCA that was stronger for men (HR 4.28) than women (HR 2.38). In a previous study from the Japan Collaborative Cohort Study for the Evaluation of Cancer (JACC Study) of over 113,000 Japanese enrolled from 1988 to 1990 and studied for a median of 13 years, the strong association of cholelithiasis with GBCA was not seen [207]. In Japan, the development of GBCA and other biliary tract cancers may be related to an abnormal pancreatobiliary junction (APBJ). This developmental abnormality of the biliary tract is found in 17 % of Japanese with GBCA, and

may represent a separate pathway from the association with cholelithiasis and typhoid infection seen in other countries [204, 208, 209]. However, it still remains unclear as to the etiology of the recent trends in increased incidence and mortality of GBCA in Japan.

2.4 Gallbladder Cancer in Africa

Few reports on the incidence and prevalence of GBCA in Africa are published in the literature. The 20-year findings in 30 patients from a tertiary referral center in Nigeria suggested an 87 % preponderance of GBCA in women, and 40 % of cases were associated with gallstones [210]. The mean age at diagnosis was 58 years of age and over 80 % presented with jaundice, an adverse prognostic factor [211]. WHO/IARC data for 1998–2002 reported a high ASR-W incidence of GBCA in females from Algeria (10.0 per 100,000) and Tunisia (3.1 per 100,000) [6]. The incidence rates from the remainder of reported nations (Egypt, Uganda, and Zimbabwe) were otherwise low, ranging from 0.4 per 100,000 in Ugandan females to 1.9 per 100,000 in Tunisian men, placing Africa as a continent of otherwise low prevalence using the available limited data [6].

2.5 Incidence and Mortality Trends: South America

In South America, similar to the United States American Indians, the incidence of GBCA varies with the degree of Amerindian admixture in the population. Again, a higher degree of Amerindian admixture, as is seen in Chile, Bolivia, Ecuador and Bolivia, closely follows a higher incidence of GBCA, than would be seen in Argentina and Brazil [181, 204, 208]. The decreased cholecystectomy rate in Chile in the 1980s coupled with a low rate of cholecystectomy in the high prevalence Mapuche Indians of South Chile led to the higher mortality rate of GBCA [204, 208]. Recent age and sex-adjusted mortality estimates from 333 counties in Chile range from 8.2 to 12.4 per 100,000 for the population from 1985 to 2002, with a higher risk in inland (10-fold) and southern-inland counties (26-fold) [212]. Trends in incidence and mortality rates for GBCA from Chile, 1985–2002, are shown in Fig. 10. Rates of GBCA were higher in counties with more Mapuche Indians in the population [212]. For female Chilean Mapuche Indians, 50 % of females older than aged 50 years harbor gallstones [213].

Differential susceptibility to gallstone formation may be a primary reason for the increased incidence of gallbladder cancer in South American, Hispanic and AIAN populations. The National Health and Nutritional Examination Survey

Fig. 9 Age-standardized (Europe) incidence and mortality of gallbladder carcinoma in Scotland, males and females, 2001–2010. Rates shown are per 100,000 person-years. *ASIR-E* age-standardized incidence ratio, Europe. *ASMR-E* age-standardized mortality ratio, Europe. *M* males. *F* females. Source Information Services Division (ISD) Scotland and NHS National Services Division [267]

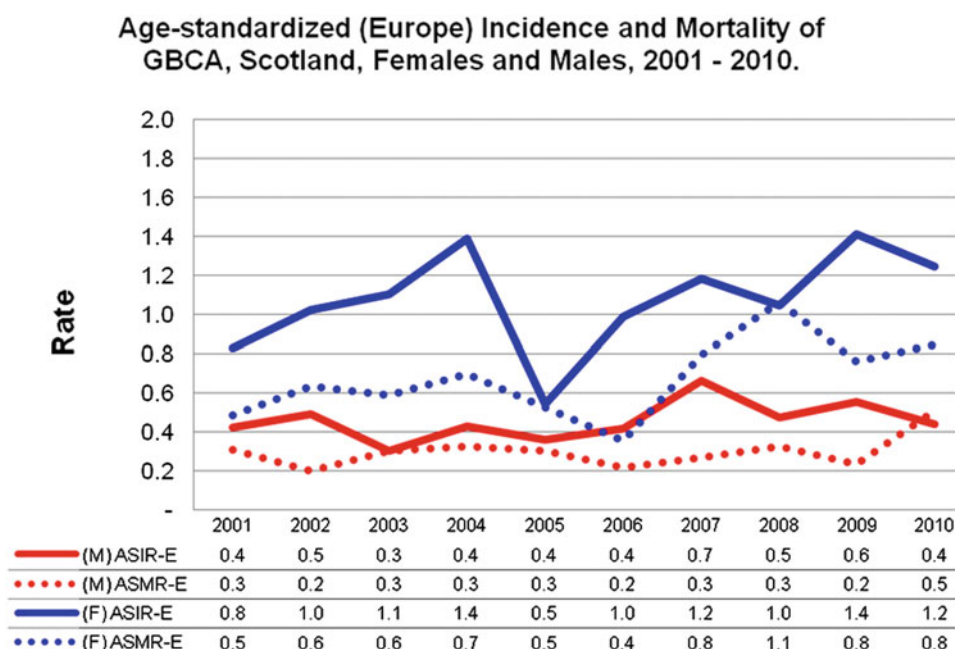
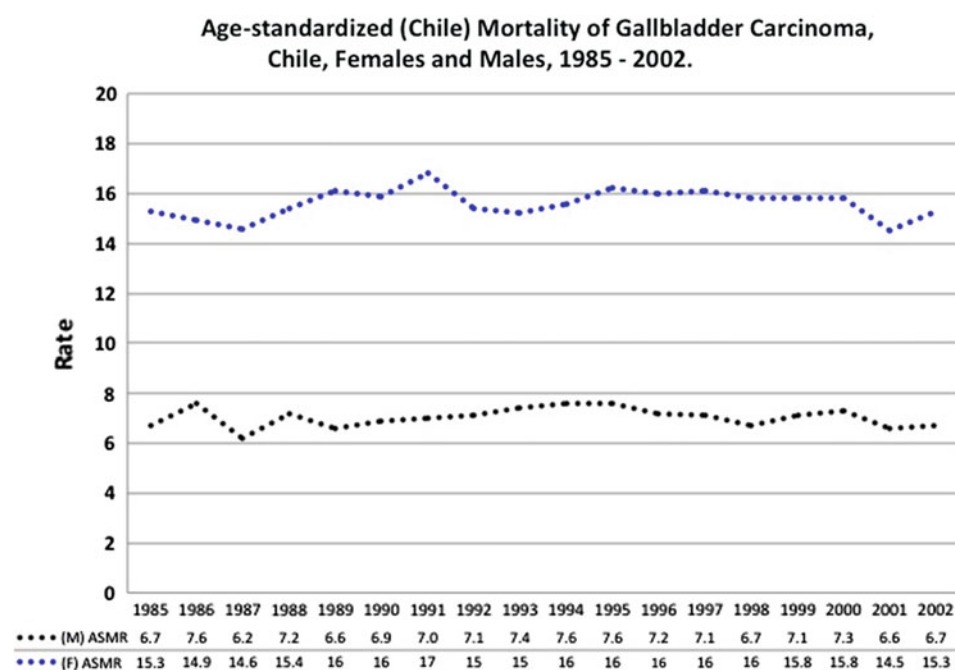


Fig. 10 Age-standardized (Chile) mortality rates (per 100,000) of GBCA, Chile, both sexes, 1985–2002 [268]. *ASMR* age-standardized mortality rate. *M* males. *F* females



(NHANES) III study established the incidence of gallbladder disease (GBD), as defined by the presence of gallstones or a history of cholecystectomy [214]. The report estimated that 20 million people in the United States had GBD. The study found a 12.8 % prevalence rate of gallstones in Mexican–American women, and a nadir of 3.9 % in American Black males, corresponding to a 26.7 and 5.3 % incidence of GBD, respectively.

There is a high incidence of larger gallstone formation at younger ages in populations with a significant Amerindian ethnic admixture, as low as 8.3 years of age in females and 10.8 years of age in males. There is a dramatic increase in the size and number of stones beginning after age 20 [169, 213]. The incidence of GBD is as high as 76 % in AIAN females aged 65 and older in the Southwestern United States, in Pima Indians older than

45 years of age, and in New Mexico Native Americans [213, 215]. However, the increased gallstone formation rates in both males and females with Amerindian ethnicity does not translate into significantly increased GBCA incidence rates for Hispanic males as compared to Hispanic females [199, 213, 216]. In the Mexican–American population, the Amerindian admixture is estimated at 30–50 % [199, 213, 216].

2.6 Cholelithiasis and Chronic Inflammation

Multiple reviews have confirmed the association of the presence of gallstones as an important risk for development of GBCA, and are present in up to 80 % of patients with this cancer [8, 168, 200, 208, 217, 218]. Worldwide, the prevalence of gallstones varies dramatically [208, 219, 220]. The prevalence of GBD, defined as the presence of gallstones or patients undergoing cholecystectomy in a population, can be as high as 64 % in American Indian Tribes of the United States, 61 % in Montreal, and 15–30 % in select areas of India, Italy and Poland [220]. The lowest prevalence areas for gallstones are in Africa (<5 %), while Asia (5–20 %) ranks as an intermediate prevalence area. Approximately 1–3 % of people harboring gallstones develop GBCA [208]. In the presence of gallstones, the odds ratio (OR) for developing GBCA was 2.4 for stones 2–2.9 cm in diameter compared to stones <1 cm, but was as high as 10.1 for stones >3 cm in diameter [168]. In a population-based study from China, the presence of gallstones was associated with a 23-fold increased risk in developing GBCA [221, 222]. Stones in this GBCA group were heavier than in the gallstone alone group [221].

The impact of gallstone size on GBCA is pronounced in American Indians, Alaskan Natives and Hispanics. In a United States study of Blacks and Whites (low prevalence) and American Indians (high prevalence) with gallstones, the age-adjusted OR for GBCA in patients with stones >2 cm in diameter was 1.5 for Indian populations and for the American Indian group, the OR for patients with stones >3 cm was 9.1. The mean gallstone size in patients with cancer compared to those who did not have GBCA was 2.5 cm versus 1.5 cm, respectively [169]. In a prospective study of 600 females with gallstones in Chile, single gallstones with a mean size of 2.5 cm were significantly associated with GBCA [200]. Patients with asymptomatic stones are more likely to have singular stones, however symptomatology and incidence of GBCA increase with greater than six gallstones [200].

The risk of GBCA in the presence of cholelithiasis may be related to the duration of stones within the gallbladder and their secondary impact as a chronic, inflammatory stimulus on the gallbladder epithelium. In populations at risk for GBCA, the increased incidence and early age at

presentation of GBCA may be related to larger stones developing earlier in life, given the similar growth rate (2.0 mm/year) across many different populations [169, 213, 216]. GBCA may proceed through a metaplasia-dysplasia-carcinoma sequence in which the chronic irritation by gallstones contributes to biliary epithelial metaplasia [170, 204, 208, 217]. The pathogenic pathway to GBCA in the presence of cholelithiasis (whether cholesterol, brown or black pigment stones) is due to alteration of the chemical composition of bile and alteration of the gallbladder wall to promote stone formation [223]. Genetic analysis of stone formation in high prevalence areas of North and South America have identified genetic predispositions to ‘lithogenic bile’ and a potential causal relationship with GBCA [208, 223, 224].

2.7 The Porcelain Gallbladder and Polypoid Lesions

The porcelain, or calcified, gallbladder confers a higher risk of GBCA, in the range of 10–60 % (Fig. 11). The risk of GBCA is higher in a partially calcified gallbladder [217, 225]. The gallbladder mucosa undergoes intestinal or biliary metaplasia prior to proceeding toward carcinoma [223].

Initial reports established a risk of GBCA in resected gallbladders with a solitary polypoid lesions of the gallbladder (PLG) >1 cm in males and in females older than 60 years of age [171, 226]. The estimated prevalence of PLG is 3–8 %, on ultrasound scanning and 2–12 % in gallbladder specimens following cholecystectomy [171]. Worrisome imaging findings include growth over an interval observation period, vascularity within the polyp, and obliteration of the fat plane between the gallbladder and liver bed, or symptomatic polyps are an indication for cholecystectomy [227] (Fig. 11). Additional reports have corroborated these findings in an attempt to stratify patients for operative resection. One recent study based on ultrasound findings suggests that cholecystectomy is indicated for PLG greater than or equal to 6 mm, and that show vascularity, growth, invasion, or cause symptoms, after reporting cancer findings in 7.4 % of their cohort [172]. Consistent high-risk features for cancer in PLG include age 50–60 years, solitary polyp, >1 cm, sessile, symptomatic, or with evidence of liver parenchymal invasion [173, 228–230].

Pathologic assessment of gallbladder specimens has clarified a decreased risk of developing GBCA from adenomas due to the low incidence of adenomas (0.14 %) in cholecystectomy specimens [170]. There is limited evidence for the progression of GBCA through an adenoma-carcinoma sequence similar to colon cancer [208]. Generally, a high index of suspicion regarding abnormalities in the gallbladder should prompt interval reassessment by ultrasound for small

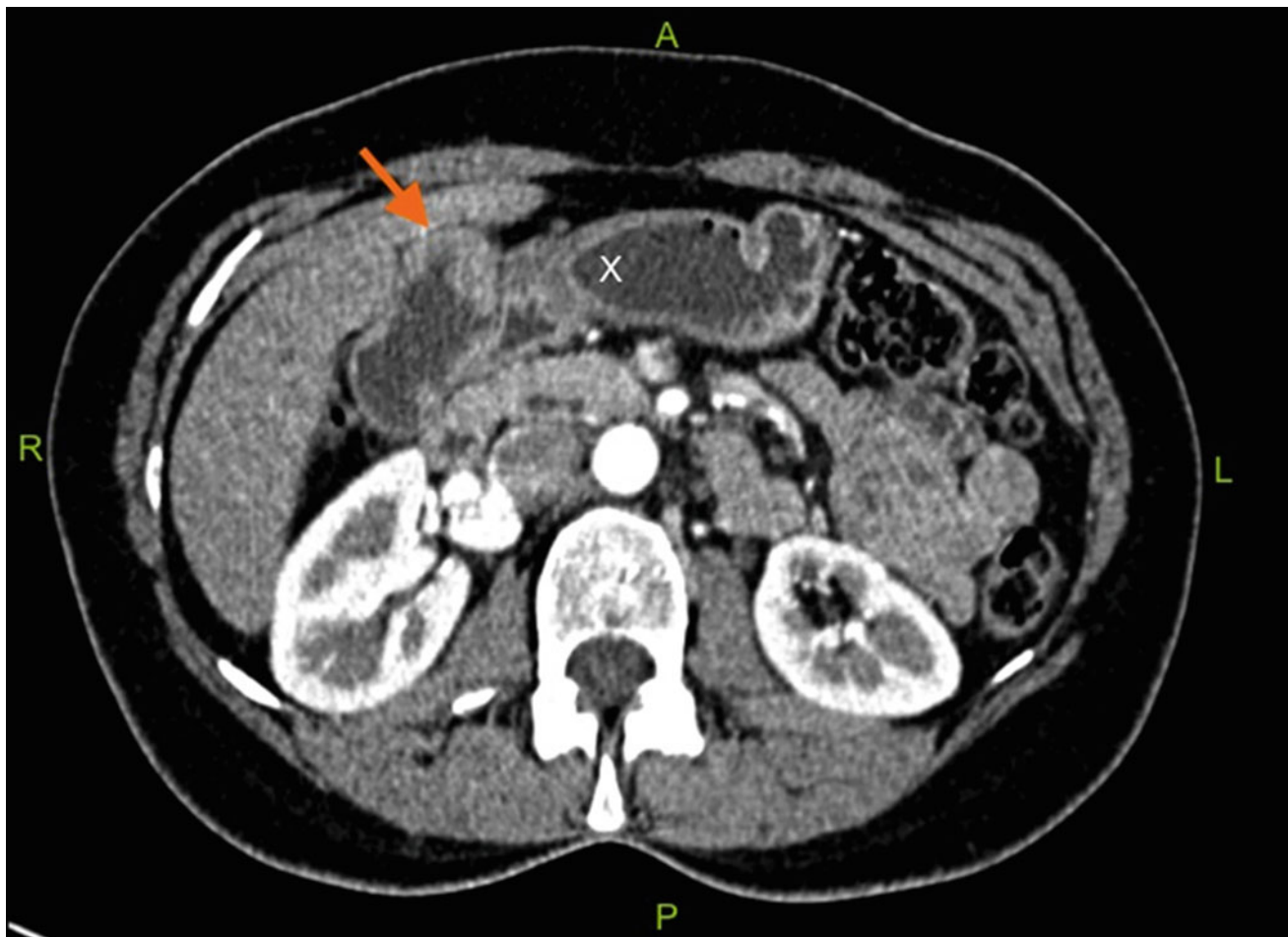


Fig. 11 a Ultrasound image of polypoid lesion of the gallbladder. 2.7 cm exophytic, mucosal enhancing polypoid lesion in fundus of gallbladder. pT2 Nx GBCA with lymphatic and vascular invasion at histologic analysis of cholecystectomy specimen. **b** Axial contrast-

enhanced CT image of gallbladder with lesion in fundus (arrow) adjacent to duodenum (X). Orientation is marked, A anterior, P posterior, R right and L left. Images: Royal Infirmary of Edinburgh, NHS Lothian ©

lesions or referral for cholecystectomy for larger lesions. Cholesterosis has not been demonstrated to have an association with GBCA in analysis from Chile [231].

2.8 The Impact of Cholecystectomy and Incidental Gallbladder Cancers

Decreased GBCA mortality of 22–30 % in Scotland, England, Wales, and in the United States during the 1970s was attributed to peaks of increased cholecystectomy rates in these countries [182, 183]. Contrastingly, excess deaths due to GBCA were reported during this time in Sweden with falling cholecystectomy rates. The data estimated that 1 in 63 excess deaths in England and Wales, and 1 in 115 excess deaths in the United States and Sweden from GBCA were prevented due to the previous year's cholecystectomies [182]. In Scotland, using the 30-year (1968–1998) GBCA incidence ($n = 1736$) and mortality ($n = 1546$) data from

Scotland, declining rates were seen in females since the 1960s and in males since the 1980s [185]. Mortality rates for GBCA closely follow incidence rates. The steeper decline in mortality realized from 1990 to 1991 and onwards coincides with the start of a second peak of increased cholecystectomy rates in Scotland and the introduction of laparoscopic cholecystectomy in Western countries [36, 185, 197].

Laparoscopic cholecystectomy accounted for a 30–60 % increased rate of cholecystectomy with the largest increases seen in women aged 45–65 years of age when it was introduced [197, 232]. Using diagnosis, treatment and survival data from the United States National Cancer Database (NCDB), patient cohorts in 1989–1990 and 1994–1995, notably before and after the introduction of laparoscopic cholecystectomy, did not show increased numbers of early stage (Stage 0, I) cancers [197]. The declining incidence in GBCA is matched by decreasing mortality, and is most marked in women. For the time period 1979–1998, the major declines in ASMR-W occurred in Scotland (41 %), England

and Wales (45 %), the United States (35 %), and France (22 %) [41]. In contrast, the ASMR rose in Japan (16 %) and Italy during the same time period (28 %) [41]. The incidence and mortality rates of GBCA and ECC have continued to fall in the last 2 decades; the etiology is likely multifactorial related to changes in lifestyle, diet, cholecystectomy rates, genetic, and other nuances. Many incidence and mortality figures report GBCA and ECC topographic codes (C23, C24) in a combined manner causing difficulty in separating which cancer accounts for the major declines noted in men and women. However, given the greater propensity of GBCA for women, and ECC for men, each cancer contributes to the steady declines seen in both sexes.

The incidental finding of GBCA, however, has become more common since the advent of laparoscopic cholecystectomy, though this has not dramatically altered incidence and prevalence data [189, 233, 234]. The impact of cholecystectomy rates on GBCA incidence rates, viewed as increasing numbers of early cancers, or incidental cancers found at cholecystectomy, for another indication has not been seen. Intestinal metaplasia, a precancerous lesion, can be seen in 10–76 % of gallbladder specimens [203]. The rate of finding an incidental gallbladder cancer is 1–10 % [235–238]. In Chile, where incidence rates are one of the highest, up to 74 % of specimens harbor a gallbladder cancer [234]. Patient survival outcomes are not adversely affected in patients with early stage tumors who have had laparoscopic cholecystectomy initially [197, 233, 234].

Major surgical resection of the liver and bile duct may be required for advanced staged tumors in order properly stage and treat patients, and guide adjuvant therapy [186, 193, 234, 235, 239–241]. Some groups have found improved survival after re-resection for T2 and T3 GBCA found in the resected pathology specimen. Modest survival benefits are seen in patients with limited nodal and regional spread in Stage III or greater GBCA [238, 241]. Interestingly, while the risk of GBCA declines with cholecystectomy, risks of liver and other biliary tract cancers increase, and the risk of EHBDcancers decreases after cholecystectomy [184, 242, 243].

2.9 The Abnormal Pancreatobiliary Junction

Biliary tract cancers, including those of the gallbladder, are demonstrably higher in the presence of an APBJ [244–246]. The estimated frequency of APBJ with GBCA ranges from 5 to 25 % [247–249]. Endoscopic retrograde cholangiopancreatography (ERCP) reports estimate the prevalence of APBJ as 0.9–2.6 % of the world's population [247, 250]. The APBJ commonly occurs associated with the development of choledochal cysts. The literature discussing these and the incidence of related biliary tract cancers originate in

Asia, namely China and Japan. The association of choledochal cysts, APBJ and biliary tract cancer are described in the section on cholangiocarcinoma. Gallbladder carcinoma is frequently encountered in APBJ with or without an associated cystic dilation of the biliary tree, and may occur more commonly in the absence of a choledochal cyst [246, 248]. The long length of the shared ‘common channel’ of the pancreatic and bile ducts, without a controlling sphincter mechanism, exposes the biliary epithelium to pancreatic amylase, intestinal bacteria and other factors that create an altered bile milieu [247, 251].

Secondary bile acids, lithocholate and deoxycholate, derived from the breakdown of bile by deconjugation, dehydroxylation, and intestinal bacteria, are considered mutagenic [223, 227, 245, 246, 248]. The gallbladder epithelium is affected by altered bile as demonstrated by increased secondary bile acid and pancreatic amylase concentrations in cholecystectomy specimens that differ from patients without an APBJ [223, 245, 246, 248].

2.10 Infectious Agents

Typhoid fever, caused by infection with *Salmonella typhi* and *S. paratyphi*, was common at the turn of the twentieth century in the United States and other developed nations, and remains an important cause of the disease in nations with poor sanitation. The history of typhoid infection or a typhoid carrier state imparts a 6-fold increased risk of death from hepatobiliary cancers as reported in 471 cases from New York (USA) in a case-control study [174]. The increased risk of death from hepatobiliary cancers was most significant in men and those who were foreign-born (67.8 %) [174]. Analysis of deaths in chronic typhoid and paratyphoid carriers in Scotland (UK) from the late 1960s confirmed the excess risk of death from hepatobiliary, or ‘bile-related’ cancers [175]. The excess risk of GBCA was most pronounced in women and was related to patients who developed a chronic typhoid carrier state rather than acute infection alone. Similar studies in high (Bolivia and Mexico) and low (Denmark) prevalence areas, also found a high incidence of the typhoid carrier state with GBCA [181, 212, 252]. In patients with gallstones, positive typhoid carrier status confers an additional increased risk factor for developing GBCA [253]. High prevalence areas for GBCA across the world (Chile, Bolivia, North India, Ecuador) are also high prevalence areas for gallstones and typhoid. The typhoid bacillus resides in the gallbladder mucosa in chronic infection [174, 212]. The chronic inflammatory environment, creation of mutagenic secondary bile acids and the relative biliary tree obstruction from stasis are postulated mechanisms of hepatobiliary cancer pathogenesis with *S. typhi*.

Bacterial DNA isolates of *Helicobacter* spp. have been retrieved from gallbladder, bile and serum specimens after cholecystectomy, with *Helicobacter pylori* more commonly found than *H. bilis* [254–256]. The role of *Helicobacter* spp. in the pathogenesis of GBCA has been looked at given its close association with bile duct injury, chronic inflammation and proliferation caused by *H. hepaticus* in the liver [257] and the role *H. pylori* plays in gastric inflammation and ulceration. Due to the high prevalence of *Helicobacter*-associated chronic cholecystitis in Chileans, it is postulated as a participatory event in gallstone formation and a possible inciting event in gallbladder epithelial metaplasia [255].

2.11 Emerging and Other Risk Factors

Quantitative summary analysis of the role of obesity, defined by the WHO as overweight (BMI 25–30) and obese (BMI ≥ 30), compared to normal weight adults (BMI 18.5–24.9) showed a 66 % increased risk of GBCA with a summary RR of 15 [180]. The report derived data from studies of populations in Chile, Bolivia, United States, Korea, Norway, Sweden, Japan, Denmark and Poland published from 1992 to 2006 demonstrated no statistically significant heterogeneity. The relationship of obesity with GBCA was stronger in women in the majority of studies. In one population-based study from China, using WHO definitions for overweight (BMI 23.0–24.9), obese (BMI ≥ 25) and normal weight (BMI 18.5–22.9) adults in Asian populations, reported a 12.6-fold increased risk of GBCA with a BMI ≥ 25 [258]. The study introduced the association of abdominal obesity with GBCA after demonstrating an increased waist-to-hip ratio was more significantly associated with GBCA for any given BMI. The WHO parameters for BMI in Asians attempt to better stratify the impact of BMI-related diseases in Asians given the demonstrated increased prevalence of BMI-related comorbidities at a lower BMI [259]. In population-based studies of biliary tract cancer in China, a significant close association of GBCA with women, diabetes, and BMI >25 has been shown [221, 260].

The familial association of GBCA was initially described in a few short reports on families from New Mexico (USA) and Brazil [166]. The Swedish Family Cancer Database provides more robust contemporary data on the impact of familial relationships on liver and biliary tract cancer [261]. From 1961 to 1998, the database contained data on 10 offspring-parent families from 1,121 offspring and over 17,000 parent cases. The standardized incidence ratio (SIR) of GBCA was increased at 3.13 in offspring from parents with liver or pancreatic cancer. The SIR of gallbladder cancer was increased in parents by offspring with liver or biliary tract cancer at 1.93. For both parents and offspring,

the SIR was highest for offspring and parents at 5.05 and 4.09, respectively, for concordant gallbladder sites. The risk of GBCA has been shown to be 57-fold higher for cholelithiasis and a family history cholelithiasis (in first-degree relatives) than the 21-fold increased risk with cholelithiasis alone for the affected patient [222]. Earlier studies on populations in Mexico and Bolivia showed the same close association of GBCA in an affected patient with a first-degree relative who had a history of cholelithiasis [181].

Additional ‘lifestyle’ and related factors such as low socioeconomic status/degrees of deprivation [185, 262, 263], alcohol consumption [207], smoking [207, 264], diets high in chili peppers [263], fried foods [263], foods baked in pork fat [181], and in patients with loose stools, more than 2 bowel movements per day or infrequent weekly stools [167], have all been associated with GBCA in populations worldwide. The association of GBCA with occupational exposure to carcinogens and toxic byproducts of industry are not clearly defined.

2.12 Summary

For the known risk factor of cholelithiasis in the development of GBCA, continued prompt treatment of gallbladder disease and symptomatic cholelithiasis should remove a known risk factor for developing GBCA. Differential availability and access to cholecystectomy (laparoscopic or open) throughout the world makes this a challenging strategy to universally implement. Controversy exists regarding the role of prophylactic cholecystectomy and cholecystectomy for asymptomatic disease due to concern for GBCA in high-risk populations [218]. Gallstones are more prevalent in populations with high Amerindian heritage in North and South America and in India, suggesting evidence of a genetic susceptibility to gallstone formation. The studies analyzing the potentially contributory lifestyle factors, such as alcohol consumption, poverty, smoking, dietary and weight factors suggest that consequences of exposure to environmental or metabolic byproducts contribute to a chronic inflammatory state that initiates gallbladder metaplasia. Indeed, the association of *S. typhi* and *Helicobacter* spp. infection with GBCA supports the pathway of chronic inflammation leading to invasive carcinoma. In areas of Asia, developmental abnormalities may play a stronger role than gallstones in the development of GBCA. Early diagnosis of gallbladder carcinoma based on a high index of suspicion still provides the best opportunity to cure the disease by surgical resection. Current studies demonstrate that cholecystectomy alone is sufficient treatment for early stage tumors, while radical surgical resection of the gallbladder, liver and extrahepatic biliary tree do little to improve survival for advanced-stage GBCA.

References

- de Groen PC et al (1999) Biliary tract cancers. *N Engl J Med* 341(18):1368–1378
- Blechacz BR, Gores GJ (2008) Cholangiocarcinoma. *Clin Liver Dis* 12(1):131–150 (ix)
- SEER, S.R.P. (2012) National Cancer Institute SEER/Stat software, version 7.0.4., *SEER Cancer Stat Rev, 1975–2009 (Vintage 2009)*
- Siegel R, Naishadham D, Jemal A (2013) Cancer statistics, 2013. *CA Cancer J Clin* 63(1):11–30
- Parkin DM et al (2002) 2002 Cancer incidence in five continents, VIII—IARC Publication No. 155, vol VIII. 2002, France (Lyon) IARC
- Curado MP et al (2007) Cancer incidence in five continents, vol IX. IARC Scientific Publication No. 160. Vol. IX. 2007, France (Lyon)
- Goodman MT, Yamamoto J (2007) Descriptive study of gallbladder, extrahepatic bile duct, and ampullary cancers in the United States, 1997–2002. *Cancer Causes Control* 18(4):415–422
- Bosman FT, Carneiro F, Hruban RH, Theise ND (eds) (2010) WHO classification of tumours of the digestive system, 4th edn. IARC, Lyon
- Hamilton SR, Aaltonen LA (eds) (2000) World Health Organization classification of tumours. Pathology and genetics of tumours of the digestive system. IARC Press, Lyon
- Alpini G, McGill JM, Larusso NF (2002) The pathobiology of biliary epithelia. *Hepatology* 35(5):1256–1268
- Sell S, Dunsford HA (1989) Evidence for the stem cell origin of hepatocellular carcinoma and cholangiocarcinoma. *Am J Pathol* 134(6):1347–1363
- O'Hara SP, Tabibian PH, Splinter PL et al (2013) The dynamic biliary epithelium. *J Hepatol* 58(3):575–582
- Albores-Saavedra J, Henson DE, Sobin LH (1991) Histological typing of gallbladder and extrahepatic bile ducts. WHO histological typing of tumors of the gallbladder and extrahepatic bile ducts. Springer, Berlin
- Klatskin G (1965) Adenocarcinoma of the hepatic duct at its bifurcation within the porta hepatis. An unusual tumor with distinctive clinical and pathological features. *Am J Med* 38(2):241–256
- Percy C, Van Holten V, Muir C (eds) (1990) International Classification of Diseases for Oncology, 2nd edn. Geneva: WHO
- Matull WR, Khan SA, Pereira SP (2007) Re: Impact of classification of hilar cholangiocarcinomas (Klatskin tumors) on incidence of intra- and extrahepatic cholangiocarcinoma in the United States. *J Natl Cancer Inst* 99(5):407–408
- Welzel TM et al (2006) Impact of classification of hilar cholangiocarcinomas (Klatskin tumors) on the incidence of intra- and extrahepatic cholangiocarcinoma in the United States. *J Natl Cancer Inst* 98(12):873–875
- Fritz A, Constance P, Jack A, Shanmugaratnam K, Sobin LH, Parkin MD (eds) (2000) International classification of diseases for oncology (ICD-O), 3rd edn. World Health Organization, Geneva
- Nakeeb A et al (1996) Cholangiocarcinoma. A spectrum of intrahepatic, perihilar, and distal tumors. *Ann Surg* 224(4):463–473 (discussion 473–475)
- IARC (1994) Schistosomes, liver flukes and helicobacter pylori. IARC Working Group on the evaluation of carcinogenic risks to humans. *IARC Monogr Eval Carcinog Risks Hum* 60:1–560 (Lyon 7–14 June 1994)
- Koga A et al (1985) Hepatolithiasis associated with cholangiocarcinoma. Possible etiologic significance. *Cancer* 55(12):2826–2829
- Rosen CB et al (1991) Cholangiocarcinoma complicating primary sclerosing cholangitis. *Ann Surg* 213(1):21–25
- Srivatanakul P et al (1991) Opisthorchis viverrini infestation and endogenous nitrosamines as risk factors for cholangiocarcinoma in Thailand. *Int J Cancer* 48(6):821–825
- Srivatanakul P et al (1991) The role of infection by Opisthorchis viverrini, hepatitis B virus, and aflatoxin exposure in the etiology of liver cancer in Thailand. A correlation study. *Cancer* 68(11):2411–2417
- Khan SA, Toledano MB, Taylor-Robinson SD (2008) Epidemiology, risk factors, and pathogenesis of cholangiocarcinoma. *HPB (Oxford)* 10(2):77–82
- Tyson GL, El-Serag HB (2011) Risk factors for cholangiocarcinoma. *Hepatology* 54(1):173–184
- Welzel TM et al (2007) Risk factors for intrahepatic and extrahepatic cholangiocarcinoma in the United States: a population-based case-control study. *Clin Gastroenterol Hepatol* 5(10):1221–1228
- Guglielmi A et al (2010) Does intrahepatic cholangiocarcinoma have better prognosis compared to perihilar cholangiocarcinoma? *J Surg Oncol* 101(2):111–115
- Nathan H et al (2007) Trends in survival after surgery for cholangiocarcinoma: a 30-year population-based SEER database analysis. *J Gastrointest Surg* 11(11):488–496 (discussion 1496–1497)
- Patel T (2002) Worldwide trends in mortality from biliary tract malignancies. *BMC Cancer* 2:10
- Patel T (2001) Increasing incidence and mortality of primary intrahepatic cholangiocarcinoma in the United States. *Hepatology* 33(6):1353–1357
- McLean L, Patel T (2006) Racial and ethnic variations in the epidemiology of intrahepatic cholangiocarcinoma in the United States. *Liver Int* 26(9):1047–1053
- Khan SA et al (2012) Rising trends in cholangiocarcinoma: is the ICD classification system misleading us? *J Hepatol* 56(4):848–854
- Alberts SR et al (2012) Occurrence of pancreatic, biliary tract, and gallbladder cancers in Alaska Native people, 1973–2007. *Int J Circumpolar Health* 71:17521
- Yang JD et al (2012) Biliary tract cancers in Olmsted County, Minnesota, 1976–2008. *Am J Gastroenterol* 107(8):1256–1262
- West J et al (2006) Trends in the incidence of primary liver and biliary tract cancers in England and Wales 1971–2001. *Br J Cancer* 94(11):1751–1758
- Taylor-Robinson SD et al (1997) Increase in primary liver cancer in the UK, 1979–1994. *Lancet* 350(9085):1142–1143
- Taylor-Robinson SD et al (2001) Increase in mortality rates from intrahepatic cholangiocarcinoma in England and Wales 1968–1998. *Gut* 48(6):816–820
- Wood R et al (2003) Do increases in mortality from intrahepatic cholangiocarcinoma reflect a genuine increase in risk? Insights from cancer registry data in Scotland. *Eur J Cancer* 39(14):2087–2092
- Coupland VH et al (2012) Incidence and survival for hepatic, pancreatic and biliary cancers in England between 1998 and 2007. *Cancer Epidemiol* 36(4):e207–e214
- Khan SA et al (2002) Changing international trends in mortality rates for liver, biliary and pancreatic tumours. *J Hepatol* 37(6):806–813

42. Engholm G et al (2010) Trends in the survival of patients diagnosed with cancer in the Nordic countries 1964–2003 followed up to the end of 2006. Materials and methods. *Acta Oncol* 49(5):545–560
43. Klint A et al (2010) Trends in survival of patients diagnosed with cancer of the digestive organs in the Nordic countries 1964–2003 followed up to the end of 2006. *Acta Oncol* 49(5):578–607
44. Jepsen P et al (2007) Incidence rates of intra- and extrahepatic cholangiocarcinomas in Denmark from 1978 through 2002. *J Natl Cancer Inst* 99(11):895–897
45. Witjes CD et al (2012) Intrahepatic cholangiocarcinoma in a low endemic area: rising incidence and improved survival. *HPB (Oxford)* 14(11):777–781
46. Alvaro D et al (2010) Descriptive epidemiology of cholangiocarcinoma in Italy. *Dig Liver Dis* 42(7):490–495
47. Lepage C et al (2011) Trends in the incidence and management of biliary tract cancer: a French population-based study. *J Hepatol* 54(2):306–310
48. Boyle P, Smales M (eds) (2008) Atlas of cancer mortality in the European Union and the European Economic Area 1993–1997—IARC Scientific Publication No. 159 IARC, France (Lyon)
49. Shaib YH et al (2004) Rising incidence of intrahepatic cholangiocarcinoma in the United States: a true increase? *J Hepatol* 40(3):472–477
50. Keiser J, Utzinger J (2005) Emerging foodborne trematodiasis. *Emerg Infect Dis* 11(10):1507–1514
51. Bouvard V et al (2009) A review of human carcinogens—Part B: biological agents. *Lancet Oncol* 10(4):321–322
52. Watanapa P, Watanapa WB (2002) Liver fluke-associated cholangiocarcinoma. *Br J Surg* 89(8):962–970
53. Watanapa P (1996) Cholangiocarcinoma in patients with opisthorchiasis. *Br J Surg* 83(8):1062–1064
54. Sripa B et al (2007) Liver fluke induces cholangiocarcinoma. *PLoS Med* 4(7):e201
55. Andoh H et al (2004) Cholangiocarcinoma coincident with schistosomiasis japonica. *J Gastroenterol* 39(1):64–68
56. Furst T, Keiser J, Utzinger J (2012) Global burden of human food-borne trematodiasis: a systematic review and meta-analysis. *Lancet Infect Dis* 12(3):210–221
57. Lun ZR et al (2005) Clonorchiasis: a key foodborne zoonosis in China. *Lancet Infect Dis* 5(1):31–41
58. Gores GJ (2003) Cholangiocarcinoma: current concepts and insights. *Hepatology* 37(5):961–969
59. Malhi H, Gores GJ (2006) Cholangiocarcinoma: modern advances in understanding a deadly old disease. *J Hepatol* 45(6):856–867
60. Marziani M, Fava G, Benedetti A (2006) Nervous and Neuroendocrine regulation of the pathophysiology of cholestasis and of biliary carcinogenesis. *World J Gastroenterol* 12(22):3471–3480
61. Shin HR et al (2010) Epidemiology of cholangiocarcinoma: an update focusing on risk factors. *Cancer Sci* 101(3):579–585
62. Shin HR et al (1996) Hepatitis B and C virus, *Clonorchis sinensis* for the risk of liver cancer: a case-control study in Pusan, Korea. *Int J Epidemiol* 25(5):933–940
63. Choi D et al (2006) Cholangiocarcinoma and *Clonorchis sinensis* infection: a case-control study in Korea. *J Hepatol* 44(6):1066–1073
64. Lim MK et al (2006) *Clonorchis sinensis* infection and increasing risk of cholangiocarcinoma in the Republic of Korea. *Am J Trop Med Hyg* 75(1):93–96
65. Shin HR et al (2010) Descriptive epidemiology of cholangiocarcinoma and clonorchiasis in Korea. *J Korean Med Sci* 25(7):1011–1016
66. Shin HR et al (2010) Comparison of incidence of intrahepatic and extrahepatic cholangiocarcinoma—focus on East and South-Eastern Asia. *Asian Pac J Cancer Prev* 11(5):1159–1166
67. Parkin DM et al (1993) Cholangiocarcinoma: epidemiology, mechanisms of carcinogenesis and prevention. *Cancer Epidemiol Biomark Prev* 2(6):537–544
68. Poomphakwaen K et al (2009) Risk factors for cholangiocarcinoma in Khon Kaen, Thailand: a nested case-control study. *Asian Pac J Cancer Prev* 10(2):251–258
69. Pattanathien P et al (2013) Survival rate of extrahepatic cholangiocarcinoma patients after surgical treatment in Thailand. *Asian Pac J Cancer Prev* 14(1):321–324
70. Hughes NR et al (2006) Liver fluke-associated and sporadic cholangiocarcinoma: an immunohistochemical study of bile duct, peribiliary gland and tumour cell phenotypes. *J Clin Pathol* 59(10):1073–1078
71. Miyakawa S et al (2009) Biliary tract cancer treatment: 5,584 results from the biliary tract cancer statistics registry from 1998 to 2004 in Japan. *J Hepatobiliary Pancreat Surg* 16(1):1–7
72. Nagakawa T et al (2002) Biliary tract cancer treatment: results from the biliary tract cancer statistics registry in Japan. *J Hepato-Biliary-Pancreatic Surg* 9(5):569–575
73. Ishiguro S et al (2008) Risk factors of biliary tract cancer in a large-scale population-based cohort study in Japan (JPHC study); with special focus on cholelithiasis, body mass index, and their effect modification. *Cancer Causes Control* 19(1):33–41
74. Todani T et al (1977) Congenital bile duct cysts: classification, operative procedures, and review of thirty-seven cases including cancer arising from choledochal cyst. *Am J Surg* 134(2):263–269
75. Yamaguchi M (1980) Congenital choledochal cyst. Analysis of 1,433 patients in the Japanese literature. *Am J Surg* 140(5):653–657
76. Soreide K et al (2004) Bile duct cysts in adults. *Br J Surg* 91(12):1538–1548
77. Edil BH et al (2008) Choledochal cyst disease in children and adults: a 30-year single-institution experience. *J Am Coll Surg* 206(5):1000–1005
78. Atkinson HD et al (2003) Choledochal cysts in adults and their complications. *HPB (Oxford)* 5(2):105–110
79. Soreide K, Soreide JA (2007) Bile duct cyst as precursor to biliary tract cancer. *Ann Surg Oncol* 14(3):1200–1211
80. Tan SS et al (2007) Management of adult choledochal cyst. *Singapore Med J* 48(6):524–527
81. Liu CL et al (2002) Choledochal cysts in adults. *Arch Surg* 137(4):465–468
82. Rossi RL et al (1987) Carcinomas arising in cystic conditions of the bile ducts. A clinical and pathologic study. *Ann Surg* 205(4):377–384
83. Nagorney DM, McIlrath DC, Adson MA (1984) Choledochal cysts in adults: clinical management. *Surgery* 96(4):656–663
84. Iwai N et al (1990) Cancer arising in a choledochal cyst in a 12-year-old girl. *J Pediatr Surg* 25(12):1261–1263
85. Visser BC et al (2004) Congenital choledochal cysts in adults. *Arch Surg* 139(8):855–860 (discussion 860–862)
86. de Vries JS et al (2002) Choledochal cysts: age of presentation, symptoms, and late complications related to Todani's classification. *J Pediatr Surg* 37(11):1568–1573
87. Fieber SS, Nance FC (1997) Choledochal cyst and neoplasm: a comprehensive review of 106 cases and presentation of two original cases. *Am Surg* 63(11):982–987
88. Ono S et al (2008) Development of bile duct cancer in a 26-year-old man after resection of infantile choledochal cyst. *J Pediatr Surg* 43(6):E17–E19

89. Iwai N et al (1992) Congenital choledochal dilatation with emphasis on pathophysiology of the biliary tract. *Ann Surg* 215(1):27–30
90. Sirica AE et al (2008) Pathobiology of biliary epithelia and cholangiocarcinoma: proceedings of the Henry M. and Lillian Stratton basic research single-topic conference. *Hepatology* 48(6):2040–2046
91. Iwai N et al (1986) Biliary manometry in choledochal cyst with abnormal cholecopancreatic ductal junction. *J Pediatr Surg* 21(10):873–876
92. Jaiswal M, LaRusso NF, Gores GJ (2001) Nitric oxide in gastrointestinal epithelial cell carcinogenesis: linking inflammation to oncogenesis. *Am J Physiol Gastrointest Liver Physiol* 281(3):G626–G634
93. Wise C et al (2008) Mechanisms of biliary carcinogenesis and growth. *World J Gastroenterol* 14(19):2986–2989
94. Tocchi A et al (2001) Late development of bile duct cancer in patients who had biliary-enteric drainage for benign disease: a follow-up study of more than 1,000 patients. *Ann Surg* 234(2):210–214
95. Hakamada K et al (1997) Late development of bile duct cancer after sphincteroplasty: a ten- to twenty-two-year follow-up study. *Surgery* 121(5):488–492
96. Strong RW (1999) Late bile duct cancer complicating biliary-enteric anastomosis for benign disease. *Am J Surg* 177(6):472–474
97. Strong RW (1999) Does sphincteroplasty predispose to bile duct cancer? *HPB Surg* 11(3):204–206
98. Bettschart V et al (2002) Cholangiocarcinoma arising after biliary-enteric drainage procedures for benign disease. *Gut* 51(1):128–129
99. Karlson BM et al (1997) Population-based study of cancer risk and relative survival following sphincterotomy for stones in the common bile duct. *Br J Surg* 84(9):1235–1238
100. Ando H et al (1996) Intrahepatic bile duct stenosis causing intrahepatic calculi formation following excision of a choledochal cyst. *J Am Coll Surg* 183(1):56–60
101. Sheen-Chen S et al (2000) Bacteriology and antimicrobial choice in hepatolithiasis. *Am J Infect Control* 28(4):298–301
102. Suzuki Y et al (2012) Predictive factors for cholangiocarcinoma associated with hepatolithiasis determined on the basis of Japanese Multicenter study. *Hepatol Res* 42(2):166–170
103. Lee CC, Wu CY, Chen GH (2002) What is the impact of coexistence of hepatolithiasis on cholangiocarcinoma? *J Gastroenterol Hepatol* 17(9):1015–1020
104. Su CH et al (1997) Hepatolithiasis associated with cholangiocarcinoma. *Br J Surg* 84(7):969–973
105. Kubo S et al (1995) Hepatolithiasis associated with cholangiocarcinoma. *World J Surg* 19(4):637–641
106. Chen MF et al (2000) Impact of concomitant hepatolithiasis on patients with peripheral cholangiocarcinoma. *Dig Dis Sci* 45(2):312–316
107. Azuma T et al (1999) The significance of hepatectomy for primary intrahepatic stones. *Surg Today* 29(10):1004–1010
108. Kim YT et al (2003) Factors predicting concurrent cholangiocarcinomas associated with hepatolithiasis. *Hepatogastroenterology* 50(49):8–12
109. Liu ZY et al (2011) Risk factors of intrahepatic cholangiocarcinoma in patients with hepatolithiasis: a case-control study. *Hepatobiliary Pancreat Dis Int* 10(6):626–631
110. Prall RT et al (2000) Current therapies and clinical controversies in the management of primary sclerosing cholangitis. *Curr Gastroenterol Rep* 2(2):99–103
111. Bergquist A et al (1998) Risk factors and clinical presentation of hepatobiliary carcinoma in patients with primary sclerosing cholangitis: a case-control study. *Hepatology* 27(2):311–316
112. Broomé U et al (1996) Natural history and prognostic factors in 305 Swedish patients with primary sclerosing cholangitis. *Gut* 38(4):610–615
113. Wiesner RH et al (1989) Primary sclerosing cholangitis: natural history, prognostic factors and survival analysis. *Hepatology* 10(4):430–436
114. Ahrendt SA et al (1999) Diagnosis and management of cholangiocarcinoma in primary sclerosing cholangitis. *J Gastrointest Surg* 3(4):357–367 (discussion 367–368)
115. Chapman R et al (2010) Diagnosis and management of primary sclerosing cholangitis. *Hepatology* 51(2):660–678
116. Blechacz B, Gores GJ (2008) Cholangiocarcinoma: advances in pathogenesis, diagnosis, and treatment. *Hepatology* 48(1):308–321
117. Brandsaeter B et al (2004) Liver transplantation for primary sclerosing cholangitis: predictors and consequences of hepatobiliary malignancy. *J Hepatol* 40(5):815–822
118. Bergquist A et al (2002) Hepatic and extrahepatic malignancies in primary sclerosing cholangitis. *J Hepatol* 36(3):321–327
119. Lazaridis KN, Gores GJ (2006) Primary sclerosing cholangitis and cholangiocarcinoma. *Semin Liver Dis* 26(1):42–51
120. Tischendorf JJ et al (2007) Characterization, outcome, and prognosis in 273 patients with primary sclerosing cholangitis: a single center study. *Am J Gastroenterol* 102(1):107–114
121. Boberg KM et al (2002) Cholangiocarcinoma in primary sclerosing cholangitis: risk factors and clinical presentation. *Scand J Gastroenterol* 37(10):1205–1211
122. Kaya M et al (2001) Treatment of cholangiocarcinoma complicating primary sclerosing cholangitis: the Mayo Clinic experience. *Am J Gastroenterol* 96(4):1164–1169
123. Brandsaeter B et al (2003) Outcome following liver transplantation for primary sclerosing cholangitis in the Nordic countries. *Scand J Gastroenterol* 38(11):1176–1183
124. de Valle MB, Björnsson E, Lindkvist B (2012) Mortality and cancer risk related to primary sclerosing cholangitis in a Swedish population-based cohort. *Liver Int* 32(3):441–448
125. Kornfeld D, Ekblom A, Ihre T (1997) Survival and risk of cholangiocarcinoma in patients with primary sclerosing cholangitis: a population-based study. *Scand J Gastroenterol* 32(10):1042–1045
126. Tanaka A et al (2008) Outcome and prognostic factors of 391 Japanese patients with primary sclerosing cholangitis. *Liver Int* 28(7):983–989
127. Rea DJ et al (2005) Liver transplantation with neoadjuvant chemoradiation is more effective than resection for hilar cholangiocarcinoma. *Ann Surg* 242(3):451–458 (discussion 458–461)
128. Brandsaeter B et al (2003) Liver transplantation for primary sclerosing cholangitis in the Nordic countries: outcome after acceptance to the waiting list. *Liver Transpl* 9(9):961–969
129. Parkin DM et al (1991) Liver cancer in Thailand. I. A case-control study of cholangiocarcinoma. *Int J Cancer* 48(3):323–328
130. Donato F et al (2001) Intrahepatic cholangiocarcinoma and hepatitis C and B virus infection, alcohol intake, and hepatolithiasis: a case-control study in Italy. *Cancer Causes Control* 12(10):959–964
131. Yamamoto S et al (2004) Hepatitis C virus infection as a likely etiology of intrahepatic cholangiocarcinoma. *Cancer Sci* 95(7):592–595

132. Shaib YH et al (2005) Risk factors of intrahepatic cholangiocarcinoma in the United States: a case-control study. *Gastroenterology* 128(3):620–626
133. El-Serag HB et al (2009) Risk of hepatobiliary and pancreatic cancers after hepatitis C virus infection: a population-based study of U.S. veterans. *Hepatology* 49(1):116–123
134. Fwu CW et al (2011) Hepatitis B virus infection and risk of intrahepatic cholangiocarcinoma and non-Hodgkin lymphoma: a cohort study of parous women in Taiwan. *Hepatology* 53(4):1217–1225
135. Lee CH et al (2009) Viral hepatitis-associated intrahepatic cholangiocarcinoma shares common disease processes with hepatocellular carcinoma. *Br J Cancer* 100(11):1765–1770
136. Lee TY et al (2008) Hepatitis B virus infection and intrahepatic cholangiocarcinoma in Korea: a case-control study. *Am J Gastroenterol* 103(7):1716–1720
137. Zhou H et al (2010) Hepatitis B virus-associated intrahepatic cholangiocarcinoma and hepatocellular carcinoma may hold common disease process for carcinogenesis. *Eur J Cancer* 46(6):1056–1061
138. Li M et al (2012) Hepatitis B virus infection increases the risk of cholangiocarcinoma: a meta-analysis and systematic review. *J Gastroenterol Hepatol* 27(10):1561–1568
139. Zhou Y et al (2012) Hepatitis Viruses infection and risk of intrahepatic cholangiocarcinoma: evidence from a meta-analysis. *BMC Cancer* 12:289
140. Tanaka M et al (2010) Risk factors for intrahepatic cholangiocarcinoma: a possible role of hepatitis B virus. *J Viral Hepat* 17(10):742–748
141. Shaib YH et al (2007) Risk factors for intrahepatic and extrahepatic cholangiocarcinoma: a hospital-based case-control study. *Am J Gastroenterol* 102(5):1016–1021
142. Blum HE et al (1983) Detection of hepatitis B virus DNA in hepatocytes, bile duct epithelium, and vascular elements by in situ hybridization. *Proc Natl Acad Sci USA* 80(21):6685–6688
143. Liorot MA et al (1999) Permissiveness of human biliary epithelial cells to infection by hepatitis C virus. *Hepatology* 29(5):1587–1595
144. Jaiswal M et al (2000) Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by a nitric oxide-dependent mechanism. *Cancer Res* 60(1):184–190
145. Cardinale V et al (2011) Multipotent stem/progenitor cells in human biliary tree give rise to hepatocytes, cholangiocytes, and pancreatic islets. *Hepatology* 54(6):2159–2172
146. Komuta M et al (2008) Clinicopathological study on cholangiolocellular carcinoma suggesting hepatic progenitor cell origin. *Hepatology* 47(5):1544–1556
147. Zhu AX, Lauwers GY, Tanabe KK (2004) Cholangiocarcinoma in association with Thorotrast exposure. *J Hepatobiliary Pancreat Surg* 11(6):430–433
148. IARC (2012) Radiation: a review of human carcinogens. Monographs 100-D. In: Monographs I (ed) Internalized α -particle emitting radionuclides, 2012, IARC, France (Lyon), pp 250–263
149. IARC (2001) Ionizing radiation, part 2: some internally deposited radionuclides. IARC monographs on the evaluation of carcinogenic risks to humans, vol 78. IARC, France (Lyon), pp 1–559 (PMID:11421248)
150. Ito Y (1988) Pathomorphological characteristics of thorotrast-related cholangiocarcinoma. *Kurume Med J* 35(2):63–69
151. Ito Y et al (1988) Pathomorphologic characteristics of 102 cases of thorotrast-related hepatocellular carcinoma, cholangiocarcinoma, and hepatic angiosarcoma. *Cancer* 62(6):1153–1162
152. Mori T, Kato Y (1991) Epidemiological, pathological and dosimetric status of Japanese thorotrast patients. *J Radiat Res* 32(2):34–45
153. van Kaick G et al (1991) Neoplastic diseases induced by chronic alpha-irradiation-epidemiological, biophysical and clinical results of the German Thorotrast Study. *J Radiat Res* 32(2):20–33
154. Liu D et al (2002) Microsatellite instability in thorotrast-induced human intrahepatic cholangiocarcinoma. *Int J Cancer* 102(4):366–371
155. Donohoe CL et al (2010) Obesity and gastrointestinal cancer. *Br J Surg* 97(5):628–642
156. Shebl FM et al (2011) Metabolic syndrome and insulin resistance in relation to biliary tract cancer and stone risks: a population-based study in Shanghai, China. *Br J Cancer* 105(9):1424–1429
157. Welzel TM et al (2007) Risk factors for intrahepatic cholangiocarcinoma in a low-risk population: a nationwide case-control study. *Int J Cancer* 120(3):638–641
158. Grainge MJ et al (2009) The antecedents of biliary cancer: a primary care case-control study in the United Kingdom. *Br J Cancer* 100(1):178–180
159. Pandey M, Shukla M (2009) Helicobacter species are associated with possible increase in risk of hepatobiliary tract cancers. *Surg Oncol* 18(1):51–56
160. Zabron A, Edwards RJ, and Khan SA (2013) The challenge of cholangiocarcinoma: dissecting the molecular mechanisms of an insidious cancer. *Disease Models & Mechanisms* 6:281–292
161. IARC (2013) Ci5. Cancer incidence in five countries project. 2013 April 2013; graphs and tables of cancer incidence in 5 countries data. Available from: <http://www.ci5.iarc.fr>
162. Levi F et al (2003) The recent decline in gallbladder cancer mortality in Europe. *Eur J Cancer Prev* 12(4):265–267
163. Hariharan D, Saied A, Kocher HM (2008) Analysis of mortality rates for gallbladder cancer across the world. *HPB (Oxford)* 10(5):327–331
164. Randi G, Franceschi S, La Vecchia C (2006) Gallbladder cancer worldwide: geographical distribution and risk factors. *Int J Cancer* 118(7):1591–1602
165. Randi G et al (2009) Epidemiology of biliary tract cancers: an update. *Ann Oncol* 20(1):146–159
166. Pandey M (2003) Risk factors for gallbladder cancer: a reappraisal. *Eur J Cancer Prev* 12(1):15–24
167. Zatonski WA et al (1997) Epidemiologic aspects of gallbladder cancer: a case-control study of the SEARCH program of the International Agency for Research on Cancer. *J Natl Cancer Inst* 89(15):1132–1138
168. Diehl AK (1983) Gallstone size and the risk of gallbladder cancer. *JAMA* 250(17):2323–2326
169. Lowenfels AB et al (1989) Gallstone growth, size, and risk of gallbladder cancer: an interracial study. *Int J Epidemiol* 18(1):50–54
170. Roa I et al (2006) Preneoplastic lesions in gallbladder cancer. *J Surg Oncol* 93(8):615–623
171. Kwon W et al (2009) Clinicopathologic features of polypoid lesions of the gallbladder and risk factors of gallbladder cancer. *J Korean Med Sci* 24(3):481–487
172. Zielinski MD et al (2009) Comparison of surgically resected polypoid lesions of the gallbladder to their pre-operative ultrasound characteristics. *J Gastrointest Surg* 13(1):19–25
173. Okamoto M et al (1999) Ultrasonographic evidence of association of polyps and stones with gallbladder cancer. *Am J Gastroenterol* 94(2):446–450

174. Welton JC, Marr JS, Friedman SM (1979) Association between hepatobiliary cancer and typhoid carrier state. *Lancet* 1(8120):791–794
175. Caygill CP et al (1994) Cancer mortality in chronic typhoid and paratyphoid carriers. *Lancet* 343(8889):83–84
176. Misra V et al (2007) *Helicobacter pylori* in areas of gastric metaplasia in the gallbladder and isolation of *H. pylori* DNA from gallstones. *Pathology* 39(4):419–424
177. Sabbaghian MS et al (2010) Identification of *helicobacter* spp. in bile and gallbladder tissue of patients with symptomatic gallbladder disease. *HPB (Oxford)* 12(2):129–133
178. Lambe M et al (1993) Parity and cancers of the gall bladder and the extrahepatic bile ducts. *Int J Cancer* 54(6):941–944
179. Pandey M, Shukla VK (2003) Lifestyle, parity, menstrual and reproductive factors and risk of gallbladder cancer. *Eur J Cancer Prev* 12(4):269–272
180. Larsson SC, Wolk A (2007) Obesity and the risk of gallbladder cancer: a meta-analysis. *Br J Cancer* 96(9):1457–1461
181. Strom BL et al (1995) Risk factors for gallbladder cancer. An international collaborative case-control study. *Cancer* 76(10):1747–1756
182. Diehl AK, Beral V (1981) Cholecystectomy and changing mortality from gallbladder cancer. *Lancet* 2(8239):187–189
183. Diehl AK (1987) Trends in cholecystectomy rates in the United States. *Lancet* 2(8560):683
184. Ekblom A et al (1993) Risk of extrahepatic bile duct cancer after cholecystectomy. *Lancet* 342(8882):1262–1265
185. Wood R et al (2003) Epidemiology of gallbladder cancer and trends in cholecystectomy rates in Scotland, 1968–1998. *Eur J Cancer* 39(14):2080–2086
186. Steinert R et al (2006) Laparoscopic cholecystectomy and gallbladder cancer. *J Surg Oncol* 93(8):682–689
187. Dixon E et al (2005) An aggressive surgical approach leads to improved survival in patients with gallbladder cancer: a 12-year study at a North American Center. *Ann Surg* 241(3):385–394
188. Kondo S et al (2008) Guidelines for the management of biliary tract and ampullary carcinomas: surgical treatment. *J Hepatobiliary Pancreat Surg* 15(1):41–54
189. Albores-Saavedra J et al (2011) Early gallbladder carcinoma: a clinicopathologic study of 13 cases of intramucosal carcinoma. *Am J Clin Pathol* 135(4):637–642
190. Bartlett DL et al (1996) Long-term results after resection for gallbladder cancer. Implications for staging and management. *Ann Surg* 224(5):639–646
191. Alberts SR et al (2005) Gemcitabine, 5-fluorouracil, and leucovorin in advanced biliary tract and gallbladder carcinoma: a North Central Cancer Treatment Group phase II trial. *Cancer* 103(1):111–118
192. Fong Y et al (2006) Evidence-based gallbladder cancer staging: changing cancer staging by analysis of data from the National Cancer Database. *Ann Surg* 243(6):767–771
193. Taner CB, Nagorney DM, Donohue JH (2004) Surgical treatment of gallbladder cancer. *J Gastrointest Surg* 8(1):83–89 (discussion 89)
194. Mayo SC et al (2010) National trends in the management and survival of surgically managed gallbladder adenocarcinoma over 15 years: a population-based analysis. *J Gastrointest Surg* 14(10):1578–1591
195. SEER, S.R.P., National Cancer Institute SEER/- Stat software, version 7.0.4. *Faststats—SEER, Surveillance Research Program, National Cancer Institute SEER/- Stat software, version 7.0.4.* 2013; Available from: <http://www.seer.cancer.gov/faststats/>
196. Kiran RP, Pokala N, Dudrick SJ (2007) Incidence pattern and survival for gallbladder cancer over three decades—an analysis of 10301 patients. *Ann Surg Oncol* 14(2):827–832
197. Donohue JH, Stewart AK, Menck HR (1998) The National Cancer Data Base report on carcinoma of the gallbladder, 1989–1995. *Cancer* 83(12):2618–2628
198. Le MD et al (2011) Is gallbladder cancer decreasing in view of increasing laparoscopic cholecystectomy? *Ann Hepatol* 10(3):306–314
199. Lemrow SM et al (2008) Gallbladder cancer incidence among American Indians and Alaska Natives, US, 1999–2004. *Cancer* 113(5):1266–1273
200. Csendes A et al (2000) Number and size of stones in patients with asymptomatic and symptomatic gallstones and gallbladder carcinoma: a prospective study of 592 cases. *J Gastrointest Surg* 4(5):481–485
201. Renard P et al (1987) Biliary tract cancers in Cote-d’Or (France): incidence and natural history. *J Epidemiol Community Health* 41(4):344–348
202. Zatonski W et al (1993) Descriptive epidemiology of gallbladder cancer in Europe. *J Cancer Res Clin Oncol* 119(3):165–171
203. Khan MR et al (2011) Gallbladder intestinal metaplasia in Pakistani patients with gallstones. *Int J Surg* 9(6):482–485
204. Wistuba II, Gazdar AF (2004) Gallbladder cancer: lessons from a rare tumour. *Nat Rev Cancer* 4(9):695–706
205. Kumar JR et al (2006) An objective assessment of demography of gallbladder cancer. *J Surg Oncol* 93(8):610–614
206. Unisa S et al (2011) Population-based study to estimate prevalence and determine risk factors of gallbladder diseases in the rural Gangetic basin of North India. *HPB (Oxford)* 13(2):117–125
207. Yagyu K et al (2008) Cigarette smoking, alcohol drinking and the risk of gallbladder cancer death: a prospective cohort study in Japan. *Int J Cancer* 122(4):924–929
208. Lazcano-Ponce EC et al (2001) Epidemiology and molecular pathology of gallbladder cancer. *CA Cancer J Clin* 51(6):349–364
209. Kimura K et al (1985) Association of gallbladder carcinoma and anomalous pancreaticobiliary ductal union. *Gastroenterology* 89(6):1258–1265
210. Alatisse OI et al (2012) Audit of management of gallbladder cancer in a Nigerian tertiary health facility. *J Gastrointest Cancer* 43(3):472–480
211. Hawkins WG et al (2004) Jaundice predicts advanced disease and early mortality in patients with gallbladder cancer. *Ann Surg Oncol* 11(3):310–315
212. Andia ME et al (2008) Geographic variation of gallbladder cancer mortality and risk factors in Chile: a population-based ecologic study. *Int J Cancer* 123(6):1411–1416
213. Carey MC, Paigen B (2002) Epidemiology of the American Indians’ burden and its likely genetic origins. *Hepatology* 36(4 Pt 1):781–791
214. Everhart JE et al (1999) Prevalence and ethnic differences in gallbladder disease in the United States. *Gastroenterology* 117(3):632–639
215. Everhart JE et al (2002) Prevalence of gallbladder disease in American Indian populations: findings from the strong heart study. *Hepatology* 35(6):1507–1512
216. Mendez-Sanchez N et al (2004) The Amerindian’s genes in the Mexican population are associated with development of gallstone disease. *Am J Gastroenterol* 99(11):2166–2170
217. Miyazaki M et al (2008) Risk factors for biliary tract and ampullary carcinomas and prophylactic surgery for these factors. *J Hepatobiliary Pancreat Surg* 15(1):15–24
218. Shrikhande SV et al (2010) Cholelithiasis in gallbladder cancer: coincidence, cofactor, or cause? *Eur J Surg Oncol* 36(6):514–519
219. Halldestam I, Kullman E, Borch K (2009) Incidence of and potential risk factors for gallstone disease in a general population sample. *Br J Surg* 96(11):1315–1322

220. Shaffer EA (2005) Epidemiology and risk factors for gallstone disease: has the paradigm changed in the 21st century? *Curr Gastroenterol Rep* 7(2):132–140
221. Hsing AW et al (2007) Gallstones and the risk of biliary tract cancer: a population-based study in China. *Br J Cancer* 97(11):1577–1582
222. Hsing AW et al (2007) Family history of gallstones and the risk of biliary tract cancer and gallstones: a population-based study in Shanghai, China. *Int J Cancer* 121(4):832–838
223. Tazuma S, Kajiyama G (2001) Carcinogenesis of malignant lesions of the gall bladder. The impact of chronic inflammation and gallstones. *Langenbecks Arch Surg* 386(3):224–229
224. Miquel JF et al (1998) Genetic epidemiology of cholesterol cholelithiasis among Chilean Hispanics, Amerindians, and Maoris. *Gastroenterology* 115(4):937–946
225. Stephen AE, Berger DL (2001) Carcinoma in the porcelain gallbladder: a relationship revisited. *Surgery* 129(6):699–703
226. Chijiwa K, Tanaka M (1994) Polypoid lesion of the gallbladder: indications of carcinoma and outcome after surgery for malignant polypoid lesion. *Int Surg* 79(2):106–109
227. Dooley J, Sherlock S (2011) *Sherlock's diseases of the liver and biliary system*, 12th edn. Wiley-Blackwell, Chichester, p 771 (xvi)
228. Kubota K et al (1995) How should polypoid lesions of the gallbladder be treated in the era of laparoscopic cholecystectomy? *Surgery* 117(5):481–487
229. Lee KF et al (2004) Polypoid lesions of the gallbladder. *Am J Surg* 188(2):186–190
230. Ito H et al (2009) Polypoid lesions of the gallbladder: diagnosis and followup. *J Am Coll Surg* 208(4):570–575
231. Roa I et al (2010) Association between cholesterosis and gallbladder cancer. *Rev Med Chil* 138(7):804–808
232. Urbach DR, Stukel TA (2005) Rate of elective cholecystectomy and the incidence of severe gallstone disease. *CMAJ* 172(8):1015–1019
233. Whalen GF et al (2001) Laparoscopic cholecystectomy does not demonstrably decrease survival of patients with serendipitously treated gallbladder cancer. *J Am Coll Surg* 192(2):189–195
234. de Aretxabala XA et al (2004) Laparoscopic cholecystectomy: its effect on the prognosis of patients with gallbladder cancer. *World J Surg* 28(6):544–547
235. Fong Y, Heffernan N, Blumgart LH (1998) Gallbladder carcinoma discovered during laparoscopic cholecystectomy: aggressive resection is beneficial. *Cancer* 83(3):423–427
236. Yamaguchi K et al (1996) Gallbladder carcinoma in the era of laparoscopic cholecystectomy. *Arch Surg* 131(9):981–984 (discussion 985)
237. Varshney S, Butturini G, Gupta R (2002) Incidental carcinoma of the gallbladder. *Eur J Surg Oncol* 28(1):4–10
238. Fuks D et al (2011) Incidental gallbladder cancer by the AFC-GBC-2009 Study Group. *World J Surg* 35(8):1887–1897
239. Reid KM, Ramos-De la Medina A, Donohue H (2007) Diagnosis and surgical management of gallbladder cancer: a review. *J Gastrointest Surg* 11(5):671–681
240. Z'Graggen K et al (1998) Incidence of port site recurrence after laparoscopic cholecystectomy for preoperatively unsuspected gallbladder carcinoma. *Surgery* 124(5):831–838
241. Misra MC, Guleria S (2006) Management of cancer gallbladder found as a surprise on a resected gallbladder specimen. *J Surg Oncol* 93(8):690–698
242. Kao WY et al (2013) Risk of hepato-biliary cancer after cholecystectomy: a nationwide cohort study. *J Gastrointest Surg* 17(2):345–351
243. Chow WH et al (1999) Gallstones, cholecystectomy and risk of cancers of the liver, biliary tract and pancreas. *Br J Cancer* 79(3–4):640–644
244. Chijiwa K, Tanaka M, Nakayama F (1993) Adenocarcinoma of the gallbladder associated with anomalous pancreaticobiliary ductal junction. *Am Surg* 59(7):430–434
245. Shimada K et al (1993) Biliary bile acids in the gall-bladder and the common bile duct of patients with anomalous pancreaticobiliary ductal junction. *J Gastroenterol Hepatol* 8(2):138–141
246. Sugiyama Y et al (2000) Altered bile composition in the gallbladder and common bile duct of patients with anomalous pancreaticobiliary ductal junction. *World J Surg* 24(1):17–20 (discussion 21)
247. Chao TC, Jan YY, Chen MF (1995) Primary carcinoma of the gallbladder associated with anomalous pancreaticobiliary ductal junction. *J Clin Gastroenterol* 21(4):306–308
248. Funabiki T et al (1997) Biliary carcinogenesis in pancreaticobiliary maljunction. *J Hep Bil Pancr Surg* 4:405–411
249. Kang CM et al (2007) Gallbladder carcinoma associated with anomalous pancreaticobiliary duct junction. *Can J Gastroenterol* 21(6):383–387
250. Hu B, Gong B, Zhou DY (2003) Association of anomalous pancreaticobiliary ductal junction with gallbladder carcinoma in Chinese patients: an ERCP study. *Gastrointest Endosc* 57(4):541–545
251. Kamisawa T et al (2002) Clinical significance of a long common channel. *Pancreatol* 2(2):122–128
252. Mellema A, Gaarslev K (1988) Risk of hepatobiliary cancer in carriers of *Salmonella typhi*. *J Natl Cancer Inst* 80(4):288
253. Dutta U et al (2000) Typhoid carriers among patients with gallstones are at increased risk for carcinoma of the gallbladder. *Am J Gastroenterol* 95(3):784–787
254. Fox JG et al (1998) Hepatic *Helicobacter* species identified in bile and gallbladder tissue from Chileans with chronic cholecystitis. *Gastroenterology* 114(4):755–763
255. Chen W et al (2003) Common presence of *Helicobacter* DNA in the gallbladder of patients with gallstone diseases and controls. *Dig Liver Dis* 35(4):237–243
256. Silva CP et al (2003) Association of the presence of *Helicobacter* in gallbladder tissue with cholelithiasis and cholecystitis. *J Clin Microbiol* 41(12):5615–5618
257. Blendis L (2005) What are *Helicobacter* doing in the hepatobiliary system? *Gastroenterology* 129(2):761–763
258. Hsing AW et al (2008) Body size and the risk of biliary tract cancer: a population-based study in China. *Br J Cancer* 99(5):811–815
259. Pan WH et al (2004) Body mass index and obesity-related metabolic disorders in Taiwanese and US whites and blacks: implications for definitions of overweight and obesity for Asians. *Am J Clin Nutr* 79(1):31–39
260. Chang SC et al (2008) Polymorphism of genes related to insulin sensitivity and the risk of biliary tract cancer and biliary stone: a population-based case-control study in Shanghai, China. *Carcinogenesis* 29(5):944–948
261. Hemminki K, Li X (2003) Familial liver and gall bladder cancer: a nationwide epidemiological study from Sweden. *Gut* 52(4):592–596
262. Weiderpass E, Pukkala E (2006) Time trends in socioeconomic differences in incidence rates of cancers of gastro-intestinal tract in Finland. *BMC Gastroenterol* 6:41
263. Serra I et al (2002) Association of chili pepper consumption, low socioeconomic status and longstanding gallstones with gallbladder cancer in a Chilean population. *Int J Cancer* 102(4):407–411
264. Chow WH et al (1995) Smoking and biliary tract cancers in a cohort of US veterans. *Br J Cancer* 72(6):1556–1558
265. SEER, S.R.P., National Cancer Institute SEER/- Stat software, version 7.0.4. *Surveillance, Epidemiology, and End Results*

- (SEER) Program (<http://www.seer.cancer.gov>) SEER*Stat Database: Incidence—SEER 13 Regs Research Data, Nov 2011 Sub (1992–2009), National Cancer Institute, DCCPS, Surveillance Research Program, Surveillance Systems Branch, released April 2013, based on the November 2012 submission. 2013 April 2013]; SEER Incidence Data]. Available from: <http://www.seer.cancer.gov>
266. SEER, S.R.P., National Cancer Institute SEER/- Stat software, version 7.0.4. *US Mortality Data 1969–2010 when Using SEER*Stat: Surveillance, Epidemiology, and End Results (SEER) Program* (<http://www.seer.cancer.gov>) SEER*Stat Database: Mortality—All COD, Aggregated With State, Total U.S. (1969–2010) <Katrina/Rita Population Adjustment> , National Cancer Institute, DCCPS, Surveillance Research Program, Surveillance Systems Branch, released April 2013. Underlying mortality data provided by NCHS (<http://www.cdc.gov/nchs>). 2013; Mortality data]. Available from: <http://www.seer.cancer.gov>
267. Scotland ISDI, Scotland NNS (2012) Cancer in Scotland. 2012 April 2013; Available from: <http://www.isdscotland.org/Health-Topics/Cancer/Cancer-Statistics/>
268. Andia KM, Gederlini GA, Ferreccio RC (2006) Gallbladder cancer: trend and risk distribution in Chile. *Rev Med Chil* 134(5):565–574

Role of Growth Factor Signaling Pathways in Biliary Tract Cancer

Kaoru Kiguchi and John DiGiovanni

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Abstract

Biliary-tract carcinomas (BTCs) are relatively infrequent but highly lethal malignancies. Novel targets for therapeutic or chemopreventive approaches are urgently needed. However, the knowledge of genomic mutations in BTC is less extensive than that of other gastrointestinal cancers. In this chapter, we will discuss the role of growth factors and their receptors (receptor tyrosine kinases, RTKs), downstream signaling pathways of these RTKs and inflammatory mediators during gallbladder carcinogenesis based on our study using a mouse model for human BTC as well as additional information in the literature.

1 Introduction

Biliary tract carcinomas (BTCs), which include cancers of the gallbladder (GBCs) and the intra- and extra-hepatic biliary tree, are relatively infrequent but highly lethal malignancies [1]. Although there have been advances in the diagnosis and management of BTCs, these cancers still prove challenging to treat due to their insensitivity to conventional therapies and the inability to prevent or detect early tumor formation. These factors render gallbladder cancer nearly incurable with a five-year survival rate of only 5–21 % [2–6]. Novel targets for therapeutic or chemopreventive approaches are urgently needed. We previously generated transgenic mice that overexpress wild-type rat erbB2 in epithelial tissues under the control of the bovine keratin 5 (BK5) promoter (BK5.erbB2 mice) [7]. Overexpression of erbB2 in basal epithelial cells of the gallbladder led to the development of adenocarcinoma of the gallbladder and cystic duct in 90 % of these transgenic mice by 2–3 months of age. This was the first direct demonstration that erbB2 overexpression could lead to the development of BTC [7]. We have shown that BK5.erbB2 transgenic mice are a valid model for investigating mechanisms underlying the development of GBCs and other BTCs. We have found

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that protein levels of erbB2 as well as protein levels of epidermal growth factor receptor (EGFR) are elevated in the gallbladder in BK5.erbB2 transgenic mice. In addition, we have found elevated levels of COX-2/PGE₂ and elevated activity of Akt, MAPK (mitogen-activated protein kinase), and mTOR (mammalian target of rapamycin) in the GBC from these mice. These molecular alterations are similar to those reported in human GBC or BTC.

In this chapter, we will discuss the role of growth factors and their receptors (receptor tyrosine kinases, RTKs), downstream signaling pathways of these RTKs, and inflammatory mediators during gallbladder carcinogenesis. Understanding the growth factor signaling pathways upregulated in GBC will provide critical clues for novel therapeutic and chemopreventive strategies using drugs and/or agents that selectively target these specific pathways.

2 Molecular Aspect of BTC

The knowledge of genomic mutations in BTC is less extensive than that of other gastrointestinal cancers. Although the molecular aspects of BTC remain poorly understood, several genetic abnormalities have been described in specimens of human GBC. Genetic alterations in p53 or K-ras may contribute to the development of certain types of GBC [8–13]. In this regard, Hanada et al. [11] found that the incidence of p53 mutations and protein expression was significantly less in the polypoid type (adenoma–carcinoma sequence) of GBC compared with the flat type (de novo development). Li et al. [14] have reported that p53 overexpression was detected in 43 % of adenomas, 60 % of dysplasias, and 57 % of GBCs in addition to frequent observation of reduced p21^{WAF1/CIP1} expression. Mutations in codon 12 of K-ras are seen infrequently in GBC, except in those cases where the carcinoma is associated with an anomalous junction of the pancreaticobiliary duct (APDJ) [8–13]. Recent studies describe the presence of p53 mutation in 92 % of invasive GBC [13]. ErbB2 overexpression has been reported in a significant percentage of GBCs [14–16] and cholangiocarcinomas [16–21]. The protein levels of both EGFR and its ligand, transforming growth factor- α (TGF α), assessed by immunostaining, are elevated in human BTC including GBC [22, 23]. Accumulating evidence suggests that COX-2, an inducible enzyme responsible for conversion of arachidonic acid to prostaglandins, may play a variety of roles in the gastrointestinal tract including pathogenic processes such as neoplasia [24]. A recent study demonstrated a relationship between erbB2 overexpression and COX-2 upregulation in human colorectal cancer cells [25]. Elevated COX-2 expression has been demonstrated in well-differentiated human hepatocellular carcinoma [26, 27] and GBC [28] compared with low or non-detectable COX-2

expression in poorly differentiated tumors. Very recently, Sirica's group reported a strong positive correlation between the immunostaining intensities of erbB2 and COX-2 in BTC. COX-2 was observed not only in the furan rat cholangiocarcinoma model, but also in human cholangiocarcinomas [29], supporting the possibility that erbB2 plays a key role in regulating COX-2 expression in neoplastic and precancerous biliary tract epithelial cells. Grossman et al. [30] reported a specific COX-2 inhibitor, but not COX-1 inhibitor, decreased mitogenesis, and increased human gallbladder cell apoptosis associated with decreased prostaglandin E₂ (PGE₂). This suggests that the COX enzymes and the prostanooids may play a role in the development of gallbladder cancer and that COX-2 inhibitors may have a therapeutic role in gallbladder neoplasms [30].

3 Role of ErbB RTKs and Their Downstream Signaling Pathways in the Development of BTC

3.1 ErbB2 and EGFR in Human BTC

To date, very few studies have addressed the molecular and cellular mechanisms underlying the development of BTC as described above; however, several lines of evidence suggest a role for the erbB receptor family. Overexpression and activation of erbB2 have been reported in a significant percentage of human BTC [15, 16, 31, 32]. In one study, 30 of 43 cases (69.6 %) and 14 of 43 cases (32.6 %) of GBCs had amplification of erbB2 DNA or overexpression of erbB2 protein, respectively [15]. In another study, 7 of 11 cases (63.6 %) of GBCs showed overexpression of erbB2 protein [16]. Yukawa et al. [17] reported erbB2 protein expression in 9 of 13 cases (69 %) of GBCs considered to be relatively early-stage tumors (all 13 cases were histologically diagnosed as well-differentiated tubular adenocarcinoma), yet erbB2 protein expression was undetectable in tumors that were more advanced. Furthermore, ErbB2 has been shown to be overexpressed in the neoplastic glandular epithelium of furan- and thioacetamide-induced intestinal-type cholangiocarcinomas in rat liver [33, 34]. It has also been reported that erbB2-transformed rat cholangiocytes, which overexpressed activated erbB2, obtained a tumorigenic feature when transplanted into isogenic rats, yielding a 100 % incidence of BTCs [34]. Overexpression and activation of epidermal growth factor receptor (EGFR) have also been reported in 30–60 % of BTC samples [31, 32, 35] and were shown to be correlated with negative clinical and pathologic features, such as distant metastasis and poor dedifferentiation [22, 36–38]. These data suggest that altered expression and activity of erbB2 and EGFR are major mechanisms underlying human BTC carcinogenesis [39].

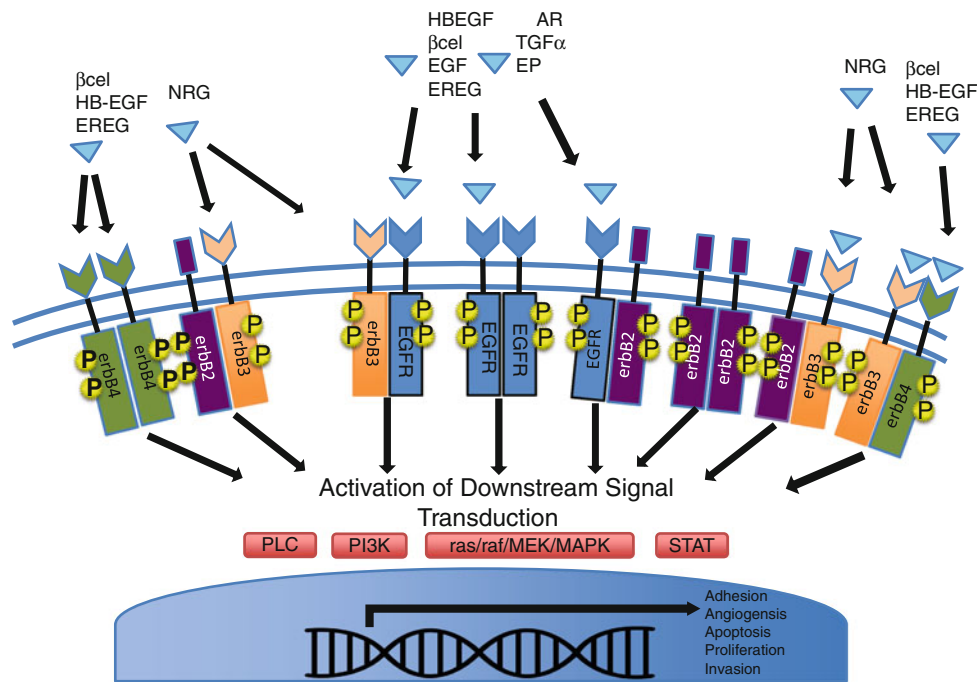


Fig. 1 ErbB family signaling system. Cross talk between erbB2/EGFR and other erbB receptor tyrosine kinase members and downstream signaling which leads to cell proliferation, survival, and migration (see text in detail)

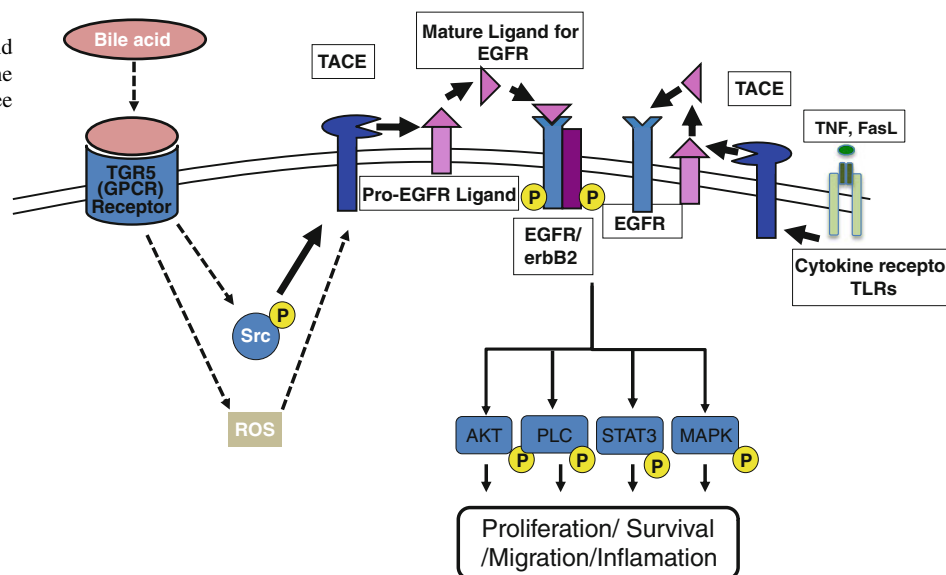
3.2 erbB RTK Family

Several lines of evidence suggest a role for the erbB receptor family as described above. A number of RTKs have been described [40–42]. Among them is the erbB family of RTKs consisting of the epidermal growth factor receptor (EGFR/erbB1), erbB2 (neu), erbB3, and erbB4 [43]. ErbB family RTKs have been shown to be important for normal development as well as in neoplasia [40, 44] (Fig. 1). Although all of the erbB family members share similarities in primary structure, receptor activation mechanism, and signal transduction patterns, they bind to different ligands. EGFR binds to and can be activated by a number of different ligands of the EGF family, including EGF, transforming growth factor- α (TGF- α), heparin-binding EGF-like growth factor (HB-EGF), amphiregulin (AR), betacellulin [45, 46], epigen (EP), and epiregulin (EREG). The neuregulin subfamily consists of various isoforms referred to as 1–4. These ligands bind to erbB4 and/or erbB3. Betacellulin, HB-EGF, and epiregulin have also been shown to bind to erbB4. Ligand-dependent activation of erbB family receptors can lead to heterodimerization, particularly of EGFR, erbB3 and erbB4 with erbB2. To date, no ligand has been identified for erbB2. ErbB3 cannot generate signals in isolation because the kinase function of this receptor is impaired, thus relying on interaction with erbB2 for signaling.

Post-receptor signaling by activated erbB family members includes signaling through Ras/MEK/MAPK/Erk (extracellular signal-regulated kinase), phospholipase C γ , signal transducer and activation of transcription (STATs), and phosphatidylinositol 3-kinase (PI3K) pathways that are common to nearly all RTKs (Fig. 1). Although the membrane-anchored peptide can be biologically active through juxtacrine signaling, in most cases, the extracellular domain is proteolytically cleaved by a metalloprotease activity present in the cell membrane. This process is known as “ectodomain shedding” and leads to the release of the soluble growth factor, which may act in an endocrine, paracrine, or autocrine fashion [47].

To allow paracrine or autocrine interaction of the EGFR ligands with the receptor, the membrane-tethered ligand precursors need to be released by a proteolytic reaction. This important step is mediated mainly by membrane-anchored metalloproteases of the ADAM (a disintegrin and metalloprotease) family [48]. ADAM17, which is also known as tumor necrosis factor- α (TNF- α)-converting enzyme, or TACE, together with ADAM10, is thought to play a central role. ADAM17 can cleave the AR, EREG, TGF- α , and HB-EGF membrane-anchored precursors, while ADAM 10 is a key sheddase for EGF and BTC, and can also cleave the HB-HGF transmembrane precursor [45, 46, 49]. Transactivation of the EGFR by ligands of G-protein-coupled receptors (GPCRs) is perhaps the best characterized example of EGFR

Fig. 2 ErbB2/EGFR transactivation through TLRs, GPCR, and TACE and cross talk between src which leads to the development of biliary tract cancer (see text in detail)



activation by heterologous ligands [48]. These include angiotensin II (ANG II), lysophosphatidic acid (LPA), endothelin-I, thrombin, IL-8, and prostaglandins such as PGE2 [48]. Different mechanisms have been proposed to mediate ADAM activation by GPCRs. Elevation of the intracellular levels of Ca^{2+} or reactive oxygen species (ROS) is likely to be involved as well as phosphorylation reactions involving protein kinase C (PKC), ERK, or c-Src [48]. As previously indicated, transactivation of the EGFR is not exclusive of GPCR-triggered signaling. Studies carried out in keratinocytes have established that the expression and release of EGFR ligands can be elicited by the cytokines $\text{TNF-}\alpha$ and interferon- γ (INF- γ) [50]. This has been recently observed also for the proapoptotic factor Fas ligand (FasL). Interestingly, it was shown that transactivation of the EGFR through the secretion of ligands such as AR contributed to mediate part of the inflammatory responses to FasL in human epidermis [51] (Fig. 2).

3.3 Animal Models for Human BTC

3.3.1 Background

As mentioned, very few studies have attempted to decipher the molecular and cellular mechanism(s) involved in the development of BTC; thus, very little is known regarding the sequence of events that lead to this disease. A limiting factor has been the lack of relevant animal models for the study of early events in BTC. Presently available animal models are based on exposure to chemical carcinogens, and in most of these models, the latency between the treatment and tumor development is long and the tumor incidence is relatively low. However, the furan rat model described by Sirica et al. gives rise to a very high incidence of BTC, intrahepatic

cholangiocarcinoma [52, 53]. In this model, treatment of rats with furan rapidly induced intestinal metaplasia and associated cholangiofibrosis in the right/caudate liver of rats [54]. Long-term treatment with furan (daily dose of 30 mg/kg of body weight, five times weekly by gavage for 9–13 weeks) resulted in the preferential development of cholangiocarcinoma [53]. The incidence of cholangiocarcinoma was 70–90 % in rats treated with furan by 16 months. The furan-induced cholangiocarcinoma in this rat model characteristically overexpressed erbB2, COX-2, and c-Met [54]. In addition to this model, combined treatment of Syrian golden hamster with dihydroxy-di-n-propyl nitrosamine and liver fluke infestation was shown to be associated with the enhancement of cholangiocarcinomas and preneoplastic lesions in the gallbladder [55].

Recently, we developed BK5.erbB2 transgenic mice, where expression of the rat erbB2 cDNA is targeted to the basal layer of multiple epithelial tissues, including the biliary tract epithelium [7, 56] (Fig. 3a). Adenocarcinoma of the gallbladder develops in 90 % of the homozygous BK5.erbB2 transgenic mice by 2–3 months of age [7]. The BK5.erbB2 transgenic mouse line represents the first genetically engineered mouse model for investigating the mechanism(s) underlying the development of GBCs and other BTCs. The remainder of this section will be devoted to a summary of this model and its initial utilization for preclinical therapeutic studies.

3.3.2 BK5.erbB2 Mouse Model of Gallbladder Cancer

Necropsy of adult BK5.erbB2 mice revealed that the gallbladder was dramatically enlarged and had a white, opaque appearance (Fig. 3bB). Enlarged gallbladders were often associated with a significantly dilated common bile duct

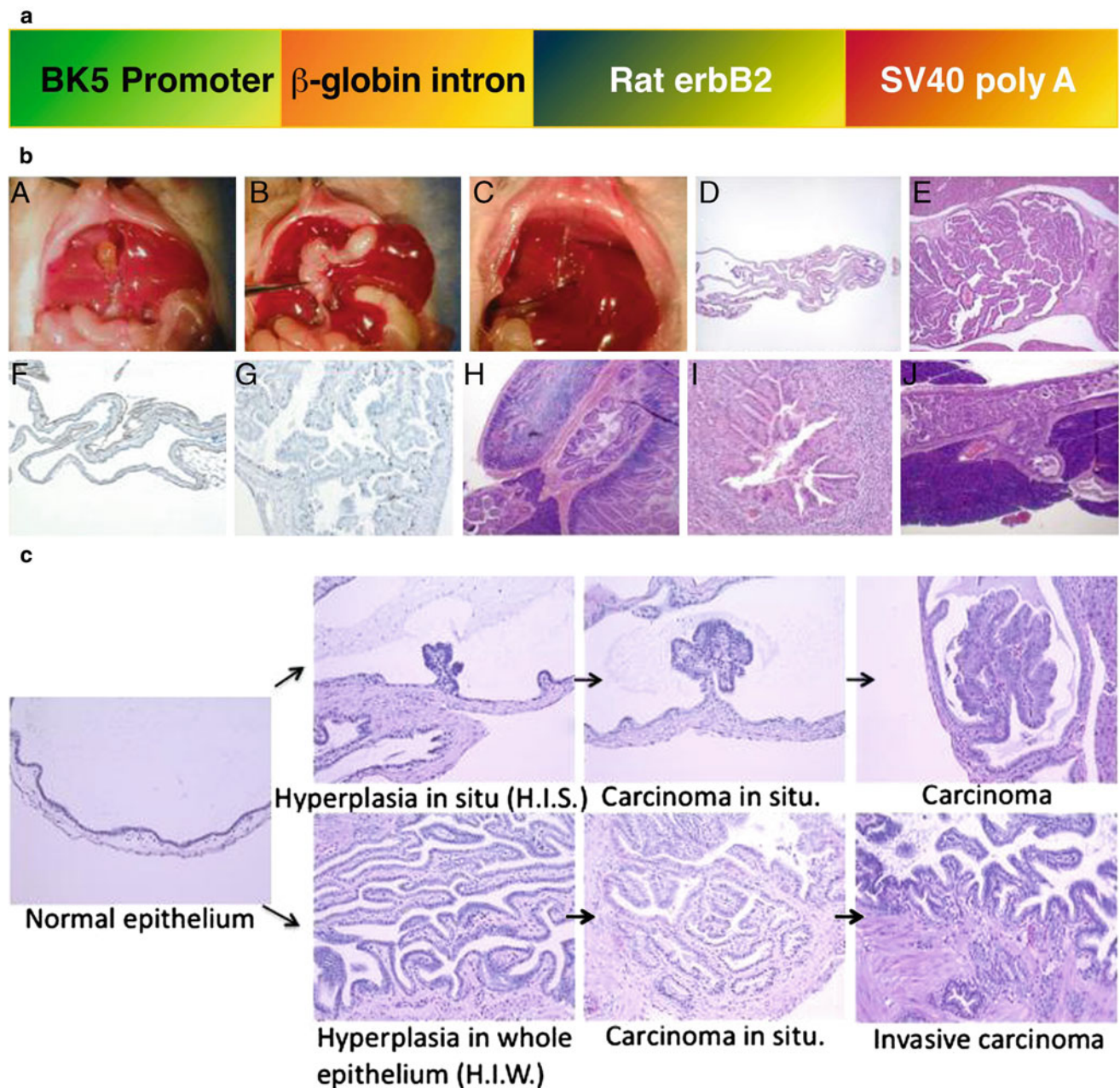


Fig. 3 **a** The DNA construct used to generate BK5.erbB2 mice. **b** Gross appearance and histological evaluations of BTC in BK5.erbB2 mice. **A** Gallbladder of wild-type mouse, **B** BK5.erbB2 mouse at 3 months of age, **C** anomalous fasciculus form of gallbladder in the early stage of gallbladder development (2 weeks of age) in BK5.erbB2 mouse, **D** H and E staining of gallbladder in wild-type mouse and **E** BK5.erbB2 mouse, **F** BrdU staining of gallbladder in wild-type mouse and **G** BK5.erbB2 mouse, **H** H and E staining of the ampulla of

Vater, **I** intrahepatic cholangiocarcinoma, and **J** the junction of the pancreaticobiliary duct (JPBD) in a 3-month-old BK5.erbB2 mouse. **c** Two different pathways of development of GBC in BK5.erbB2 mice. (*Upper figures*) Carcinoma arising from hyperplasia in situ shown in an adenoma/hyperplasia/carcinoma sequence. (*Lower figures*) Carcinoma arising from hyperplasia shown in a de novo sequence. (*Figure on left*) Normal gallbladder from wild-type control mouse. Some of figures are adopted from Kiguchi K et al. [7]

(Fig. 3bB). This enlarged hepatic duct from the liver and the cystic duct from the gallbladder unite to form the enlarged common bile duct, which extends posteriorly through the pancreas and intestinal wall, where it opens to the mucosal surface of the duodenum as the ampulla of

Vater (Fig. 3B and H). Most of the gallbladders in young BK5.erbB2 mice (<3 weeks) possess an anomalous fasciculus structure (Fig 3bC). The majority of the GBCs completely filled the lumen (Fig. 3E), although some showed focal lesions.

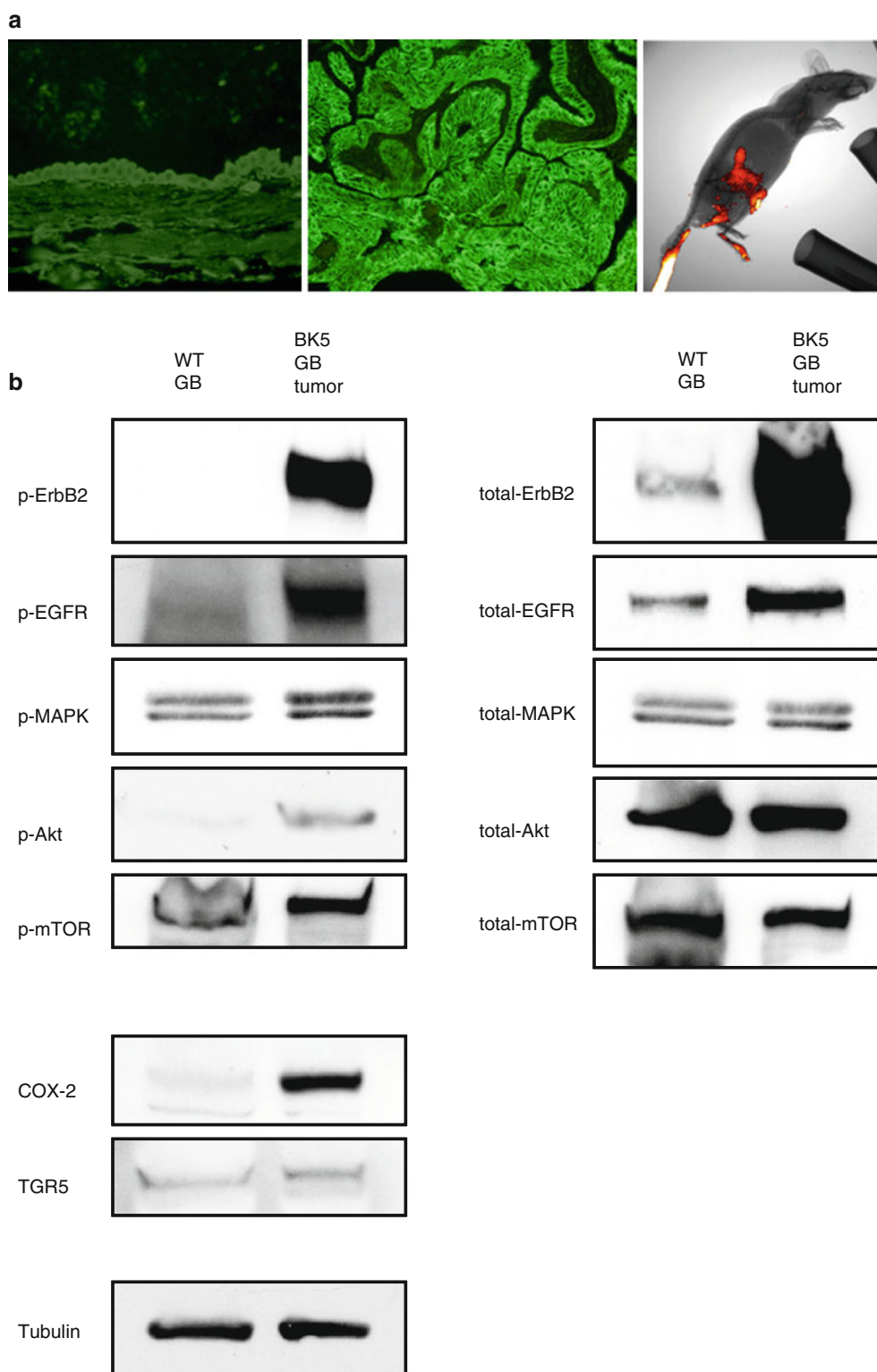


Fig. 4 a Expression of ErbB2: immunostaining for erbB2 in gallbladder from wild-type mouse (*left*) and BK5.erbB2 mouse (*middle*) of 3-month-old mouse. Image of GBC detected by Kodak in-vivo Imaging System FX-Pro, 48 h after EGF-labeled probe injection (*right, arrow*). High-intensity area in tail is injection site

(*arrow head*). **b** Analysis of protein statuses and kinase activation in gallbladder from wild-type and BK5.erbB2 mice. Whole tissue lysates from wild-type and BK5.erbB2 mice were analyzed via Western blot with antibodies against indicated molecules. Tubulin was used as an internal control

Analysis of the mucosa adjacent to the GBC observed in the mice allowed segregation into two categories based on etiology: carcinoma arising from hyperplasia *in situ* (HIS, 14 cases out of 34 GBCs from BK5.erbB2 mice, 41 %) or hyperplasia in whole mucosa (HIW, 59 %) (Fig. 3c). GBC tumors arising from HIW were more likely to be invasive (70 %, $p < 0.01$) compared to those arising from HIS (14 %). Tumors were characterized by branching structures with finger-like projections covered with high columnar epithelium and hyperchromatic nuclei. Most of the tumors were diagnosed as well-differentiated adenocarcinomas. Carcinoma cells frequently invaded into the surrounding connective tissues. In addition, hypervascularization was a characteristic feature of these tumors. Staining with CD31, a marker for endothelial cells, revealed extensive vascularization in the adenocarcinomas from BK5.erbB2 mice [7]. Adenocarcinomas from BK5.erbB2 mice exhibited a significantly elevated labeling index (a marker of proliferation) compared to normal gallbladder epithelium as determined by staining with antibromodeoxyuridine (BrdU) antibody (Fig. 3bG). Tumor cells of the common bile duct often invaded into the pancreatic duct (Fig. 3bJ). The ampulla of Vater was dilated, and hyperplasia of the epithelium was observed in transgenic mice. Pronounced congestion of bile, inflammation, necrosis, hyperplasia of biliary duct cells, and/or tumor development was also frequently observed in intrahepatic biliary ducts of transgenic mice (Fig. 3bI).

3.3.3 Status of EGFR and ErbB2 in GBC of BK5.erbB2 Mice

Persistent expression of the erbB2 transgene was observed in the epithelia of both gallbladder and intrahepatic biliary duct as well as in gallbladder adenocarcinoma (Fig. 4a) and cholangiocarcinomas [7]. Endogenous erbB2 expression was only weakly detectable in both the intrahepatic biliary duct and gallbladder from wild-type mice (Fig. 4a). Western blot analysis of gallbladder tissue lysates showed that the level of erbB2 protein was significantly elevated in BK5.erbB2 mice compared to that of wild-type mice, as expected (Fig. 4b). ErbB2 was also hyperphosphorylated after adjustment for total erbB2 protein level (Fig. 4b). Interestingly, the level of EGFR protein (but not erbB3 or erbB4 protein) was elevated and hyperphosphorylated on tyrosine residues in gallbladder tissue from BK5.erbB2 mice (Fig. 4b). Additional analyses by immunoprecipitation of EGFR and erbB2 followed by Western blot analysis for erbB2 and EGFR, respectively, confirmed elevated heterodimer formation between erbB2 and EGFR in gallbladder tissue of BK5.erbB2 mice [7]. Furthermore, to detect gallbladder tumors in BK5.erbB2 mice *in vivo*, we utilized a molecular imaging system. BK5.erbB2 mice were injected via tail vein with either EGF-labeled NHS ester conjugate with infrared dye 800CW (IRDye 800CW EGF

probe) or IRDye 800CW Carbonate as control. The distribution of the IRDye 800CW EGF was visualized by the Kodak *in vivo* Imaging System FX-Pro (Carestream Health Inc., Rochester, NY). 48 h after the injection, the EGF probe accumulated in the gallbladder (Fig. 4a). The background signal in the gallbladders of mice injected with IRD 800CW Carbonate as well as the gallbladder of wild-type mice injected with the EGF probe was undetectable (data not shown). This preliminary experiment indicates that the level of EGFR and/or erbB2 is significantly high in the gallbladder of BK5.erbB2 mice and that this bioimaging technique can be a useful tool for tracking tumor size in longitudinal *in vivo* experiments.

3.3.4 MAPK, Akt, and mTOR in Gallbladder Tissue of BK5.erbB2 Mice

The activation status of signaling molecules downstream of erbB2/EGFR and the status of other proteins were also examined. Although total protein levels of MAPK were not changed (Fig. 4b), the level of phosphorylation of MAPK was increased in the gallbladder of transgenic mice. Furthermore, phospho-Akt, but not total Akt level, was elevated in the gallbladder of BK5.erbB2 mice as assessed by Western blot analysis (Fig. 4b). We have reported that mTOR and other signaling molecules both immediately upstream (Akt, MAPK) and downstream (p70S6 K) of mTOR are hyperphosphorylated in gallbladder tissues from BK5.erbB2 mice compared to corresponding tissue from wild-type mice [39]. We also found that cyclin D1, bcl-2, c-Met, E-cadherin, and b-catenin were upregulated in the gallbladder tissue of BK5.erbB2 compared to wild-type mouse by Western blot analysis [7].

3.3.5 Increased COX-2 Protein and mRNA Expression, PGE2 Synthesis, and Phosphorylation of PLA2 in GBC of BK5.erbB2 Mice

The protein level (determined by immunohistochemistry and Western blot) and mRNA expression (determined by RT-PCR) of COX-2 were significantly elevated in the gallbladder tissue of BK5.erbB2 mice compared to wild-type mice (Fig. 4b) and [7]. The level of PGE2 was also found to be elevated in the tissue [47]. These results suggest that elevated prostaglandins, particularly PGE2, may play an important role in the development of GBC in BK5.erbB2 mice. Phosphorylated form of phospholipase A2 (PLA2), but not total PLA2, was also elevated in the gallbladder tissue of BK5.erbB2 mice (not shown).

3.3.6 Therapeutic Studies with Specific Molecular Targeting Agents Using BK5.erbB2 Mice

Similarities in molecular alterations, such as overexpression and/or activation of erbB2, EGFR, Akt, and COX-2 between BTCs in BK5.erbB2 mice and humans, make

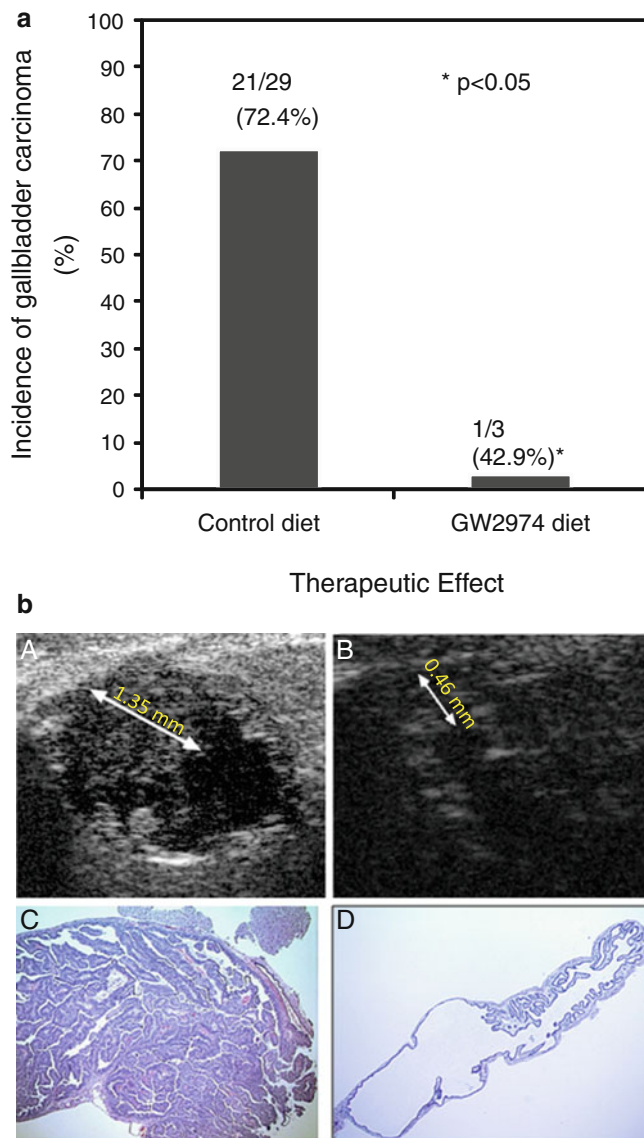


Fig. 5 Therapeutic effect of tyrosine kinase inhibitor, GW2974, and HDAC inhibitor, PCI-24781, on the incidence of GBC in BK5.erbB2 mice. **a** Incidence of GBC in BK5.erbB2 mice. Two month-old BK5.erbB2 mice were treated with AIN76 control diet or AIN76 diet containing 200 ppm GW2974 for one month. *, $P < 0.01$. **b** Effects of GW2974 detected by ultrasound and **b** histological analyses. Regression of GBC by GW2974 treatment as detected by ultrasound biomicroscopy. All images are from a single animal depicting the response representative of the treatment group. **A** Ultrasound image of GBC (maximum size: 1.35 mm) before GW2974 treatment. **B** Ultrasound image of gallbladder on the 23rd day of treatment; this image indicates regression of the carcinoma (observed size: 0.46 mm). **C** Gallbladder of BK5.erbB2 mouse receiving AIN76 control diet. **D** Typical histological features of the gallbladder from BK5.erbB2 mice treated with AIN76 diet containing 200 ppm GW2974. Figures are adopted from Kiguchi K et al. [56]

BK5.erbB2 transgenic mice a unique animal model for further mechanistic studies regarding the role of erbB2/EGFR and their downstream signaling in the development and growth of BTC, as well as a promising tool for the development of new treatment and/or prevention modalities. We have used this model successfully in several pre-clinical therapeutic studies using tyrosine kinase inhibitors [56], a COX-2 inhibitor [57], an mTOR inhibitor [58], and histone deacetylase (HDAC) inhibitor [59]. Figure 5 shows the therapeutic effect of GW2974, a dual specific erbB2/EGFR inhibitor [60]. In this experiment, BK5.erbB2 mice received 200 ppm GW2974 in the diet for 1 month. Treatment with GW2974 resulted in a significant decrease in the incidence of GBC to 3 % (Fig. 5a). These reductions corresponded to a 95 % decrease in tumor incidence compared with BK5.erbB2 mice receiving the control diet, which had a GBC incidence of 72 % as determined by histopathological examination. The impact of treatment is very clearly seen in the ultrasound images in Fig. 5b. H and E staining in the right panels clearly shows that the dramatic regression of the tumor with only hyperplasia is still evident (Fig 5b). The labeling index determined by BrdU staining was also reduced in the gallbladder of mice receiving GW2974.

Treatment with GW2974 resulted in decreased levels of both erbB2 and EGFR. Furthermore, levels of p-erbB2 and p-EGFR were markedly reduced [56]. Nearly complete inhibition of tumor development by GW2974 suggests a level of erbB2 dependency during gallbladder tumor development in BK5.erbB2 mice. Treatment of BK5.erbB2 mice with the HDAC inhibitor, PCI-24781, for 1 month prevented 79 % of GBCs cases from progression and showed a clinical effect in 47 % of cases. This effect was associated with downregulation of erbB2 mRNA, ErbB2 protein/activity, and EGFR activity and upregulation of acetylated histone and acetylated tubulin [58]. These results indicate that the significant therapeutic/inhibitory effect that this HDAC has on the development of gallbladder tumors is due to its ability to block the activation of both erbB2 and EGFR.

We also examined the effects of a COX-2 inhibitor, CS-706, on the development of GBCs using the BK5.erbB2 mouse model. Ultrasound image analysis as well as histological evaluation revealed a significant therapeutic effect of CS-706 on the GBCs, either as reversion to a milder phenotype or as inhibition of tumor progression. The antitumor effect was associated with inhibition of prostaglandin E2 synthesis. CS-706 treatment also downregulated the activation of erbB2 and EGFR, resulting in decreased levels of phosphorylated Akt and COX-2 in GBCs of BK5.erbB2

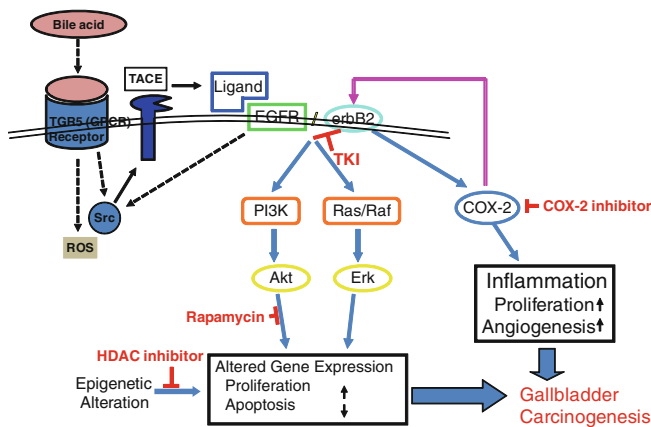


Fig. 6 Proposed pathway in which erbB2/EGFR, COX-2, bile acid, and src may play a role during the BTC carcinogenesis. ErbB2 overexpression/activation may accelerate the transactivation cascade of during the development of BTC. Figure is adopted from Kiguchi et al. [75]

mice. Based on our results, targeting COX-2 could provide a potentially new and effective therapy alone or in combination with other therapeutic agents for patients with BTC [57].

In addition, BK5.erbB2 mice were treated with rapamycin by i.p. injection (5 mg/kg BW, once daily for 14 days). Rapamycin significantly reduced the incidence and severity of GBCs in BK5.erbB2 mice in a dose-dependent manner. Tumors responsive to treatment exhibited a higher number of apoptotic cells. Furthermore, rapamycin treatment led to decreased levels of phosphorylated p70 S6 kinase (Thr389) in gallbladder tissue as assessed by both Western blot and immunofluorescence analyses. Immunofluorescence staining revealed elevated phosphorylated Akt (Ser473) and phosphorylated mammalian target of rapamycin (mTOR; Ser2448) in human GBC compared with normal gallbladder tissue. Based on the fact that the Akt/mTOR pathway is activated in human GBC, rapamycin and related drugs may be effective therapeutic agents for the treatment of human GBC with activated Akt/mTOR pathway [58]. Proposed inhibitory effect of each therapeutic compound on the signaling pathways in GBC of BK5.erbB2 mice is shown in Fig. 6.

4 The Role of Bile Acid During BTC Carcinogenesis

Exposure to high levels or abnormal composition of bile acid is associated with an increased incidence of cancer of the laryngopharyngeal tract, esophagus, stomach, pancreas, small intestine, and colon [61]. Bile acids, which are synthesized from cholesterol, have long been recognized as essential for dietary lipid absorption; however, an important role for bile acids as signaling molecules has emerged in recent years [62–64]. Bile acids activate EGFR, MAPK, and

PI3-K/Akt signaling pathways in hepatocytes [65, 66]. More recent evidence suggests that bile acids may activate RTKs and downstream signaling molecules, indirectly, in a G-protein-coupled receptor (GPCR)-dependent manner [67] mediated by ADAM family peptidases [68, 69]. The role of cell signaling by these organic acids in the development of human biliary tract cancer remains unknown. A recent study from our laboratory [70] demonstrated that the secondary conjugated bile acid, taurochenodeoxycholic acid (TCDC), increased proliferation of primary cultured gallbladder epithelial cells from BK5.erbB2 mice and human BTC cells. TCDC treatment activated erbB2/EGFR and downstream signaling molecules in both primary cultured cells and human BTC cells. TCDC also increased the expression of EGFR ligands and TACE activity in human BTC cells. These results suggest that during the development of BTC, bile acid may act as a promoter when erbB2 is activated in gallbladder epithelial cells.

Previous lines of evidence have suggested that the non-receptor tyrosine kinase, c-Src, elicits cross talk between TGR5 (a GPCR) and EGFR to transduce bile acid signaling for activation of EGFR [71–74]. Figure 6 shows the proposed role of erbB2/EGFR and other key molecules as well as possible cross talk in the development of BTC in BK5.erbB2 mice.

5 Conclusion and Future Direction

The dismal outcomes that generally result from gallbladder carcinoma and other BTCs explain the pessimism that surrounds treatment for these cancers. Nevertheless, more aggressive surgical technique, advanced oncologic, and radiation therapy have led many institutions to report an increase in long-term survival rates. Although these treatments are progressive, the efforts directed toward early detection and novel treatment derived from basic research to determine the mechanisms involved in BTC development may play a key role in the improvement of patients' survival. New drugs that selectively target specific augmented molecule(s) such as erbB2 and COX-2 in BTC and associated risk conditions may serve as potentially effective adjunct therapeutic strategies for this cancer, for which there is currently no effective medical treatment. In addition, identification of novel candidate gene(s) or protein(s), which regulate these mechanisms, may provide not only potential therapeutic targets, but also novel tumor markers for this lethal disease. The BK5.erbB2 transgenic mouse model provides a unique opportunity to study the mechanisms involved in the development of this cancer.

Acknowledgments The authors would like to thank Christie Nguyen and Lauren Pascale for technical assistance.

References

- Wistuba II, Gazdar AF (2004) Gallbladder cancer: lessons from a rare tumour. *Nat Rev Cancer* 4(9):695–706
- Carriaga MT, Henson DE (1995) Liver, gallbladder, extrahepatic bile ducts, and pancreas. *Cancer* 75(1 Suppl):171–190
- Cuberta-fond P, Gainant A, Cucchiario G (1994) Surgical treatment of 724 carcinomas of the gallbladder. Results of the French Surgical Association Survey. *Ann Surg* 219(3):275–280.
- Ruckert JC, Ruckert RI, Gellert K, Hecker K, Muller JM (1996) Surgery for carcinoma of the gallbladder. *Hepato-gastroenterology* 43(9):527–533
- Oertli D, Herzog U, Tondelli P (1993) Primary carcinoma of the gallbladder: operative experience during a 16 year period. *Eur J Surg* 159(8):415–420
- Ogura Y, Mizumoto R, Isaji S, Kusuda T, Matsuda S, Tabata M (1991) Radical operations for carcinoma of the gallbladder: present status in Japan. *World J Surg* 15(3):337–343
- Kiguchi K, Carbajal S, Chan K, Beltran L, Ruffino L, Shen J, Matsumoto T, Yoshimi N, DiGiovanni J (2001) Constitutive expression of ErbB-2 in gallbladder epithelium results in development of adenocarcinoma. *Cancer Res* 61(19):6971–6976
- Itoi T, Watanabe H, Ajioka Y, Oohashi Y, Takel K, Nishikura K, Nakamura Y, Horil A, Saito T (1996) APC, K-ras codon 12 mutations and p53 gene expression in carcinoma and adenoma of the gall-bladder suggest two genetic pathways in gall-bladder carcinogenesis. *Pathol Int* 46(5):333–340
- Watanabe H, Date K, Itoi T, Matsubayashi H, Yokoyama N, Yamano M, Ajioka Y, Nishikura K (1999) Histological and genetic changes in malignant transformation of gallbladder adenoma. *Ann Oncol Official J Eur Soc Med Oncol/ESMO* 10(Suppl 4):136–139
- Hanada K, Itoh M, Fujii K, Tsuchida A, Ooishi H, Kajiyama G (1997) K-ras and p53 mutations in stage I gallbladder carcinoma with special attention to growth patterns. *Europ J Cancer* 33:1136–1140
- Hanada K, Tsuchida A, Iwao T, Eguchi N, Sasaki T, Morinaka K, Matsubara K, Kawasaki Y, Yamamoto S, Kajiyama G (1999) Gene mutations of K-ras in gallbladder mucosae and gallbladder carcinoma with an anomalous junction of the pancreaticobiliary duct. *Am J Gastroenterol* 94(6):1638–1642
- Hidaka E, Yanagisawa A, Seki M, Takano K, Setoguchi T, Kato Y (2000) High frequency of K-ras mutations in biliary duct carcinomas of cases with a long common channel in the papilla of Vater. *Cancer Res* 60(3):522–524
- Wee A, Teh M, Raju GC (1994) Clinical importance of p53 protein in gall bladder carcinoma and its precursor lesions. *J Clin Pathol* 47(5):453–456
- Li X, Hui AM, Shi YZ, Takayama T, Makuuchi M (2001) Reduced p21(WAF1/CIP1) expression is an early event in gallbladder carcinogenesis and is of prognostic significance for patients with carcinomas of the gallbladder. *Hum Pathol* 32(8):771–777
- Suzuki T, Takano Y, Kakita A, Okudaira M (1993) An immunohistochemical and molecular biological study of c-erbB-2 amplification and prognostic relevance in gallbladder cancer. *Pathol Res Pract* 189(3):283–292
- Chow NH, Huang SM, Chan SH, Mo LR, Hwang MH, Su WC (1995) Significance of c-erbB-2 expression in normal and neoplastic epithelium of biliary tract. *Anticancer Res* 15(3):1055–1059
- Yukawa M, Fujimori T, Hirayama D, Idei Y, Ajiki T, Kawai K, Sugiura R, Maeda S, Nagasako K (1993) Expression of oncogene products and growth factors in early gallbladder cancer, advanced gallbladder cancer, and chronic cholecystitis. *Hum Pathol* 24(1):37–40
- Suzuki H, Isaji S, Pairojkul C, Uttaravichien T (2000) Comparative clinicopathological study of resected intrahepatic cholangiocarcinoma in northeast Thailand and Japan. *J Hepatobiliary Pancreat Surg* 7(2):206–211
- Ito Y, Takeda T, Sasaki Y, Sakon M, Yamada T, Ishiguro S, Imaoka S, Tsujimoto M, Higashiyama S, Monden M, Matsuura N (2001) Expression and clinical significance of the erbB family in intrahepatic cholangiocellular carcinoma. *Pathol Res Pract* 197(2):95–100
- Aishima SI, Taguchi KI, Sugimachi K, Shimada M, Sugimachi K, Tsuneyoshi M (2002) c-erbB-2 and c-Met expression relates to cholangiocarcinogenesis and progression of intrahepatic cholangiocarcinoma. *Histopathology* 40(3):269–278
- Ukita Y, Kato M, Terada T (2002) Gene amplification and mRNA and protein overexpression of c-erbB-2 (HER-2/neu) in human intrahepatic cholangiocarcinoma as detected by fluorescence in situ hybridization, in situ hybridization, and immunohistochemistry. *J Hepatol* 36(6):780–785
- Lee CS, Pirdas A (1995) Epidermal growth factor receptor immunoreactivity in gallbladder and extrahepatic biliary tract tumours. *Pathol Res Pract* 191(11):1087–1091
- Lee CS (1998) Transforming growth factor alpha immunoreactivity in human gallbladder and extrahepatic biliary tract tumours. *Eur J Surg Oncol* 24(1):38–42
- DuBois RN, Eberhart CF, Williams CS (1996) Introduction to eicosanoids and the gastroenteric tract. *Gastroenterol Clin N Am* 25(2):267–277
- Vadlamudi R, Mandal M, Adam L, Steinbach G, Mendelsohn J, Kumar R (1999) Regulation of cyclooxygenase-2 pathway by HER2 receptor. *Oncogene* 18(2):305–314
- Koga H, Sakisaka S, Ohishi M, Kawaguchi T, Taniguchi E, Sasatomi K, Harada M, Kusaba T, Tanaka M, Kimura R, Nakashima Y, Nakashima O, Kojiro M, Kurohiji T, Sata M (1999) Expression of cyclooxygenase-2 in human hepatocellular carcinoma: relevance to tumor dedifferentiation. *Hepatology* 29(3):688–696
- Kondo M, Yamamoto H, Nagano H, Okami J, Ito Y, Shimizu J, Eguchi H, Miyamoto A, Dono K, Umeshita K, Matsuura N, Wakasa K, Nakamori S, Sakon M, Monden M (1999) Increased expression of COX-2 in nontumor liver tissue is associated with shorter disease-free survival in patients with hepatocellular carcinoma. *Clin Cancer Res* 5(12):4005–4012
- Ghosh M, Kawamoto T, Koike N, Fukao K, Yoshida S, Kashiwagi H, Kapoor VK, Agarwal S, Krishnani N, Uchida K, Miwa M, Todoroki T (2000) Cyclooxygenase expression in the gallbladder. *Int J Mol Med* 6(5):527–532
- Endo K, Yoon BI, Pairojkul C, Demetris AJ, Sirica AE (2002) ERBB-2 overexpression and cyclooxygenase-2 up-regulation in human cholangiocarcinoma and risk conditions. *Hepatology* 36(2):439–450
- Grossman EM, Longo WE, Panesar N, Mazuski JE, Kaminski DL (2000) The role of cyclooxygenase enzymes in the growth of human gall bladder cancer cells. *Carcinogenesis* 21(7):1403–1409
- Kim YW, Huh SH, Park YK, Yoon TY, Lee SM, Hong SH (2001) Expression of the c-erb-B2 and p53 protein in gallbladder carcinomas. *Oncol Rep* 8(5):1127–1132
- Kawamoto T, Krishnamurthy S, Tarco E, Trivedi S, Wistuba II, Li D, Roa I, Roa JC, Thomas MB (2007) HER receptor family: novel candidate for targeted therapy for gallbladder and extrahepatic bile duct cancer. *Gastrointest Cancer Res* 1(6):221–227
- Radaeva S, Ferreira-Gonzalez A, Sirica AE (1999) Overexpression of C-NEU and C-MET during rat liver cholangiocarcinogenesis: a link between biliary intestinal metaplasia and mucin-producing cholangiocarcinoma. *Hepatology* 29(5):1453–1462
- Yeh CN, Maitra A, Lee KF, Jan YY, Chen MF (2004) Thioacetamide-induced intestinal-type cholangiocarcinoma in rat: an animal model recapitulating the multi-stage progression of human cholangiocarcinoma. *Carcinogenesis* 25(4):631–636

35. Leone F, Cavalloni G, Pignochino Y, Sarotto I, Ferraris R, Piacibello W, Venesio T, Capussotti L, Risio M, Aglietta M (2006) Somatic mutations of epidermal growth factor receptor in bile duct and gallbladder carcinoma. *Clin Cancer Res* 12(6):1680–1685
36. Kaufman M, Mehrotra B, Limaye S, White S, Fuchs A, Lebowicz Y, Nissel-Horowitz S, Thomas A (2008) EGFR expression in gallbladder carcinoma in North America. *Int J Med Sci* 5:285–291
37. Sasaki T, Hiroki K, Yamashita Y (2013) The role of epidermal growth factor receptor in cancer metastasis and microenvironment. *Biomed Res Int* 546318–546325
38. Ogo Y, Nio Y, Yano S, Toga T, Koike M, Hashimoto K, Itakura M, Maruyama R (2006) Immunohistochemical expression of HER-1 and HER-2 in extrahepatic biliary carcinoma. *Anticancer Res* 26(1B):763–770
39. Kiguchi K, DiGiovanni J (2009) Role of growth factors and signal transduction pathways in biliary tract cancer. In: Clifton Fuller M, Charles Thomas M (eds) *Biliary tract and gallbladder cancer: diagnosis and therapy*. Demos Medical Publishing
40. Aaronson SA (1991) Growth factors and cancer. *Science* 254:1146–1153
41. Schlessinger J, Ullrich A (1992) Growth factor signaling by receptor tyrosine kinases. *Neuron* 9:389–391
42. Ullrich A, Schlessinger J (1990) Signal transduction by receptors with tyrosine kinase activity. *Cell* 61:203–212
43. Dougall W, Qian X, Peterson N, Miller M, Samanta A, Greene M (1994) The neu-oncogene: signal transduction pathways, transformation mechanisms and evolving therapies. *Oncogene* 9:2109–2123
44. Gullick WJ (1991) Prevalence of aberrant expression of the epidermal growth factor receptor in human cancers. *Br Med Bull* 47:87–98
45. Wieduwilt MJ, Moasser MM (2008) The epidermal growth factor receptor family: biology driving targeted therapeutics. *Cell Mol Life Sci (CMLS)* 65(10):1566–1584
46. Yarden Y, Shilo BZ (2007) SnapShot: EGFR signaling pathway. *Cell* 131(5):1018
47. Schneider MR, Wolf E (2009) The epidermal growth factor receptor ligands at a glance. *J Cell Physiol* 218(3):460–466
48. Ohtsu H, Dempsey PJ, Eguchi S (2006) ADAMs as mediators of EGF receptor transactivation by G protein-coupled receptors. *Am J Physiol Cell Physiol* 291(1):C1–10
49. Berasain C, Nicou A, Garcia-Irigoyen O, Latasa MU, Urtasun R, Elizalde M, Salis F, Perugorria MJ, Prieto J, Recio JA, Corrales FJ, Avila MA (2012) Epidermal growth factor receptor signaling in hepatocellular carcinoma: inflammatory activation and a new intracellular regulatory mechanism. *Dig Dis* 30(5):524–531
50. Pastore S, Mascia F, Mariani V, Girolomoni G (2008) The epidermal growth factor receptor system in skin repair and inflammation. *J Invest Dermatol* 128(6):1365–1374
51. Farley SM, Purdy DE, Ryabinina OP, Schneider P, Magun BE, Jordanov MS (2008) Fas ligand-induced proinflammatory transcriptional responses in reconstructed human epidermis. Recruitment of the epidermal growth factor receptor and activation of MAP kinases. *J Biol Chem* 283(2):919–928
52. Sirica AE, Lai GH, Zhang Z (2001) Biliary cancer growth factor pathways, cyclo-oxygenase-2 and potential therapeutic strategies. *J Gastroenterol Hepatol* 16(4):363–372
53. Elmore LW, Sirica AE (1993) “Intestinal-type” of adenocarcinoma preferentially induced in right/caudate liver lobes of rats treated with furan. *Cancer Res* 53(2):254–259
54. Elmore LW, Sirica AE (1991) Phenotypic characterization of metaplastic intestinal glands and ductular hepatocytes in cholangiofibrotic lesions rapidly induced in the caudate liver lobe of rats treated with furan. *Cancer Res* 51(20):5752–5759
55. Thamavit W, Moore MA, Hiasa Y, Ito N (1988) Enhancement of DHPN induced hepatocellular, cholangiocellular and pancreatic carcinogenesis by *Opisthorchis viverrini* infestation in Syrian golden hamsters. *Carcinogenesis* 9(6):1095–1098
56. Kiguchi K, Ruffino L, Kawamoto T, Ajiki T, DiGiovanni J (2005) Chemopreventive and therapeutic efficacy of orally active tyrosine kinase inhibitors in a transgenic mouse model of gallbladder carcinoma. *Clin Cancer Res* 11(15):5572–5580
57. Kiguchi K, Ruffino L, Kawamoto T, Franco E, Kurakata S, Fujiwara K, Hanai M, Rumi M, DiGiovanni J (2007) Therapeutic effect of CS-706, a specific cyclooxygenase-2 inhibitor, on gallbladder carcinoma in BK5.ErbB-2 mice. *Mol Cancer Ther* 6(6):1709–1717
58. Wu Q, Kiguchi K, Kawamoto T, Ajiki T, Traag J, Carbajal S, Ruffino L, Thames H, Wistuba I, Thomas M, Vasquez KM, DiGiovanni J (2007) Therapeutic effect of rapamycin on gallbladder cancer in a transgenic mouse model. *Cancer Res* 67(8):3794–3800
59. Kitamura T, Connolly K, Ruffino L, Ajiki T, Lueckgen A, DiGiovanni J, Kiguchi K (2012) The therapeutic effect of histone deacetylase inhibitor PCI-24781 on gallbladder carcinoma in BK5.erbB2 mice. *J Hepatol* 57(1):84–91
60. Rusnak DW, Affleck K, Cockerill SG, Stubberfield C, Harris R, Page M, Smith KJ, Guntrip SB, Carter MC, Shaw RJ, Jowett A, Stables J, Topley P, Wood ER, Brignola PS, Kadwell SH, Reep BR, Mullin RJ, Alligood KJ, Keith BR, Crosby RM, Murray DM, Knight WB, Gilmer TM, Lackey K (2001) The characterization of novel, dual ErbB-2/EGFR, tyrosine kinase inhibitors: potential therapy for cancer. *Cancer Res* 61(19):7196–7203
61. Bernstein H, Bernstein C, Payne CM, Dvorakova K, Garewal H (2005) Bile acids as carcinogens in human gastrointestinal cancers. *Mutat Res* 589(1):47–65
62. Raufman JP, Zimniak P, Bartoszko-Malik A (1998) Lithocholyltaurine interacts with cholinergic receptors on dispersed chief cells from guinea pig stomach. *Am J Physiol* 274(6 Pt 1):G997–1004
63. Parks DJ, Blanchard SG, Bledsoe RK, Chandra G, Consler TG, Kliewer SA, Stimmel JB, Willson TM, Zavacki AM, Moore DD, Lehmann JM (1999) Bile acids: natural ligands for an orphan nuclear receptor. *Science* 284(5418):1365–1368
64. Makishima M, Okamoto AY, Repa JJ, Tu H, Learned RM, Luk A, Hull MV, Lustig KD, Mangelsdorf DJ, Shan B (1999) Identification of a nuclear receptor for bile acids. *Science* 284(5418):1362–1365
65. Qiao L, Studer E, Leach K, McKinsty R, Gupta S, Decker R, Kukreja R, Valerie K, Nagarkatti P, El Deiry W, Molkentin J, Schmidt-Ullrich R, Fisher PB, Grant S, Hylemon PB, Dent P (2001) Deoxycholic acid (DCA) causes ligand-independent activation of epidermal growth factor receptor (EGFR) and FAS receptor in primary hepatocytes: inhibition of EGFR/mitogen-activated protein kinase-signaling module enhances DCA-induced apoptosis. *Mol Biol Cell* 12(9):2629–2645
66. Rao YP, Studer EJ, Stravitz RT, Gupta S, Qiao L, Dent P, Hylemon PB (2002) Activation of the Raf-1/MEK/ERK cascade by bile acids occurs via the epidermal growth factor receptor in primary rat hepatocytes. *Hepatology* 35(2):307–314
67. Hylemon PB, Zhou H, Pandak WM, Ren S, Gil G, Dent P (2009) Bile acids as regulatory molecules. *J Lipid Res* 50(8):1509–1520
68. Mifune M, Ohtsu H, Suzuki H, Nakashima H, Brailoiu E, Dun NJ, Frank GD, Inagami T, Higashiyama S, Thomas WG, Eckhart AD, Dempsey PJ, Eguchi S (2005) G protein coupling and second messenger generation are indispensable for metalloprotease-dependent, heparin-binding epidermal growth factor shedding through angiotensin II type-1 receptor. *J Biol Chem* 280(28):26592–26599
69. Higashiyama S, Nanba D (2005) ADAM-mediated ectodomain shedding of HB-EGF in receptor cross-talk. *Biochim Biophys Acta* 1751(1):110–117

70. Kitamura T, Srivastava J, DiGiovanni J, Kiguchi K (2013) Bile Acid Accelerates ErbB2-Induced Pro-Tumorigenic Activities in Biliary Tract Cancer. *Mol Carcinog* (on line)
71. Maruyama T, Miyamoto Y, Nakamura T, Tamai Y, Okada H, Sugiyama E, Nakamura T, Itadani H, Tanaka K (2002) Identification of membrane-type receptor for bile acids (M-BAR). *Biochem Biophys Res Commun* 298(5):714–719
72. Dent P, Fang Y, Gupta S, Studer E, Mitchell C, Spiegel S, Hylemon PB (2005) Conjugated bile acids promote ERK1/2 and AKT activation via a pertussis toxin-sensitive mechanism in murine and human hepatocytes. *Hepatology* 42(6):1291–1299
73. Kawamata Y, Fujii R, Hosoya M, Harada M, Yoshida H, Miwa M, Fukusumi S, Habata Y, Itoh T, Shintani Y, Hinuma S, Fujisawa Y, Fujino M (2003) A G protein-coupled receptor responsive to bile acids. *J Biol Chem* 278(11):9435–9440
74. Vassileva G, Golovko A, Markowitz L, Abbondanzo SJ, Zeng M, Yang S, Hoos L, Tetzloff G, Levitan D, Murgolo NJ, Keane K, Davis HR, Jr., Hedrick J, Gustafson EL (2006) Targeted deletion of Gpbar1 protects mice from cholesterol gallstone formation. *Biochem J* 398(3):423–430
75. Kiguchi K (2014) Molecular aspects of cholangiocarcinoma. *J Hepatobiliary Pancreat Sci* (In Press)

Pathology of Gallbladder Cancer

Vikram Deshpande and Andrea Primiani

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Abstract

Gallbladder cancer is an uncommon cancer of gastrointestinal tract. Adenocarcinoma is the most common type of cancer to arise within the gallbladder and is typically associated with two precursor lesions: flat dysplasia and adenomas. Intracystic papillary neoplasms and mucinous cystic neoplasms are less common precursor lesions. The prognosis of patients with gallbladder carcinoma is highly dependent on tumor stage. Non-invasive and minimally invasive adenocarcinomas are typically cured with cholecystectomy and hence precise pathologic staging is critical for management and prognostication. Other less common variants of gallbladder carcinoma include adenosquamous carcinoma, squamous cell carcinoma, carcinosarcoma, and undifferentiated carcinoma and are typically associated with a worse prognosis. Neuroendocrine neoplasms, lymphomas, and mesenchymal neoplasms of the gallbladder, as well as metastases, are extremely rare.

1 Introduction

Although gallbladder carcinoma is an uncommon malignancy in North America, it is, nevertheless, the seventh most common gastrointestinal cancer and the most common malignancy of the biliary tract [1, 2]. The disease tends to present in an advanced stage, and the survival is abysmal, except for the minority of cases that are identified early, often incidentally on a cholecystectomy performed for gallstones.

There are significant geographic variations in the prevalence of the disease, with the highest rates in Chile and India [3, 4]. The majority of the current pathology literature pertains to the disease in these countries with a high incidence of gallbladder carcinoma. Therefore, little is known about the pathology and genetics of this disease in countries

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with a low incidence of gallbladder carcinoma, such as the United States.

2 Precursor Lesions of Gallbladder Carcinoma

There are two distinct types of precursor lesions: (1) flat lesions that could show either low-grade dysplasia or high-grade dysplasia and (2) mass-forming lesions (adenomas) [5, 6]. The majority of gallbladder carcinomas arise from flat dysplasia while mass-forming precursor lesions are identified only in a minority of cases. In a systematic analysis of 606 invasive gallbladder carcinomas from Chile, only 6.4 % of patients were associated with mass-forming precursor lesions [6].

2.1 Flat Dysplasia of the Gallbladder

2.1.1 Definition

The term flat dysplasia of the gallbladder refers to a non-invasive, neoplastic lesion characterized by unequivocal cytological features of malignancy [7]. By definition, it lacks a polypoidal character, and therefore, it is seldom recognized on gross evaluation. Nevertheless, some forms of “flat” dysplasia may demonstrate a grossly appreciable papillary architecture and thus show overlapping features with adenoma. Recently, the term biliary intraepithelial neoplasia (BilIN) has been proposed as an alternative term for biliary dysplasia, analogous to that used in other organs such as pancreatic intraepithelial neoplasia (PanIN), a pre-invasive neoplasia of the pancreas.

The American Joint Committee on Cancer (AJCC) recognizes the term carcinoma in situ, and hence, pathologists are required to distinguish high-grade dysplasia from carcinoma in situ. Unfortunately, there are no well-defined histologic criteria to make this distinction, and thus, the separation, in most instances, is an arbitrary one. Furthermore, biologically, there appear to be no differences between the lesions characterized as high-grade dysplasia and carcinoma in situ [8].

2.1.2 Incidence

Between 1 and 3.5 % of cholecystectomies performed for gallstones harbor dysplasia, in most instances low-grade dysplasia, whereas high-grade dysplasia is distinctly uncommon [9–11]. There are also geographic variations in the incidence of dysplasia, being significantly higher in countries such as Chile that have a high incidence of gallbladder carcinoma.

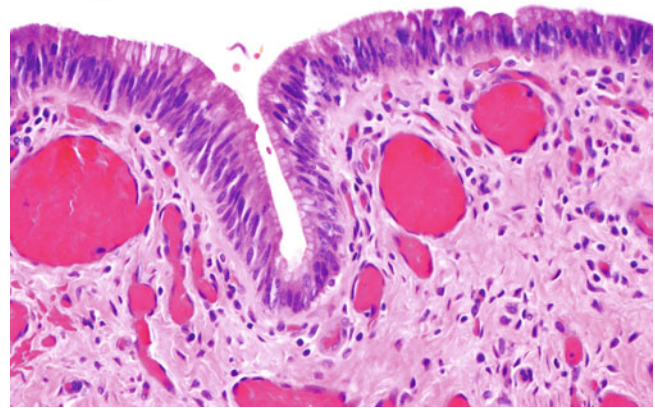


Fig. 1 Gallbladder mucosa showing low-grade dysplasia. The epithelium shows mild nuclear atypia, nuclear stratification, and hyperchromasia. However, the polarity of the cells is maintained, thus arguing against the diagnosis of high-grade dysplasia

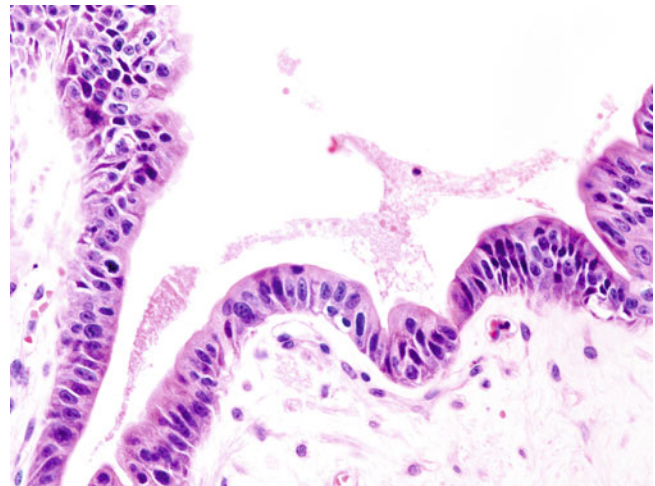


Fig. 2 A high-powered view of the lining epithelium of the gallbladder. The image shows high-grade dysplasia. Note the marked nuclear atypia, occasional prominent nucleoli, and mitotic figures, as well as the loss in cells polarity

2.1.3 Pathology of Flat Dysplasia

The cells lining dysplasia of the gallbladder show cytological features of neoplasia in the form of nuclear enlargement, nuclear hyperchromasia, pseudostratified nuclei, and mitotic activity. As with other forms of dysplasia in the gastrointestinal tract, two grades of dysplasia are recognized, low grade (Fig. 1) and high grade (Fig. 2), the latter typically associated with a loss of cell polarization as well as significantly greater degree of cytologic and architectural atypia. The adjacent gallbladder mucosa invariably shows metaplasia, with pseudopyloric- and

intestinal-type metaplasia representing the two most common forms of metaplasia.

There is overlap between these two grades of dysplasia, and, although untested, there is likely to be a significant interobserver variability. Proponents of the term BilIN, generally used to designate dysplasia in the biliary tree, recognize three grades of dysplasia—BilIN-1, BilIN-2, and BilIN-3, corresponding to low-, intermediate- and high-grade dysplasia [12]. However, this grading system also suffers from similar shortcomings—a relatively high degree of interobserver variability, likely even higher than a two-tier system of grading.

2.1.4 Clinical Significance and Natural History

No further therapy is generally required when dysplasia is identified during routine cholecystectomy [13]. As this recommendation is predicated on the absence of invasive carcinoma, it is important that the pathologist thoroughly evaluates the resected specimen; this especially applies to cases with extensive high-grade dysplasia. However, data from the Surveillance, Epidemiology, and End Results Program of the National Cancer Institute suggest that one-third of patients with carcinoma in situ of the gallbladder died of tumor after 10 years [14]. This may be the result of an unsampled invasive component of the tumor. However, a more likely scenario is the genesis of a second malignancy of the biliary tract, a consequence of a field defect within the biliary system.

2.2 Mass-Forming Precursor Lesions of Gallbladder Carcinoma (Adenoma)

There are several analogies that could be drawn between adenomas of the colon and those of the gallbladder; most significantly, both lesions may progress to invasive carcinoma. However, in contrast to colonic carcinoma, only a minority of gallbladder carcinomas arise from adenomas [6].

Adenomas of the gallbladder are a poorly characterized entity, primarily because of their low prevalence. A wide array of terms have been used to designate adenomas of the gallbladder including “pyloric gland adenoma,” “papillary adenoma,” “tubular papillary adenoma,” “papillary neoplasm,” “papillary carcinoma,” and “intracystic papillary neoplasm.” A selection of these terms is based on growth patterns, while others are based on the morphological appearance of the lining epithelium and its resemblance to epithelium lining the gastrointestinal tract. In a recent analysis of 123 gallbladder adenomas, Adsay and colleagues suggest the unifying term intracholecystic papillary-tubular neoplasm (ICPN) to encompass all neoplastic mass-forming precursor lesions of gallbladder [6].

This argument has merit since all of these mass-forming neoplasms of the gallbladder are associated with a significant risk of invasion. However, there appeared to be some minor variations in the risk of invasive carcinoma among the various subcategories of gallbladder adenomas. It is important to emphasize that some de novo invasive gallbladder carcinomas may present as a polypoid mass. Therefore, pathologists must draw a clear distinction between this entity, polypoid carcinoma, and a precursor neoplastic polyp associated with an invasive carcinoma; the latter category is associated with a slightly better prognosis.

2.2.1 Gross Features of Gallbladder Polyps

The polyps could be either sessile or pedunculated; the latter are often easily detached from the underlying wall and thus may be mistaken for stones. Interestingly, only about 20 % of neoplastic gallbladder polyps are associated with gallstones.

2.2.2 Morphologic Variants of Gallbladder Adenomas

Based on the architectural pattern of growth, adenomas could be classified into (1) tubular, (2) papillary, and (3) tubulopapillary [6]. In comparison with the tubular pattern, adenomas with a papillary growth pattern are associated with an increased risk of invasion.

By definition, all neoplastic gallbladder polyps show low-grade dysplasia. However, a substantial population of polyps also reveal high-grade dysplasia, characterized by marked cytological atypia and/or architectural abnormalities, such as crowding of glands and a cribriform pattern of growth. Polyps with extensive high-grade dysplasia are more likely to show invasive carcinoma.

2.2.3 Variants Based on Cell Lineage

Analogies have been drawn between intraductal papillary mucinous neoplasms (IPMNs) of the pancreas and “adenomas” of the gallbladder. Similar to IPMNs, four variants of gallbladder polyps are recognized [6, 15].

However, unlike IPMNs of the pancreas, the majority of which are easily classified into one of these four lineages (gastric, intestinal, pancreatobiliary, and oncocytic), these preneoplastic lesions in the gallbladder are often difficult to classify, and many of these polyps exhibit more than one cell lineage.

1. Biliary: The epithelium lining these polyps resembles biliary epithelium. The biliary subtype appears to be associated with a higher incidence of invasive carcinoma.
2. Gastric and pyloric types of adenomas: These polyps are lined by gastric-type epithelium, characterized by basal nuclei and abundant apical pale cytoplasm. The pyloric

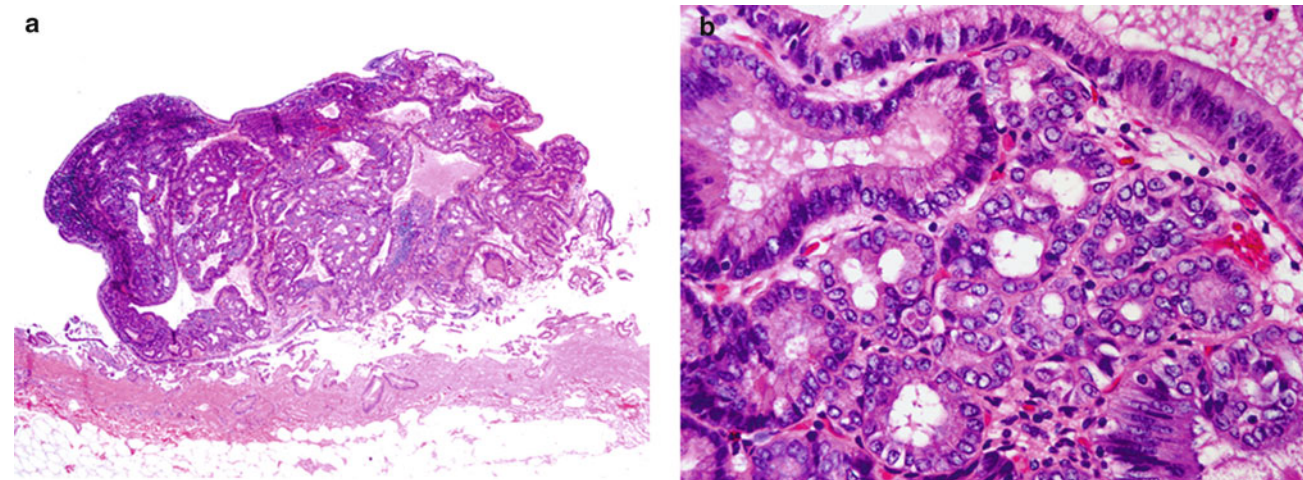


Fig. 3 **a** Low-power view of a gallbladder adenoma. The adenoma is composed of predominantly small glands, although occasional larger glandular structures are also present. Attachment to the underlying gallbladder wall is not identified in this image. **b** High-powered view

of a gallbladder adenoma with mild dysplasia. The glands are lined by cuboidal to columnar cells with pale eosinophilic cytoplasm, an appearance that resembles pyloric glands. This lesion could be classified as a pyloric gland adenoma

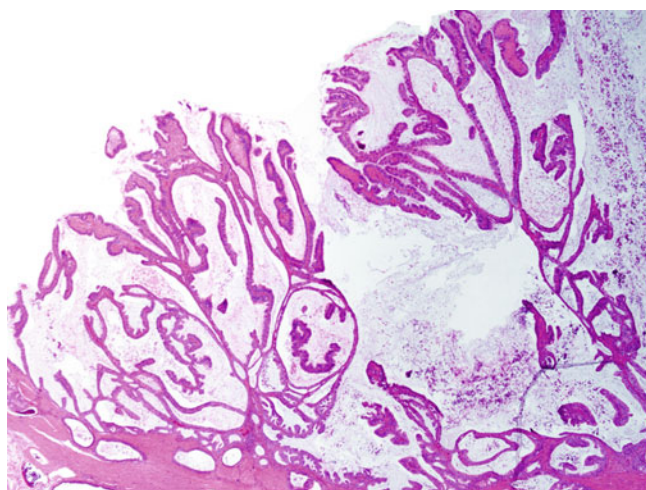


Fig. 4 Intracystic papillary neoplasm of the gallbladder

variant shows small caliber glands lined by cells containing abundant intracellular mucin (Fig. 3). This phenotype is characterized by the presence of strong and diffuse reactivity for Muc6.

3. Intestinal type: These polyps are lined by epithelium that is similar to colonic adenomas. Immunohistochemically, these polyps are characterized by the presence of keratin 20 and CDX2 reactivity. Absence of keratin 7 is also characteristic of this phenotype.
4. Oncocytic variant: This is the least common of the four subtypes and is characterized by the presence of tumor cells containing abundant pink cytoplasm.

2.2.4 Prognosis

Adenomas of the gallbladder should be viewed as a pre-malignant lesion. The prognosis of patients with polypoid precursor lesions of the gallbladder is significantly better than invasive gallbladder carcinoma. In one recent analysis, non-invasive adenomas and papillary carcinomas had a 3-year and 5-year survival of 90 % and 78 %, respectively [6].

2.3 Intracystic Papillary Neoplasms

The World Health Organization's classification of tumors of the digestive system recognizes an intracystic papillary neoplasm as a distinct entity and distinguishes them from adenomas of the gallbladder [16]. Intracystic papillary neoplasms show a prominent intraluminal proliferation of papillary epithelium, generally with high-grade atypia (Fig. 4). However, in many cases, there is no clear distinction between this entity and an adenoma of the gallbladder. Similar to the adenomas of the gallbladder, intracystic papillary neoplasms that lack invasion are associated with an excellent prognosis [17].

2.4 Mucinous Cystic Neoplasms

Mucinous cystic neoplasms are seen in adult females. This neoplasm is more frequently seen in the pancreas; the hepatic and gallbladder counterparts are extremely uncommon [18]. Histologically, these cystic neoplasms are

lined by mucinous epithelium with varying grades of atypia. These neoplasms are defined by the presence of ovarian-type stroma. Similar to the pancreas, non-invasive lesions are associated with an excellent outcome, while invasive adenocarcinomas arising in mucinous cystic neoplasms are associated with a risk of metastasis and death.

2.5 Other Polypoid Lesions of the Gallbladder

Neoplastic polyps constitute only a minority of gallbladder polyps; the majority of gallbladder polyps are non-neoplastic. The two most common non-neoplastic polyps of the gallbladder are cholesterol polyp and adenomyoma.

2.5.1 Cholesterol Polyp

Cholesterol polyps are either sessile or, less commonly, pedunculated. They generally measure less than 1 cm in size [19]. Grossly, the lesion has a bright yellow cut surface. Microscopically, the polyps are lined by benign-appearing biliary epithelium that is often organized in a papillary architecture. The papillary structures are characteristically occupied by sheets of foamy histiocytes, which contain abundant lipid. These polyps have no neoplastic potential.

2.5.2 Adenomyomatous Polyps

Adenomyomatous polyps generally do not form an intraluminal nodule; instead, this lesion presents as a mural thickening or a well-defined mural nodule. Histologically, the nodule is composed of dilated glandular structures that often resemble Rokitansky–Aschoff sinuses. The glands are surrounded by fibrocollagenous tissue and interspersed smooth muscle [20]. Like the cholesterol polyp, adenomyomatous polyps are benign and have not been reported to undergo malignant degeneration. However, high-grade dysplasia may involve the lesion and, thus, may mimic an invasive carcinoma.

2.5.3 Polypoidal Pyloric Gland Hyperplasia

Pyloric gland hyperplasia is a common form of metaplasia of the gallbladder. Occasionally, circumscribed aggregates of pyloric glands may mimic an adenoma, specifically a pyloric-type adenoma. Similar to the lesions listed above, this metaplastic lesion is not associated with an elevated risk of gallbladder malignancy.

2.6 Variants of Cholecystitis Associated with Risk of Gallbladder Carcinoma

2.6.1 Porcelain Gallbladder

Porcelain gallbladder describes the gross appearance of a gallbladder with extensive calcification, resulting in a brittle

Table 1 Neoplasms of the gallbladder [16]

Epithelial tumors	Mesenchymal tumors
<i>Benign</i>	<i>Benign</i>
Cholesterol polyp	Granular cell tumor
Adenomyoma	Leiomyoma
<i>Premalignant lesions</i>	<i>Malignant</i>
Adenoma (tubular, papillary, or tubulopapillary type)	Rhabdomyosarcoma
Biliary intraepithelial neoplasia (BilIN)	Leiomyosarcoma
Intracystic papillary neoplasm	<i>Lymphoma</i>
Mucinous cystic neoplasm	<i>Metastases</i>
<i>Malignant</i>	
Adenocarcinoma, biliary type	
Adenocarcinoma, gastric foveolar type	
Adenocarcinoma, intestinal type	
Clear cell adenocarcinoma	
Hepatoid adenocarcinoma	
Mucinous adenocarcinoma	
Signet ring cell carcinoma	
Cribriform carcinoma	
Adenosquamous carcinoma	
Squamous cell carcinoma	
Carcinosarcoma	
Undifferentiated carcinoma	
<i>Neuroendocrine neoplasms</i>	
Neuroendocrine tumor (NET)	
NET, grade 1 (carcinoid)	
NET, grade 2	
Neuroendocrine carcinoma (NEC)	
Large cell NEC	
Small cell NEC	
Mixed adenoneuroendocrine carcinoma	
Goblet cell carcinoid	
Tubular carcinoid	

consistency and blue discoloration. There is much variation in the literature about the frequency with which invasive carcinoma arises in these gallbladders; however, it seems that the pattern of gallbladder wall calcification correlates with the risk of developing carcinoma such that approximately 5 % of gallbladders with diffuse, transmural calcification harbor carcinoma [21].

2.6.2 Hyalinizing Cholecystitis

Hyalinizing cholecystitis is an uncommon variant of cholecystitis that is characterized by a dense, paucicellular hyaline-type fibrosis of the gallbladder wall, which thins the

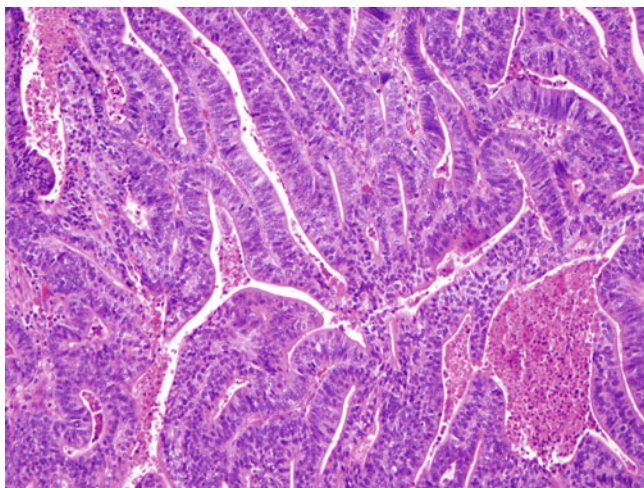


Fig. 5 Invasive adenocarcinoma of the gallbladder

wall and effaces the normal histologic structures. In contrast to porcelain gallbladders, only focal mucosal or intramural calcifications are typically present and about 15 % of cases are associated with invasive carcinoma [22]. These carcinomas are often subtle and difficult to diagnose as only few widely spaced malignant glands are present and the surface mucosa can be denuded; in these cases, extensive sampling of the gallbladder is recommended.

3 Gallbladder Carcinoma

Most malignant epithelial neoplasms of the extrahepatic biliary tract arise within the gallbladder. The majority of gallbladder carcinomas are adenocarcinomas, most commonly of the biliary type, although several other histologic subtypes have been defined by the World Health Organization [16] (Table 1).

3.1 Gross Features

Most gallbladder carcinomas arise within the gallbladder fundus, with the body and neck being affected less often. However, as these tumors are often ill-defined, the location of origin and boundaries of the tumor can be difficult to determine grossly. Gallbladder carcinomas appear as a firm, infiltrative gray-white mass within the wall of the gallbladder. Mucinous and signet ring carcinomas can have a mucoid or gelatinous cut surface. The gallbladder may be enlarged secondary to the presence of the mass or, when obstructed by a proximal mass, may be collapsed. Occasionally, carcinomas (most notably signet ring cell adenocarcinoma) may cause diffuse thickening of the gallbladder wall, mimicking fibrosis associated with chronic

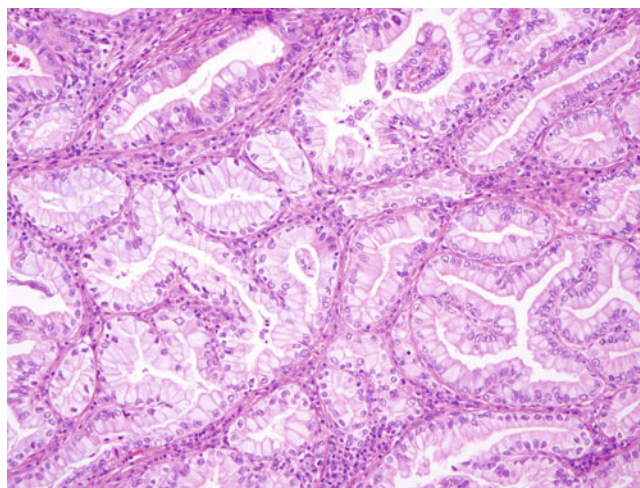


Fig. 6 Invasive gallbladder carcinoma with a gastric phenotype

cholecystitis. Carcinomas may also show a polypoid or exophytic component, which, when present, is typically soft, granular, and friable.

3.2 Histologic Features

3.2.1 Adenocarcinoma

Most gallbladder adenocarcinomas are composed of irregularly dispersed, infiltrative glands. Histologic grading of adenocarcinomas depends on the degree of glandular differentiation; however, most tumors are well to moderately differentiated. The glands are often separated by prominent desmoplastic stroma, which may also contain an inflammatory infiltrate. These glands most commonly resemble biliary epithelium and are lined by cuboidal to columnar cells. There is often marked nuclear atypia, out of proportion to the degree of glandular differentiation (Fig. 5). Cytoplasmic and luminal mucin may be present. Goblet, neuroendocrine, and Paneth cells may also be seen in varying numbers, although they are more commonly present in adenocarcinomas with intestinal differentiation. Intestinal-type adenocarcinomas of the gallbladder may also be composed of tubules lined by pseudostratified, elongated, and hyperchromatic nuclei and may be accompanied by “dirty” necrosis, thereby resembling colonic adenocarcinoma. Gastric foveolar-type adenocarcinoma, which is less common than the biliary and intestinal types, characteristically shows well-differentiated glands composed of columnar cells with basally located nuclei and mucin-filled cytoplasm (Fig. 6).

Poorly differentiated adenocarcinomas can show various growth patterns, including cords or nests of cells or single infiltrating cells. Artifactual clefts surrounding nests of tumor cell can be seen in micropapillary adenocarcinomas.

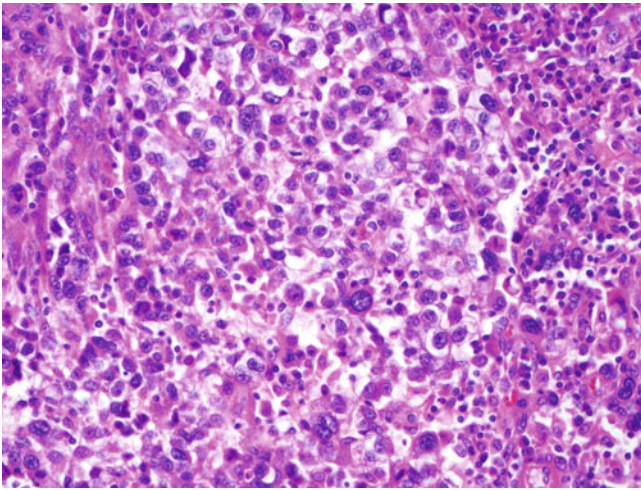


Fig. 7 Poorly differentiated invasive adenocarcinoma of the gallbladder. The tumor lacks evidence of glandular differentiation

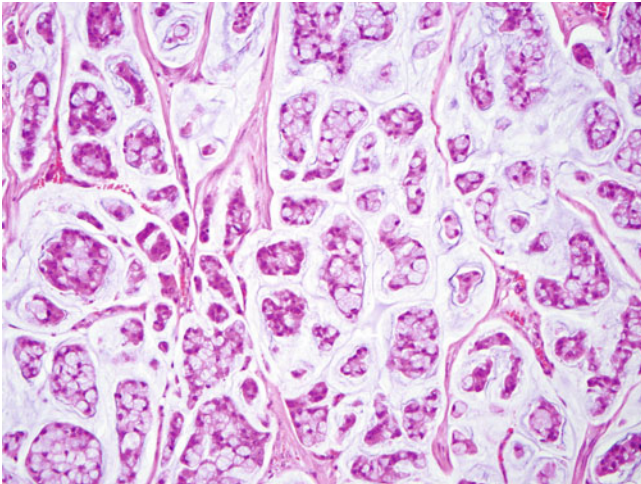


Fig. 8 Mucinous adenocarcinoma of the gallbladder. Note the abundant extracellular mucin within which cohesiveness of neoplastic cells is seen

Some poorly differentiated adenocarcinomas have features of medullary carcinoma with a syncytial-like growth of large, pleomorphic tumor cells with indistinct cell borders, and prominent nucleoli. These tumors can show marked nuclear pleomorphism, bizarre nuclei, and multinucleated giant cells (Fig. 7).

3.2.2 Variants of Gallbladder Adenocarcinoma

Less common gallbladder adenocarcinoma variants include mucinous (colloid) adenocarcinoma, signet ring adenocarcinoma, cribriform carcinoma, clear cell adenocarcinoma, and hepatoid adenocarcinoma. Mucinous adenocarcinomas contain abundant extracellular mucin in >50 % of the tumor [23] (Fig. 8). Signet ring cell adenocarcinoma is characterized by a predominance of tumor cells with intracytoplasmic

mucin that displaces the nucleus to the periphery. These tumors can be accompanied by extracellular mucin and be extensively infiltrative. Cribriform carcinoma is a rare, aggressive carcinoma subtype in the gallbladder that typically affects younger patients. These tumors, which mimic primary breast carcinoma, are composed of back-to-back glands made of uniform cells with hyperchromatic nuclei that line round, regular luminal spaces; high-grade lesions can show comedonecrosis [24]. Clear cell adenocarcinoma is defined by the presence of glycogen-rich clear cells with well-defined cell borders arranged in sheets, nests, trabeculae, or glands. These tumors resemble and must be distinguished from metastatic clear cell renal cell carcinoma by clinical history and ancillary tests, such as immunohistochemistry. Hepatoid adenocarcinoma is extremely rare and diagnosed when >50 % of the tumor is composed of hepatoid cells, typically arranged in a trabecular pattern [25]. As these tumors are reminiscent of liver parenchyma, a primary hepatocellular carcinoma must be considered.

3.2.3 Adenosquamous Carcinoma

Adenosquamous carcinoma is composed of both glandular and squamous malignant components. While focal squamous differentiation may be present in conventional adenocarcinomas, the diagnosis of adenosquamous carcinoma is made only when the squamous component makes up at least 25 % of the tumor. The degree of differentiation varies, although both components are usually moderately differentiated. The glandular elements may contain mucin, and keratin pearls may be seen within the squamous component.

3.2.4 Squamous Cell Carcinoma

Pure squamous cell carcinomas of the gallbladder are exceptionally rare. Thorough sampling of the gallbladder is necessary when a squamous cell carcinoma is suspected because the presence of any degree of glandular differentiation in a tumor warrants the diagnosis of adenosquamous carcinoma [26]. The degree of differentiation and keratinization within squamous cell carcinomas varies widely. Poorly differentiated squamous cell carcinomas may show spindle cell areas, which may be confused with a sarcoma.

3.2.5 Carcinosarcoma

Carcinosarcoma is defined as a biphasic tumor with a malignant glandular component and a sarcomatous component. Typically, the glandular component predominates. The sarcomatous component may contain heterologous elements, such as osteoid and cartilage formation [27].

3.2.6 Undifferentiated Carcinoma

Undifferentiated carcinomas typically contain no or few glandular structures. One of the variants of an undifferentiated carcinoma contains large numbers of non-neoplastic

osteoclast-type cells—undifferentiated carcinoma with osteoclast-type cells [28].

3.3 Immunohistochemical Findings

Most gallbladder adenocarcinomas are positive for carcinoembryonic antigen (CEA), keratin 7, MUC1, MUC2, carbohydrate antigen 19-9 (CA 19-9), and p53. Adenocarcinomas with intestinal differentiation are also immunoreactive for CDX2 and are more commonly positive for keratin 20. Those with gastric foveolar differentiation label with MUC5AC.

Hepatoid adenocarcinomas are positive for HepPar1 and, occasionally, alpha-fetoprotein. Areas of squamous differentiation in gallbladder tumors, either within adenosquamous carcinomas or within squamous cell carcinoma, stain with p63.

3.4 Prognosis

The prognosis for patients with gallbladder adenocarcinoma depends significantly on tumor stage and histologic subtype. Most non-invasive and minimally invasive adenocarcinomas seldom metastasize, and cholecystectomy appears to be curative [29]. However, patients with invasive adenocarcinomas that extend through the wall of the gallbladder have a 10-year survival of 30 %. The 10-year survival of patients with spindle cell and giant cell types of undifferentiated carcinoma is <1 % [16].

Adenosquamous and squamous cell carcinomas of the gallbladder are highly aggressive neoplasms. When compared to patients with gallbladder adenocarcinoma, the survival of patients with gallbladder adenosquamous carcinoma or squamous cell carcinoma is much worse; this comparison holds true when these tumors types are matched for tumor stage [26, 30].

4 Neuroendocrine Neoplasms of the Gallbladder

Neuroendocrine tumors (carcinoids; NETs) are rare and represent <1 % of all gallbladder neoplasms. They are associated with von Hippel–Lindau syndrome and multiple endocrine neoplasia (MEN-1) syndrome. Neuroendocrine carcinomas (NECs) of the gallbladder represent approximately 4 % of malignant tumors of the gallbladder; small cell and large cell variants exist.

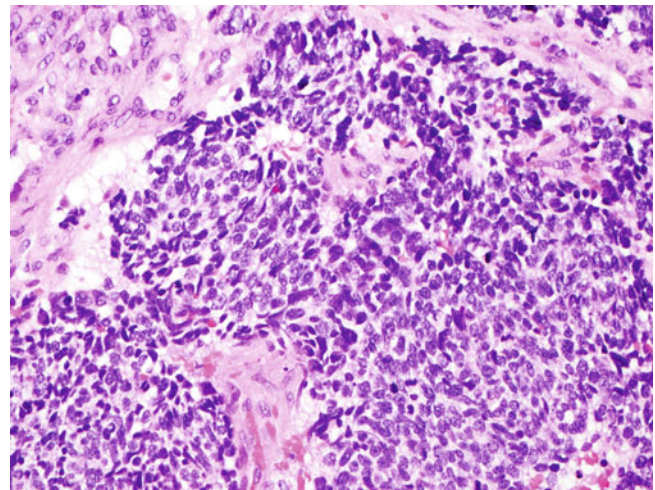


Fig. 9 Neuroendocrine carcinoma with small cell features. The tumor cells are small with hyperchromatic nuclei and nuclear molding. This tumor closely resembles a small cell carcinoma of the lung

4.1 Gross Features

Neuroendocrine neoplasms of the gallbladder can occur throughout the gallbladder and are typically small (<2 cm), uniform, gray-white submucosal nodules or polyps. NECs are usually larger in size but may also have a polypoid appearance.

4.2 Histologic Features

4.2.1 Well-Differentiated Neuroendocrine Tumor (Carcinoid Tumor)

NETs are characteristically composed of small, round cells with a moderate amount of eosinophilic cytoplasm, uniform nuclei, and inconspicuous nucleoli arranged in solid nests, trabeculae, or tubular structures. Clear cell features have been associated with von Hippel–Lindau syndrome [31]. Notably, carcinoid tumors are low-grade neoplasms with few mitoses (<10 per 10 high-power fields).

4.2.2 Small Cell Neuroendocrine Carcinoma

Small cell carcinomas typically display submucosal growth. These tumors are histologically identical to small cell carcinoma of the lung; they are composed of round cells with scant cytoplasm, hyperchromatic nuclei, nuclear molding, and inconspicuous nucleoli arranged in sheets or nests (Fig. 9). Tubule formation or squamous differentiation is occasionally present. Necrosis and apoptosis are often present, and mitotic figures are frequent (>20 per 10 high power fields) [32].

4.2.3 Large Cell Neuroendocrine Carcinoma

Large cell NEC of the gallbladder is uncommon. These tumors are histologically similar to large cell NEC of the lung and show large cells with prominent nucleoli, coarse chromatin, and a variable amount of cytoplasm. The tumor cells are arranged in an organoid pattern, occasionally with rosette formation. Necrosis is often seen, and numerous mitotic figures are present, generally >20 per 10 HPF [33].

4.3 Immunohistochemical Findings

Neuroendocrine neoplasms are immunoreactive for synaptophysin, chromogranin A, and keratin AE1/AE3. The small cell and large cell NECs are variably positive for chromogranin and synaptophysin; some examples may stain for only one of these markers, typically synaptophysin.

4.4 Prognosis

Unlike the rest of the gastrointestinal tract, there is no formal grading and staging system for neuroendocrine tumors of the gallbladder, in part because these tumors are rare. The presence of metastases or signs of local aggressiveness (i.e., infiltration of the gallbladder wall or perineural invasion) defines malignant behavior of NETs. Furthermore, malignant neuroendocrine gallbladder tumors tend to be >2 cm in size. The 5-year survival of metastatic gallbladder NETs is approximately 40 %. In contrast, survival of patients with NECs, including both the small cell and large cell variants, is poor; about half the patients present with metastatic disease [16].

5 Mesenchymal Gallbladder Tumors

Although mesenchymal tumors are rare in the gallbladder, a wide variety of mesenchymal neoplasms have been described to involve the gallbladder, including both benign tumors (such as granular cell tumors, lipomas, hemangiomas, lymphangiomas, and ganglioneuromas) and malignant tumors (such as rhabdomyosarcoma, Epstein–Barr virus-associated leiomyosarcoma, undifferentiated pleomorphic sarcoma, myxofibrosarcoma, and angiosarcoma, among other sarcomas) [34]. These tumors histologically resemble their counterparts at other sites. The most common gallbladder mesenchymal tumors are discussed here.

5.1 Granular Cell Tumor

Granular cell tumors are the most common benign non-epithelial tumor of the gallbladder and are typically incidental findings. These tumors are characterized by sheets of large, polygonal cells with abundant granular cytoplasm and small nuclei. The tumor cells are immunoreactive for S100.

5.2 Rhabdomyosarcoma

Rhabdomyosarcoma is the most common malignant mesenchymal tumor of the extrahepatic biliary tract in children. Grossly, the tumor forms intraluminal polypoid projections. Histologic evaluation characteristically reveals a cambium layer, a condensation of sarcoma cells beneath the epithelium. The tumor cells show muscle differentiation recognizable by the presence of abundant eosinophilic cytoplasm, although immunohistochemical markers, such as desmin, myogenin, and myoD1, are often required to confirm the line of differentiation.

6 Other Gallbladder Tumors

6.1 Lymphoma

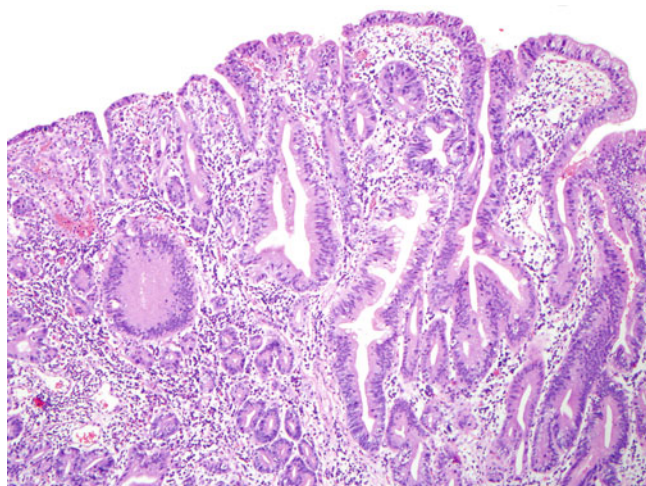
Primary lymphomas of the gallbladder are extremely rare. Most patients with gallbladder lymphoma present with cholelithiasis, cholecystitis, or obstructive jaundice. The most common types of gallbladder lymphoma are diffuse large B-cell lymphoma and extranodal marginal zone lymphoma (MALT lymphoma); both occur in elderly patients. Similar to other gastrointestinal sites, other types of lymphoma, including follicular lymphoma, B-lymphoblastic lymphoma, extracavitary primary effusion lymphoma, and plasmablastic lymphoma, occur less frequently [35]. Histologic, immunohistochemical, and genetic features of these lymphomas are similar to those seen in other gastrointestinal sites. Secondary involvement of the gallbladder by systemic lymphoma is also very rare, occurring in about 2 % of cases.

6.2 Metastases

Metastasis to the gallbladder is uncommon. Although any malignant tumor can spread to the gallbladder, melanoma makes up >50 % of metastases to the gallbladder. Primary

Table 2 Staging of the gallbladder neoplasms [40]

<i>Primary tumor (T)</i>	
Tx	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor invades lamina propria or muscular layer
T1a	Tumor invades lamina propria
T1b	Tumor invades muscular layer
T2	Tumor invades perimuscular connective tissue; no extension beyond serosa or into liver
T3	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
T4	Tumor invades main portal vein or hepatic artery or invades two or more extrahepatic organs or structures
<i>Regional lymph nodes (N)</i>	
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastases
N1	Metastases to nodes along the cystic duct, common bile duct, hepatic artery, and/or portal vein
N2	Metastases to periaortic, pericaval, superior mesentery artery, and/or celiac artery lymph nodes
<i>Distant metastasis (M)</i>	
M0	No distant metastasis
M1	Distant metastasis

**Fig. 10** The gallbladder mucosa shown in this image is composed of glands lined by high-grade nuclear atypia. In this example of early gallbladder carcinoma, the marked architectural atypia suggests invasion of the lamina propria

gallbladder melanoma is even rarer than melanoma metastatic to the gallbladder and should only be considered when metastatic melanoma is excluded. Microscopic features of

metastases to the gallbladder resemble features of the primary tumor and metastases seen elsewhere in the body.

7 Controversies in Pathologic Staging of Gallbladder Carcinoma

The surgical management of patients with gallbladder carcinoma depends on the depth of penetration through the wall of the gallbladder and involvement of adjacent structures (Table 2). The wall of the gallbladder consists of mucosa, lamina propria, a muscular layer (muscularis propria), and perimuscular connective tissue. A portion of the gallbladder is covered by serosa; the other half of the gallbladder lacks a serosal covering and instead abuts the underlying liver parenchyma. The gallbladder, unlike most other organs of the gastrointestinal tract, lacks a muscularis mucosa. Furthermore, the muscularis propria of the gallbladder is seldom as well organized as seen in the tubular gut. The epithelium of the gallbladder invariably extends into the muscularis propria, as the so-called Rokitansky–Aschoff sinuses. The lack of well-defined anatomic layers is accentuated in individuals with chronic cholecystitis. As a consequence, pathologists frequently experience difficulty in distinguishing extensive high-grade dysplasia involving the mucosa (Tis) from tumors that invade the lamina propria (T1a) [36]. Furthermore, since dysplasia frequently extends into the underlying Rokitansky–Aschoff sinuses, the distinction of high-grade dysplasia from tumors that invade the muscularis propria may also be problematic [37]. This has led to the concept of “early gallbladder carcinoma,” a term that incorporates lesions staged a pTis as well as T1a and T1b (Fig. 10) [38]. In studies performed in high-risk regions, the long-term survival of early gallbladder carcinomas was 90 % at 10 years, with little difference between pTis, T1a, and T1b [8, 39]. In spite of this fairly compelling data, pathologists should continue to attempt to distinguish between these subgroups of early gallbladder carcinoma [13, 36].

References

1. Siegel R, Ma J, Zou Z, Jemal A (2014) Cancer statistics 2014. *CA Cancer J Clin* 64(1):9–29
2. Konstantinidis IT, Deshpande V, Genevay M, Berger D, Fernandez-del Castillo C, Tanabe KK, Zheng H, Lauwers GY, Ferrone CR (2009) Trends in presentation and survival for gallbladder cancer during a period of more than 4 decades: a single-institution experience. *Arch Surg* 144(5):441. Retrieved from <http://archsurg.ama-assn.org/cgi/reprint/144/5/441.pdf>
3. Eslick GD (2010) Epidemiology of gallbladder cancer. *Gastroenterol clin North Am* 39(2):307–330. doi:10.1016/j.gtc.2010.02.011

4. Goldin RD, Roa JC (2009) Gallbladder cancer: a morphological and molecular update. *Histopathology* 55(2):218–229. doi:10.1111/j.1365-2559.2008.03192.x
5. Adsay NV (2007) Neoplastic precursors of the gallbladder and extrahepatic biliary system. *Gastroenterol Clin North Am* 36(4):889–900. doi:10.1016/j.gtc.2007.08.011
6. Adsay V, Jang K-T, Roa JC, Dursun N, Ohike N, Bagci P et al (2012) Intracholecystic papillary-tubular neoplasms (ICPN) of the gallbladder (neoplastic polyps, adenomas, and papillary neoplasms that are ≥ 1.0 cm): clinicopathologic and immunohistochemical analysis of 123 cases. *Am J Surg Pathol* 36(9):1279–1301. doi:10.1097/PAS.0b013e318262787c
7. Albores-Saavedra J, Alcántra-Vazquez A, Cruz-Ortiz H, Herrera-Goepfert R (1980) The precursor lesions of invasive gallbladder carcinoma. Hyperplasia, atypical hyperplasia and carcinoma in situ. *Cancer* 45(5):919–927
8. de Aretxabala X, Roa I, Hepp J, Maluenda F, Mordojovich G, Leon J, Roa JC (2009) Early gallbladder cancer: is further treatment necessary? *J Surg Oncol* 100(7):589–593. doi:10.1002/jso.21389
9. Chan KW (1988) Review of 253 cases of significant pathology in 7,910 cholecystectomies in Hong Kong. *Pathology* 20:20–23
10. Aldridge MC, Bismuth H (1990) Gallbladder cancer: the polyp-cancer sequence. *Br J Surg* 77(4):363–364. Retrieved from <http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=2187556&retmode=ref&cmd=prlinks>
11. Kozuka S, Tsubone M, Yasui A, Hachisuka K (1982) Relation of adenoma to carcinoma in the gallbladder. *Cancer* 50(10):2226–2234. Retrieved from <http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=7127263&retmode=ref&cmd=prlinks>
12. Zen Y, Adsay NV, Bardadin K, Colombari R, Ferrell L, Haga H et al (2007) Biliary intraepithelial neoplasia: an international interobserver agreement study and proposal for diagnostic criteria. *Mod Pathol* 20(6):701–709. doi:10.1038/modpathol.3800788
13. Zhu AX, Hong TS, Hezel AF, Kooby DA (2010) Current management of gallbladder carcinoma. *Oncologist* 15(2):168–181. doi:10.1634/theoncologist.2009-0302
14. Albores-Saavedra J, Murakata L, Krueger JE, Henson DE (2000) Noninvasive and minimally invasive papillary carcinomas of the extrahepatic bile ducts. *Cancer* 89(3):508–515
15. Furukawa T, Klöppel G, Adsay NV, Albores-Saavedra J, Fukushima N, Horii A et al (2005) Classification of types of intraductal papillary-mucinous neoplasm of the pancreas: a consensus study. *Virchows Archiv* 447(5):794–799. doi:10.1007/s00428-005-0039-7
16. Bosman FT, Carneiro F, Hruban RH, Theise ND (Eds) (2010) WHO classification of tumours of the digestive system, 4edn. Retrieved from <http://www.cabdirect.org/abstracts/20113051318.html>
17. Albores-Saavedra J, Tuck M, McLaren BK, Carrick KS, Henson DE (2005) Papillary carcinomas of the gallbladder: analysis of noninvasive and invasive types. *Arch Pathol Lab Med* 129(7):905–909
18. Zen Y, Pedica F, Patcha VR, Capelli P, Zamboni G, Casaril A et al (2011) Mucinous cystic neoplasms of the liver: a clinicopathological study and comparison with intraductal papillary neoplasms of the bile duct. *Mod Pathol* 24(8):1079–1089. doi:10.1038/modpathol.2011.71
19. Konstantinidis IT, Bajpai S, Kambadakone AR, Tanabe KK, Berger DL, Zheng H et al (2011) Gallbladder lesions identified on ultrasound. lessons from the last 10 years. *J Gastrointest Surg* 16(3):549–553. doi:10.1007/s11605-011-1696-2
20. Albores-Saavedra J, Keenportz B, Bejarano PA, Alexander AAZ, Henson DE (2007) Adenomyomatous hyperplasia of the gallbladder with perineural invasion: revisited. *Am J Surg Pathol* 31(10):1598–1604. doi:10.1097/PAS.0b013e31804fa10e
21. Stephen AE, Berger DL (2001) Carcinoma in the porcelain gallbladder: a relationship revisited. *Surgery* 129(6):699–703
22. Patel S, Roa JC, Tapia O, Dursun N, Bagci P, Basturk O et al (2011) Hyalinizing cholecystitis and associated carcinomas: clinicopathologic analysis of a distinctive variant of cholecystitis with porcelain-like features and accompanying diagnostically challenging carcinomas. *Am J Surg Pathol* 35(8):1104–1113. doi:10.1097/PAS.0b013e31822179cc
23. Dursun N, Escalona OT, Roa JC, Basturk O, Bagci P, Cakir A, Cheng J, Sarmiento J, Losada H, Kong SY et al (2012) Mucinous carcinomas of the gallbladder: clinicopathologic analysis of 15 cases identified in 606 carcinomas. *Arch Pathol Lab Med* 136(11):1347–1358. doi:10.5858/arpa.2011-0447-OA
24. Albores-Saavedra J, Henson DE, Moran-Portela D, Lino-Silva S (2008) Cribriform carcinoma of the gallbladder: a clinicopathologic study of 7 cases. *Am J Surg Pathol* 32(11):1694–1698. doi:10.1097/PAS.0b013e3181706237
25. Karayiannakis AJ, Kakolyris S, Giatromanolaki A, Courcousakis N, Bolanaki H, Chelis L et al (2011) Hepatoid adenocarcinoma of the gallbladder : case report and literature review. *J Gastrointest Cancer*. doi:10.1007/s12029-011-9326-5
26. Roa JC, Tapia O, Cakir A, Basturk O, Dursun N, Akdemir D et al (2011) Squamous cell and adenosquamous carcinomas of the gallbladder: clinicopathological analysis of 34 cases identified in 606 carcinomas. *Mod Pathol* 24(8):1069–1078. doi:10.1038/modpathol.2011.68
27. Pu JJ, Wu W (2011) Gallbladder carcinosarcoma. *BMJ Case Rep* 2011(mar31 1): bcr0520103009–bcr0520103009. doi:10.1136/bcr.05.2010.3009
28. Albores-Saavedra J, Grider DJ, Wu J, Henson DE, Goodman ZD (2006) Giant cell tumor of the extrahepatic biliary tree: a clinicopathologic study of 4 cases and comparison with anaplastic spindle and giant cell carcinoma with osteoclast-like giant cells. *Am J Surg Pathol* 30(4):495–500
29. Jayaraman S, Jarnagin WR (2010) Management of gallbladder cancer. *Gastroenterol clin North Am* 39(2):331–342. doi:10.1016/j.gtc.2010.02.006
30. Kim WS, Jang KT, Choi DW, Choi SH, Heo JS, You DD, Lee HG (2011) Clinicopathologic analysis of adenosquamous/squamous cell carcinoma of the gallbladder. *J Surg Oncol* 103(3):239–242
31. Sinkre PA, Murakata L, Rabin L, Hoang MP, Albores-Saavedra J (2001) Clear cell carcinoid tumor of the gallbladder: another distinctive manifestation of von Hippel-Lindau disease. *Am J Surg Pathol* 25(10):1334–1339
32. Maitra A, Tascilar M, Hruban RH, Offerhaus GJ, Albores-Saavedra J (2001) Small cell carcinoma of the gallbladder: a clinicopathologic, immunohistochemical, and molecular pathology study of 12 cases. *Am J Surg Pathol* 25(5):595–601
33. Papotti M, Cassoni P, Sapino A, Passarino G, Krueger JE, Albores-Saavedra J (2000) Large cell neuroendocrine carcinoma of the gallbladder: report of two cases. *Am J Surg Pathol* 24(10):1424–1428
34. Al-Daraji WI, Makhlof HR, Miettinen M, Montgomery EA, Goodman ZD, Marwaha JS, Fanburg-Smith JC (2009) Primary gallbladder sarcoma: a clinicopathologic study of 15 cases, heterogeneous sarcomas with poor outcome, except pediatric botryoid rhabdomyosarcoma. *Am J Surg Pathol* 33(6):826–834
35. Mani H, Climent F, Colomo L, Pittaluga S, Raffeld M, Jaffe ES (2010) Gall bladder and extrahepatic bile duct lymphomas: clinicopathological observations and biological implications. *Am J Surg Pathol* 34(9):1277–1286. doi:10.1097/PAS.0b013e3181e9bb8b

36. Adsay NV, Bagci P, Tajiri T, Oliva I, Ohike N, Balci S et al (2012) Pathologic staging of pancreatic, ampullary, biliary, and gallbladder cancers: pitfalls and practical limitations of the current AJCC/UICC TNM staging system and opportunities for improvement. *Semin Diagn Pathol* 29(3):127–141. doi:[10.1053/j.semdp.2012.08.010](https://doi.org/10.1053/j.semdp.2012.08.010)
37. Albores-Saavedra J, Shukla D, Carrick K, Henson DE (2004) In situ and invasive adenocarcinomas of the gallbladder extending into or arising from Rokitansky-Aschoff sinuses: a clinicopathologic study of 49 cases. *Am J Surg Pathol* 28(5):621–628
38. Albores-Saavedra J, Chable-Montero F, Angeles-Albores D, Schwartz A, Klimstra DS, Henson DE (2011) Early gallbladder carcinoma: a clinicopathologic study of 13 cases of intramucosal carcinoma. *Am J Clin Pathol* 135(4):637–642. doi:[10.1309/AJCPFRKCFEDLV03Y](https://doi.org/10.1309/AJCPFRKCFEDLV03Y)
39. Roa I, Araya JC, Villaseca M, De Aretxabala X, Riedemann P, Endoh K, Roa J (1996) Preneoplastic lesions and gallbladder cancer: an estimate of the period required for progression. *Gastroenterology* 111(1):232–236. doi:[10.1053/gast.1996.v111.pm8698204](https://doi.org/10.1053/gast.1996.v111.pm8698204)
40. AJCC Cancer Staging Manual (2010) AJCC cancer staging manual, 7th edn. Springer, New York

Histopathology and Molecular Pathogenesis of Cholangiocarcinoma

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Abstract

Cholangiocarcinomas (CCs) are primary hepatobiliary carcinomas of increasing importance and with major biological and therapeutic challenges. CCs may occur in any segment along the biliary tract and are presently also classified into two major categories according to their anatomic location. CCs arising from small bile duct and ductules to segmental large bile ducts are designated as intrahepatic or peripheral CC (ICC). Tumors originating from large bile ducts at the hilum (right or left hepatic bile duct or at their junction) or along the extrahepatic biliary tree are designated as extrahepatic bile duct carcinomas (BDC) or extrahepatic CC. Features of cholangiocyte differentiation characterize them; traditionally, they are thought to derive from the malignant transformation of bile duct epithelial cells and histologically classified as adenocarcinoma and rare variants. Recent data emphasized the significant degree of CCs' heterogeneity in terms of their epidemiology and risk factors, pathological and molecular features, pathogenesis, and clinical behaviors and treatment and underlined the role of hepatic stem/progenitor cells as cell origin of a proportion of CC and their possible overlap with the major primary malignant tumor of the liver, namely hepatocellular carcinoma (HCC); precursor lesions and early lesions have been characterized underlining the existence of multistep carcinogenesis process. Overall, these data result in proposal of new histological or molecular classifications that could soon replace current anatomic-based classification and have major impact on establishment of prognosis and on development of novel target treatment approaches.

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1 Introduction

Hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC) are the two major adult hepatic malignant tumors. Although the prevalence of CC disease is increasing worldwide, it remains a rather rare malignant tumor and is far less prevalent than HCC.

Until recently, HCC and CC were believed to derive through dedifferentiation from mature hepatocytes and cholangiocytes, respectively, and were separated one from the other according to this different histogenesis. HCC mostly occurs on a background of advanced chronic liver diseases as cirrhosis, while CC principally arises in the context of a normal liver. In contrast to HCC, only a few high-risk factors for CC have been identified (essentially for extrahepatic bile duct carcinoma), and an established screening system for CC does not exist.

While all this remains mostly true, new insights in regards to combined hepatocellular cholangiocarcinoma (CHC), a histopathologic intermediate tumoral entity between HCC and CC, suggests nowadays-possible histological phenotypic overlap between these two tumors. Recent data on pathogenesis have put forward the fact that hepatic stem/progenitor cells (which can differentiate into either hepatic or biliary cells) may, through maturation arrest phenomenon, give origin to HCC, CHC, or CC, as to a spectrum of liver carcinomas with heterogeneous phenotypic overlaps between these defined entities. Furthermore, if still most CC occurs without underlining liver disease, the incidence of CC is increasing in non-endemic areas of parasitic biliary infection and often in relation to non-biliary chronic liver disease, chronic hepatitis C virus (HCV) infection being a major risk factor for intrahepatic CC. This redefines the borders of HCC and CC and has extended knowledge of their histogenesis.

Finally, nomenclature of bile duct tumors is still a matter of debate. It has been proposed that the term “cholangiocarcinoma” be limited for intrahepatic peripheral tumors and tumors arising from large bile ducts both at the hilum and along the extrahepatic biliary tree and be designated “bile duct carcinomas.” Intrahepatic CC is also assigned as peripheral CC, a term that tends to be discouraged today.

2 Pathology

CCs are currently classified into two major categories according to their anatomic location along the biliary tract:

1. Intrahepatic cholangiocarcinoma (ICC), accounting for 20 % of CC, develops within the hepatic parenchyma and most often appears as a mass without major bile duct obstruction or jaundice.
2. Extrahepatic bile duct carcinoma (EBDC), representing 80 % of CC, encompasses tumors arising from large hepatic hilar bile duct (or Klatskin tumor) to more distal extrahepatic bile ducts but excluding those occurring from ampulla. However, classification of Klatskin tumor is object of some debate. Notably, because a tumor can extend from hilum to the intrahepatic perihilar parenchyma, complicating determination of their anatomic origin, it has been classified in the literature either as intrahepatic or as extrahepatic CC. Moreover, recent studies have highlighted that hilar CC shows similar profile of mucin-producing subtype of ICC [1]. Based on its location and presentation, today's consensus is to classify Klatskin tumor as EBDC but is recurrently considered a form of EBDC separately from the more distal EBDC. Bismuth classification for Klatskin tumor is broadly used to guide surgical treatment [2].

This current anatomical-based classification of CC causes notable conflict in accurate assessment of epidemiological background, carcinogenesis, and patients' outcome of CC. ICC and EBDC can be further classified according to their pathology and molecular features (see below).

A significant limitation to exploring risk factors of CC resides in the classification systems that have been used in the literature: (1) Most cancer registries combine CC with other hepatobiliary malignancies; therefore, it is unclear whether CC also includes HCC and gallbladder cancer. (2) When ICC and EBDC are reported separately, sometimes, HCC is included with ICC and gallbladder cancer is included with EBDC. (3) Classification of Klatskin tumor as ICC resulted in an overestimation of the incidence of ICC and an underestimation of EBDC. (4) Most CC studies do not distinguish site (e.g., ductal, hilar, and peripheral) or histology subtype and heterogeneity. Specific risk factors for different types of CC are, therefore, likely to be missed, depending on the distribution of these types in a given study. (5) In studies where the distinction between ICC and EBDC was used, some potential risk factors seem to have a differential effect on CC, depending on the site. The consistent use of a more refined notably histological classification would allow a better understanding of risk factors for CC.

2.1 Intrahepatic Cholangiocarcinoma

ICC is primarily adenocarcinomas with biliary differentiation arising in any segment of the intrahepatic biliary tree, from the peripheral ductules and small portal bile ducts to the perihilar segmental ducts [3]; ICC may also arise from intrahepatic peribiliary glands.

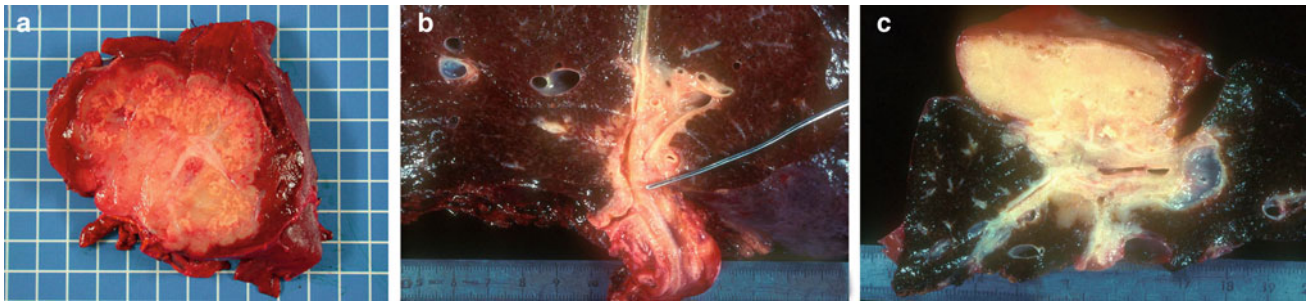


Fig. 1 Different macroscopical types of cholangiocarcinomas. **a** the mass forming type. **b** the periductal infiltrating type. **c** the mixed type

2.1.1 Macroscopic Pattern

According to its macroscopic appearance, the Liver Cancer Study Group of Japan has subdivided ICC into four categories:

1. The mass-forming type (MF), corresponding to a gray-white, well-delimited, firm and solid, non-encapsulated, polylobulated mass within the liver at distance from the hilum and with no connection with a bile duct macroscopically visible (Fig. 1a).
2. The periductal infiltrating type (PI), characterized by a tumoral growth spreading along intrahepatic portal tracts, associated with stenosis of the involved ducts and upstream bile duct obstructive dilatation and cholangitis (Fig. 1b).
3. The intraductal-growing type (IG), defined as a polypoid or papillary tumor mass growing within the lumen of a dilated large bile duct.
4. The mixed pattern (Fig. 1c).

Among these forms, the MF type is the most prevalent gross type, accounting for about 65 % of all ICC types, while PI type and IG type are rare, representing 6 and 4 % of all ICC, respectively. The predominant mixed pattern (around 25 % of ICC) combines to the PI type.

ICC originating from malignant transformation of peripheral ductules and small portal bile ducts usually results in a MF type with no connection with a bile duct macroscopically visible, while perihilar segmental ducts may result in any of the four types and are often associated with intrahepatic biliary fibrosis and cholangitis in the surrounding liver parenchyma. ICC from large intrahepatic bile ducts are often associated with noninvasive intraductal papillary neoplasm (IPN), which may result in mixed pattern, combining a MF to a IG type and extend superficially along the surrounding bile duct epithelium.

At advanced stages, intrahepatic metastases appear consisting on various sized nodules, which may coalesce; regional lymph nodes as lung metastases may develop.

2.1.2 Microscopic Pattern

Histologically, ICCs were, until recently, classified in classic adenocarcinomas and rare histological variants such as adenosquamous and squamous carcinoma, mucinous carcinoma

(often with mucin visible at cut surface and intraductal growth pattern, occasionally associated with intestinal-type goblet cells), signet-ring cell carcinoma, clear cell carcinoma (with abundant cytoplasm), lymphoepithelioma-like carcinoma, and neuroendocrine type or may have sarcomatous area, mimicking a spindle cell sarcoma (Table 1).

Based on recent knowledge in carcinogenesis of CC, notably the existence of cholangiocytes' heterogeneity along the different levels of the biliary tree and the role of hepatic stem cell (HSC), additional histological classifications of ICC have been proposed. These include subdividing ICC in conventional subtype (most often occurring without underlining liver disease) and unconventional subtype (most likely developing on the background of a non-biliary chronic liver disease and cirrhosis) or in mucin-producing or non-producing CC [3–6].

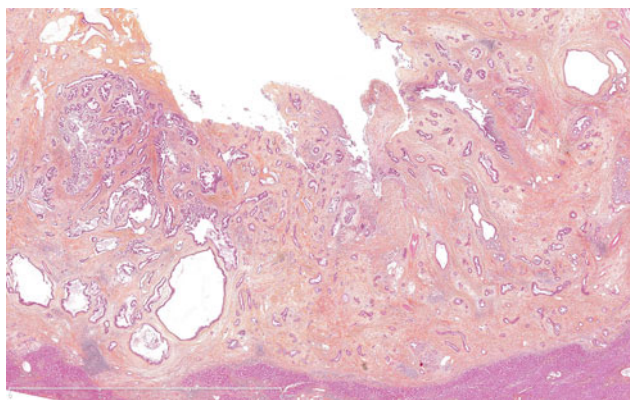
Histologically, conventional ICC is classic mucin-producing adenocarcinomas with biliary features and displays three major (occasionally overlapping) architectural patterns (Fig. 2):

1. Well-differentiated fairly regular tubular glands with lumen of variable size, with or without micropapillary features. Tumoral cells are only slightly atypical and pleomorphic; they look like bile cells with small-to-medium size, cuboidal shape, with small nuclei and nucleoli surrounded by pale and occasionally vacuolated cytoplasm. Occasionally, neutrophil aggregates are intermingled within the tumoral glands.
2. Moderately differentiated, more irregular, and tortuous tubular glands. Tumoral cells are mostly columnar shaped and moderately pleomorphic with a hyperchromatic nuclei and regular mitotic figures, surrounded by an eosinophilic cytoplasm.
3. Poorly marked irregular glands or solid, nested, or cribriform growth pattern, occasionally associated with tumor necrosis. Tumoral cells are small and monomorphic cells with scanty cytoplasm and dark nuclei.

For unconventional ICC, several histological subtypes (both at architectural or cytological level) have been newly defined and subject to intense studies notably on their histogenesis.

Table 1 Classifications of intrahepatic cholangiocarcinoma

Traditional classification	New classification of ICC
<i>Adenocarcinoma</i>	<i>Adenocarcinoma conventional type</i>
Well differentiated	Well differentiated
Moderately differentiated	Moderately differentiated
Poorly differentiated	Poorly differentiated
	<i>Adenocarcinoma unconventional type</i>
	Trabecular
	Perihilar
	Ductular
<i>Rare variants</i>	<i>Rare variants</i>
Squamous cell type	Squamous cell type
Adenosquamous cell type	Adenosquamous cell type
Mucinous carcinoma	Mucinous carcinoma
Signet-ring cell carcinoma	Signet-ring cell carcinoma
Clear cell carcinoma	Clear cell carcinoma
Sarcomatous carcinoma	Sarcomatous carcinoma

**Fig. 2** Microscopical pattern of a well differentiated CC composed of tubular glands and abundant desmoplastic stroma along a large intrahepatic bile duct

1. Trabecular subtype, made of polygonal eosinophilic tumoral cells arranged in thick, occasionally anastomosing trabeculae, mimicking an HCC. They however differ from HCC by indistinct nucleoli, absence of bile production, presence of central fibrosis with sparse tumoral cells, presence of calcification, and immunostaining characteristics.
2. Hilar subtype mimicking typical hilar extrahepatic BDC. Large bile duct shows luminal spread of carcinoma and ulceration surrounded by periductal invasion similar to conventional ICC; peribiliary glands are often invaded. It most likely corresponds to CC originating from large

bile ducts that have progressed into MF CC, peribiliary gland carcinoma, or a conventional ICC with secondary involvement of intrahepatic large bile ducts.

3. Intraductal neoplasia of the intrahepatic bile duct
 - (a) IPN of bile duct, characterized by a spectrum of lesion ranging from preneoplastic intraductal papillary neoplasm of the bile duct (IPNB) (see below) to well-differentiated papillary, noninvasive and invasive, adenocarcinoma. The invasive component often is a mucinous carcinoma. They typically correspond macroscopically to the intraductal growth type, and intraductal superficial intraepithelial tumoral spread may occur along large and even small bile ducts.
 - (b) Intraductal tubular neoplasm of bile duct (ITBN), rare and principally composed of tumor tubular glands, rarely papillary, without mucin, that cast and obstructs the dilated biliary duct.
 - (c) Superficial spreading type.
4. Cholangiolocellular carcinoma (CLC), composed of proliferation of very regular and well-differentiated ductular structures within fibrosis, mimicking a ductal plate malformation [7]. It was previously categorized into a subtype of ICC (bile ductular adenocarcinoma) and is still as yet in Japanese literature. Today, because it is thought to originate from the hepatic progenitor cells (HPCs) located in ductules/canals of Hering [8, 9], it is classified in the latest edition (2010) of WHO tumor classification as a subtype of CHC and will be treated in that section (see below).
5. ICC with predominant “ductal plate malformation” pattern characterized irregularly dilated neoplastic glands associated with an important desmoplastic fibrosis.

A general hallmark of ICC is histological heterogeneity (which may be responsible for misclassification at preoperative biopsy because of sampling problem) and an often, abundant desmoplastic fibrosis, variably distributed within the tumor. The center is often more densely fibrotic intermingled sparse tumoral cells, with occasionally focal calcifications. The periphery has more abundant and proliferating tumoral cells that infiltrate the surrounding parenchyma either by compression and infiltration along the sinusoids or by directly replacing hepatocytes in their cords. Portal tract is co-opted within the mass. Portal venules, lymphatic vessels, and intrahepatic nerves are often invaded, already at an early stage.

ICCs are positive at immunohistochemistry for biliary subtype of cytokeratin, namely cytokeratins 7 and 19, but no specific markers still exist in order to distinguish such tumors from HCC or metastases. ICC histological heterogeneity is also underlined by immunohistochemical profiles and gene expression profiling [1].

2.2 Extrahepatic Bile Duct Carcinoma

EBDC is defined as carcinoma arising either (1) from the common hepatic duct (proximal to cystic duct) to right or from left hepatic bile duct (segment assessed as extrahepatic from their junction in hepatic hilum up to the secondary bifurcation). This EBDC is also named hilar or Klatskin tumor or (2) from extrahepatic distal bile duct (segment distal to cystic duct), excluding ampulla of Vater.

2.2.1 Macroscopic Pattern

Distinguishing between perihilar ICC and hilar EBDC relies essentially on macroscopic examination and may be difficult or even arbitrary particularly at advanced stage. Peripheral ICC may have secondarily infiltrated large hilar bile duct, or on the contrary, a hilar EBDC may extend to form a mass with the liver parenchyma. This may explain some discrepancies in the literature notably concerning the epidemiology and incidence of this tumor.

Bismuth subclassification for Klatskin tumors is widely used for surgery. Type I tumor involves the common hepatic duct distal to the biliary confluence; type II tumor involves the biliary confluence; type IIIa tumor involves the biliary confluence plus the right hepatic duct; type IIIb tumor involves the biliary confluence plus the left hepatic duct; and type IV multifocal or tumor involves the confluence and both the right and left hepatic ducts.

According to their macroscopic appearance, BDC may also be further divided into four categories, with, beside the first one, often overlap between the types:

1. The polypoid type (Fig. 3a), with endoluminal mass.
2. The sclerosing (scirrhous) constricting type, the most common, characterized by diffuse bile duct thickening due to extensive tumor infiltration and fibrosis spreading in periductal tissue.
3. The nodular type.
4. The diffusely infiltrating type, spreading linearly along the wall of the bile duct.

2.2.2 Microscopic Pattern

Histologically, EBDC is carcinoma with various patterns of differentiation that can occasionally coexist within the same tumor:

1. Adenocarcinoma of biliary type, characterized by tubular glands bordered by cuboidal to columnar tumoral cells, resembling biliary epithelium, with mucosecreting cytoplasm, embedded in a desmoplastic reaction.
2. Adenocarcinoma of foveolar type.
3. Adenocarcinoma of intestinal type, where the tumoral glands share morphological and immunohistochemical characteristics with colonic adenocarcinoma (colonic columnar and goblet cells, positive to CK20 and CDX2).

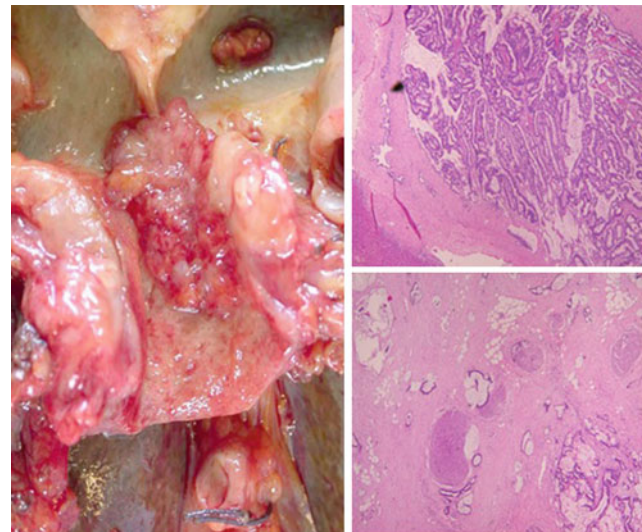


Fig. 3 **a** Macroscopical polypoid types of extrahepatic bile duct carcinoma. **b** Microscopical papillary pattern of a moderate differentiated carcinoma, with **c** invasive component

4. Squamous cell carcinoma.

Klatskin tumor is characterized either by cords and tubules, or by larger, irregular dilated gland-lined atypical cells with and hyperchromatic nuclei. Extensive portal infiltration, perineural invasion, mucin production, papillary structures, and obvious features of intraductal dysplasia are classically observed.

3 Pathogenesis

Mechanisms implicated in CC carcinogenesis today are not fully established. They are variable, underlined by the differences between ICC and EBDC, as illustrated by geographic and risk factor variations.

Study of the pathogenesis of CC illustrates the major role of infection, chronic epithelial inflammation, and bile stasis in malignant transformation of cholangiocytes. Recent data have broadened the pathogenesis of ICC to the malignant transformation of hepatic progenitor cells (HPC). HPC-derived tumors can show varying hepatocytic and/or cholangiocytic differentiation pattern within the same tumor. Several risk factors and common molecular characteristics have been observed in CC and HCC, underlining the concept of a common origin in a subset of cases of these tumors.

3.1 Precursor Lesions of CC

It is now established that CC develops through multistep carcinogenesis; two types of precursor lesions have today been morphologically identified both in ICC and in EBDC:

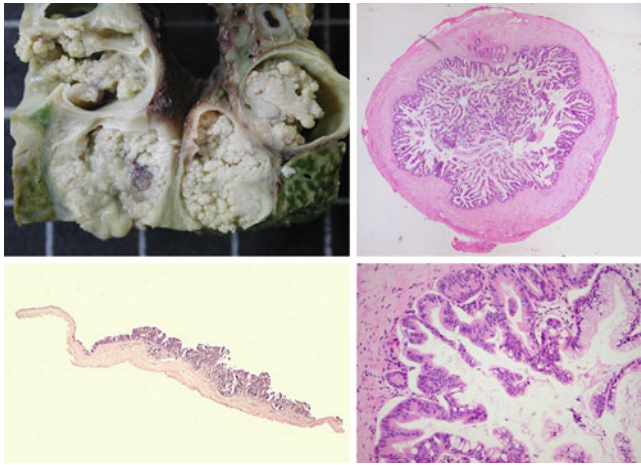


Fig. 4 Macroscopic and microscopical pattern intraductal papillary neoplasm (IPNB)

(1) a flat intraepithelial biliary neoplasia or Bilin (discernible only at level of microscope) and (2) an intraductal papillary lesion or IPNB (previously named papillomatosis) (discernible on radiologic imaging or at macroscopic examination, Fig. 4) [10]. They usually arise in large intrahepatic, hilar, and extrahepatic bile ducts and are only rarely present in the septal or interlobular bile ducts.

Both occur more commonly in relation to chronic inflammatory biliary diseases, such as hepatolithiasis, primary sclerosing cholangitis, infestation by liver flukes, as well as congenital biliary diseases. Bilin has been reported in chronic HCV hepatic disease. Bilin more likely progresses to conventional invasive ICC (tubular adenocarcinoma), whereas IPN-B is associated either with colloid carcinoma (mucinous carcinoma) or with conventional ICC.

IPNB is increasingly accepted as the biliary counterpart of intraductal papillary mucinous neoplasm (IPMN) of the pancreas, while intraductal tubular neoplasm of bile duct (ITNB) corresponds to the pancreatic counterpart, intraductal tubular neoplasm of the pancreas. Both biliary and pancreatic ducts derive embryonically from the foregut.

3.2 Risk Factors of Intrahepatic Cholangiocarcinoma

ICC most often occurs without underlining liver disease; nevertheless, it may also develop on the background of a chronic liver disease and cirrhosis. The incidence of ICC is increasing in non-endemic areas of parasitic biliary infection and often in relation to non-biliary chronic liver disease.

Chronic HCV infection is a major risk factor for ICC. Epithelial damage of small intrahepatic bile duct and bile duct dysplasia are observed in chronic HCV infection. However, how HCV is involved in CC carcinogenesis is

presently indeterminate. Metabolic diseases such as hemochromatosis and alpha-1-antitrypsin deficiency may also predispose to CC [11, 12].

ICC developing in the context of a non-biliary chronic liver disease is often characterized by ductular morphology, possibly underlining its hepatic progenitor cell origin, and more likely correspond to CHC than conventional ICC.

3.3 Risk Factors of Extrahepatic Bile Duct Carcinoma

Established predisposing factors for CC principally concern EBDCs and are correlated to chronic inflammation of the biliary tract such as primary sclerosing cholangitis in Western countries; liver fluke infestation and hepatolithiasis (recurrent pyogenic cholangitis) in Asian countries; various types of biliary malformations such as choledochal cysts, Caroli disease, congenital hepatic fibrosis, polycystic disease, and von Meyenburg complexes; and Thorotrast exposure.

3.4 Molecular Mechanisms of CC

Molecular mechanisms at the basis of the development of CC are still far from being completely understood. Notably, in the literature, it is recurrently difficult to distinguish specific molecular alterations of ICC versus EBDC, as tumoral location is not always precisely indicated. Molecular data underlining genetic differences in ICC and EBDC are thus rare and today need still to be better studied [13].

A variety of mutations in oncogenes, as well as tumor suppressor genes, have been described in ICC. This includes mutations in oncogenes KRAS, BRAF, and EGFR, as in tumor suppressor genes as p53 and bcl-2 (Table 2) [13, 14]. Other particular molecular characteristics of CC reported in few studies are chromosomal aberrations, epigenetic changes, and the process of epithelial-to-mesenchymal transition (EMT) associated with the malignant transformation. Upregulation of different tyrosine kinase receptor-related pathways may also support the use of tyrosine kinase inhibitors as a new therapeutic option [15].

During chronic inflammatory processes, different cytokines such as IL-6, TGF- β , IL-8, and TGF- α released into the biliary microenvironment are responsible for the induction of malignant transformation of cholangiocytes. All of these cytokines produced by cholangiocytes, hepatocytes, and non-parenchymal cells play a fundamental role in the development and growth of biliary tract cancers. In particular, several studies have shown that mitogenic property of IL-6 is mediated by the upregulation of STAT-3, which increases Mcl-1 expression, a key antiapoptotic Bcl-2 family member protein. This suggests a critical role of antiapoptotic signaling

Table 2 Oncogene mutations in ICC

Gene	Abnormalities in ICC
K-ras	Mutated in 2–57 %
P53	Allelic loss or mutation in 1–40 %
BRAF	Activating mutations in 1–22 %
Bcl-2	Expressed in 60–70 %
HER2	Expressed in 30 % (without gene amplification)
EGFR	Expressed in 13 % (without mutation or amplification)
MET	Expressed in 30 % (without amplification)

in biliary malignant transformation [13]. Furthermore, not surprisingly as it has been demonstrated that ICC, combined hepatocellular cholangiocarcinoma and poorly differentiated HCC could originate from stem/progenitor cells, some of these tumors share common genomic alterations [16]. This is also supported by the recent data obtained in mice showing that CC may originate from hepatocytes [17].

A recent study on a gene expression profile, high-density single-nucleotide polymorphism array, and mutation analyses using formalin-fixed ICC samples has identified two main biological classes of ICC that could result in different treatment approaches [18]. These two main classes are (1) the inflammation class (representing 38 % of ICCs) typified by activation of inflammatory signaling pathways, overexpression of cytokines, and STAT3 activation and (2) the proliferation class (62 %), characterized by activation of oncogenic signaling pathways (including RAS, mitogen-activated protein kinase, and MET), DNA amplifications at 11q13.2, deletions at 14q22.1, mutations in KRAS and BRAF, copy number variations (high-level amplifications in 5 regions, including 1p13 (9 %) and 11q13.2 (4 %), and several focal deletions, such as 9p21.3 (18 %) and 14q22.1 (12 % in coding regions for the SAV1 tumor suppressor) and gene expression signatures previously associated with poor outcomes for patients with HCC.

A major histological characteristic of ICC is abundant desmoplastic fibrosis associated with inflammatory cells, notably macrophages. The role of this cancer microenvironment in particular in promoting cancer growth or in prognosis is under active study.

4 Differential Diagnosis

4.1 Intrahepatic Cholangiocarcinoma

4.1.1 Combined Hepatocellular Cholangiocarcinoma

The latest WHO classification defines combined hepatocellular non cholangiocarcinoma as a tumor composed of definite unequivocal area of both HCC and CC, both closely

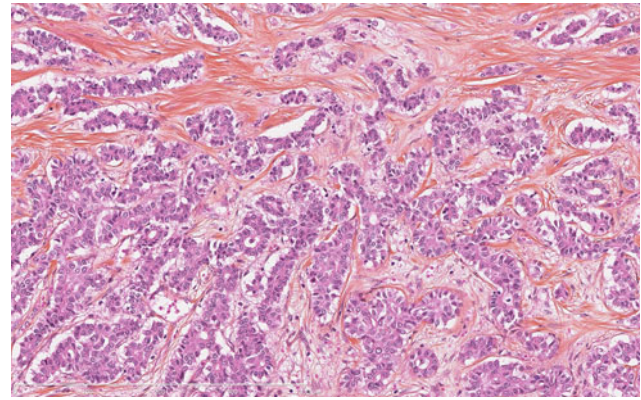


Fig. 5 Microscopical pattern of combined hepatocellular cholangiocarcinoma

intermingled. This entity is to be distinguished from collision tumor where both separated HCC and CC coexist within the same liver (either at distance or as close tumors). Macroscopically, combined HCC CC may mimic ICC.

CLC, a subtype of CHC, significantly mimics ICC (Fig. 5); it is characterized by proliferation of tumoral cells that look like cholangioles (bile ductules) and share CC immunomarkers such as CK7 and CK19. CLC expresses N-CAM (HPC marker) and albumin mRNA (HCC differentiation marker), helpful for distinguishing CLC from classical well-differentiated ICC. HPC origin of CLC is also emphasized by regular concomitance of CLC with conventional HCC and/or ICC components.

Today, CLC is most likely underdiagnosed, to some extent because when HCC and/or ICC areas are present, the CLC component is often overlooked and, while a specific marker for ICC is currently not available, CK7 expression is used as diagnostic marker of ICC and overdiagnosed ICC.

4.1.2 Hepatocellular Carcinoma

While ICC may have trabecular architecture, HCC may show a pseudoglandular architecture, potentially complicating their histological diagnostic distinctions. However, unlike ICC, HCC fails to show true glands or mucin, while it produces bile, and has often prominent nucleoli. The differential diagnosis is even more problematic when a fibrotic stroma is present in HCC.

Immunohistochemically, ICC fails to express the specific hepatocytic markers as HepPar1 and AFP as glypican-3 or CK8 and 18 (commonly expressed by HCC), while, contrary to HCC, they show diffuse cytoplasmic labeling with polyclonal CEA, monoclonal CEA, and CA19–9.

A focal expression of CK7 and of CK19 is observed in 15 % and 10–27 % of HCC, respectively. HCC expressing HPC or ductular markers like CK19 has a more aggressive clinical course. CK19 expression in HCC highlights a

subcategory of HCC with stemness features and is a significant predictor of worse overall survival and of early postoperative recurrence in these patients [19, 20].

4.1.3 Scirrhous HCC

HCC with large area (>50 % of area) of fibrosis (scirrhous pattern) may be misdiagnosed as an ICC both on diagnostic imaging and on gross appearance [21]. Macroscopically, it is often white-grayish, solid, and well demarcated but no encapsulated subcapsular mass with area of stellate-shaped fibrosis. Histologically, non-scirrhous HCCs are characterized as a diffuse band of fibrosis along sinusoid-like blood spaces intermingled with tumoral cell trabeculae of varying grades of thickness. Key element for diagnosis is the tumor cell morphology that is no different from regular HCC. Immunohistochemistry studies illustrated a significantly higher expression of cytokeratin 7 (>60 % of cases) and a significantly lower expression of hepatocyte paraffin 1 in scirrhous HCC than in ordinary HCC, underlining a peculiar histogenesis of this HCC variants, occurring probably also as CLC from stem/progenitor cells and the limited use of immunohistochemistry for the differential diagnosis of ICC.

4.1.4 Metastases

Diagnosis of ICC is established by exclusion of metastatic adenocarcinoma. The basic immunohistochemical panel combining CK7, CK20, CDX-2, TTF-1, ER, PR, BRST-2, and PSA selected according to the clinical setting contribute to rule out hepatic metastases from common primary sites including colon, lung, breast, and prostate. ICC is usually diffusely positive for CK7, while negative or slightly positive for CK20 and other previously cited markers. However, metastatic carcinoma from gallbladder, pancreas, or upper gastrointestinal tract can be distinguished neither morphologically nor by immunomarkers from ICC.

4.1.5 Bile Duct Adenoma

Bile duct adenoma (BDA) may be object of incidental finding, often confused with peripheral ICC. Being benign, it is not a true neoplasm and is currently regarded as a peribiliary gland hamartoma or a localized reactive ductular proliferation due to previous unknown injury. It is usually subcapsular and measures from 1 to 20 mm and is macroscopically firm, gray-white, tan or yellow, and well circumscribed but non-encapsulated round most often solitary mass. Histologically, benign, non-cystic ductules and variable degrees of inflammation and fibrosis characterize BDA. The immunophenotype of these ductules was similar to that of interlobular bile ducts. The absence of bile and cystic changes and lack of association with polycystic disease of the liver and kidneys are the main features distinguishing BDA from von Meyenburg complex.

4.2 Extrahepatic Bile Duct Carcinoma

4.2.1 Endobiliary Metastases

Intraepithelial spread along bile ducts of colorectal adenocarcinoma is a recognized behavior of hepatic metastases [22]. Morphologically, this pattern closely resembled high-grade dysplasia (i.e., carcinoma in situ) of the extrahepatic and intrahepatic bile ducts. A definite diagnosis of metastatic carcinoma is established by medical history, thorough evaluation of the morphologic features and histologic comparison with the primary colon cancer.

4.2.2 Rare Variants

Neuroma, granular cell tumor, endocrine tumor are rare variants of endobiliary tumors.

5 Conclusion

CC is a heterogeneous malignancy that consists of two different anatomically distinguishable categories (namely ICC and EBDC), according to several macroscopic and histologic subtypes. CC is characterized by a poor prognosis and a limited response to conventional anticancer therapies. Currently, there is limited understanding of the pathogenesis of this cancer. A universal consensus on the anatomical definition of intrahepatic CC versus extrahepatic bile duct cancer will facilitate improvement in the design of future clinical trials. Recent studies have made significant progress in the clarification of the intracellular pathways and molecular mechanisms involved in ICC pathogenesis. Cholestasis and chronic inflammation trigger genomic and epigenetic damage, therefore leading to a malignant transformation of cholangiocytes. Advances in the complete characterization of the molecular abnormality involved in the pathogenesis will help to better classify CC and develop new specific molecular targets for therapies.

References

1. Komuta M, Govaere O, Vandecaveye V, Akiba J, Van Steenberghe W, Verslype C et al (2012) Histological diversity in cholangiocellular carcinoma reflects the different cholangiocyte phenotypes. *Hepatology* 55(6):1876–1888
2. Bismuth H, Nakache R, Diamond T (1992) Management strategies in resection for hilar cholangiocarcinoma. *Ann Surg* 215(1):31–38
3. Sempoux C, Jibara G, Ward SC, Fan C, Qin L, Roayaie S et al (2011) Intrahepatic cholangiocarcinoma: new insights in pathology. *Semin Liver Dis* 31(1):49–60
4. Nakanuma Y, Sato Y, Harada K, Sasaki M, Xu J, Ikeda H (2010) Pathological classification of intrahepatic cholangiocarcinoma based on a new concept. *World J Hepatol* 2(12):419–427
5. Nakanuma Y, Xu J, Harada K, Sato Y, Sasaki M, Ikeda H et al (2011) Pathological spectrum of intrahepatic cholangiocarcinoma

- arising in non-biliary chronic advanced liver diseases. *Pathol Int* 61(5):298–305
6. Nakanuma Y, Curado MP, Franceschi S, Gores G, Paradis V, Sripa B et al (2010) Intrahepatic cholangiocarcinoma. WHO Classification of Tumours, vol 3
 7. Sempoux C, Fan C, Singh P, Obeidat K, Roayaie S, Schwartz M et al (2011) Cholangiolocellular carcinoma: an innocent-looking malignant liver tumor mimicking ductular reaction. *Semin Liver Dis* 31(1):104–110
 8. Woo HG, Lee JH, Yoon JH, Kim CY, Lee HS, Jang JJ et al (2010) Identification of a cholangiocarcinoma-like gene expression trait in hepatocellular carcinoma. *Cancer Res* 70(8):3034–3041
 9. Komuta M, Spee B, Borght SV, De Vos R, Verslype C, Aerts R et al (2008) Clinicopathological study on cholangiolocellular carcinoma suggesting hepatic progenitor cell origin. *Hepatology* 47(5):1544–1556
 10. Zen Y, Fujii T, Itatsu K, Nakamura K, Minato H, Kasashima S et al (2006) Biliary papillary tumors share pathological features with intraductal papillary mucinous neoplasm of the pancreas. *Hepatology* 44(5):1333–1343
 11. Morcos M, Dubois S, Bralet MP, Belghiti J, Degott C, Terris B (2001) Primary liver carcinoma in genetic hemochromatosis reveals a broad histologic spectrum. *Am J Clin Pathol* 116(5):738–743
 12. Zhou H, Fischer HP (1998) Liver carcinoma in PiZ alpha-1-antitrypsin deficiency. *Am J Surg Pathol* 22(6):742–748
 13. Fava G, Lorenzini I (2012) Molecular pathogenesis of cholangiocarcinoma. *Int J Hepatol* 2012:630543
 14. Sia D, Tovar V, Moeini A, Llovet JM (2013) Intrahepatic cholangiocarcinoma: pathogenesis and rationale for molecular therapies. *Oncogene* 32(41):4861–4870
 15. Andersen JB, Spee B, Blechacz BR, Avital I, Komuta M, Barbour A et al (2012) Genomic and genetic characterization of cholangiocarcinoma identifies therapeutic targets for tyrosine kinase inhibitors. *Gastroenterology* 142(4):1021–1031 e15
 16. Coulouarn C, Cavard C, Rubbia-Brandt L, Audebourg A, Dumont F, Jacques S et al (2012) Combined hepatocellular-cholangiocarcinomas exhibit progenitor features and activation of Wnt and TGFbeta signaling pathways. *Carcinogenesis* 33(9):1791–1796
 17. Fan B, Malato Y, Calvisi DF, Naqvi S, Razumilava N, Ribback S et al (2012) Cholangiocarcinomas can originate from hepatocytes in mice. *J Clin Invest* 122(8):2911–2915
 18. Sia D, Hoshida Y, Villanueva A, Roayaie S, Ferrer J, Tabak B et al (2013) Integrative molecular analysis of intrahepatic cholangiocarcinoma reveals 2 classes that have different outcomes. *Gastroenterology* 144(4):829–840
 19. Durnez A, Verslype C, Nevens F, Fevery J, Aerts R, Pirenne J et al (2006) The clinicopathological and prognostic relevance of cytokeratin 7 and 19 expression in hepatocellular carcinoma. A possible progenitor cell origin. *Histopathology* 49(2):138–151
 20. Park YN (2011) Update on precursor and early lesions of hepatocellular carcinomas. *Arch Pathol Lab Med* 135(6):704–715
 21. Matsuura S, Aishima S, Taguchi K, Asayama Y, Terashi T, Honda H et al (2005) ‘Scirrhou’ type hepatocellular carcinomas: a special reference to expression of cytokeratin 7 and hepatocyte paraffin 1. *Histopathology* 47(4):382–390
 22. Riopel MA, Klimstra DS, Godellas CV, Blumgart LH, Westra WH (1997) Intrahepatic growth of metastatic colonic adenocarcinoma: a pattern of intrahepatic spread easily confused with primary neoplasia of the biliary tract. *Am J Surg Pathol* 21(9):1030–1036

Novel Biomarkers for Cholangiocarcinoma

Ross C. Smith

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Abstract

The development of cholangiocarcinoma is an uncommon event apart from countries where liver fluke is prevalent. It most commonly occurs as a consequence of chronic inflammation and, therefore, markers of the onset of malignant change need to distinguish between the process of chronic inflammation and neoplastic transformation. Access to samples of tumour is difficult because of its small size but biomarkers have been recognised in plasma, bile and brushings of strictures. The most available biomarkers are derived from the mucus produced by biliary epithelium, where although carbohydrate antigen 19-9 (CA 19-9) and carcinoembryonic antigen (CEA) are frequently relied on for advanced cases where their sensitivity and specificity is about 90 % and 98 %, the diagnostic accuracy is much poorer in early disease. Other mucoproteins have similar results but these markers do not distinguish between other forms of GI cancer. Markers of genetic alterations associated with neoplasia, such as aneuploidy and mutations of *P53*, have been shown to improve the cytological assessment of brushing samples from biliary strictures. Future understanding of the neoplastic mechanism through gene sequencing promises to give a more accurate picture. Proteomic analysis of serum has demonstrated the presence of some interesting proteins with *m/z* of 4462 and 11535, which add to the diagnostic value of CA19-9 and CEA to diagnose cholangiocarcinoma from patients with other benign diseases and from healthy volunteers. Leucin- rich alpha-2-glycoprotein, LRG1 is an interesting protein identified by the MALDI technique which has been shown to be concentrated in cholangiocarcinoma tissue and in the serum of these patients. When a serum protein panel combines this novel biomarker with CA19-9 and with the inflammatory marker IL-6 the ROC AUC was 0.98. A multiplexmeasure of biomarkers will be required to bring these novel findings into clinical practice.

“Novel” indicates a new kind of nature: strange; previously unknown.

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In some tumors, the understanding of abnormal biochemical pathways and genetic alterations allows for the discovery of interesting new markers to establish the diagnosis and to monitor treatment. Furthermore, these may lead to new and specific therapies; for example, the presence of c-Kit staining in gastrointestinal (GI) stromal tumors indicates that Glivec® should be an efficacious treatment. This chapter reviews some of the current knowledge about our progress with cholangiocarcinoma, an uncommon cancer but of increasing incidence in the Western world. However, in Southeast Asia, there are regions of high incidence due to the prevalence of chronic biliary inflammatory conditions.

Cholangiocarcinoma is generally thought to arise on a background of prolonged inflammatory events in the biliary tree. This inflammation may result from the presence of gallstones, choledochal cyst/s [1], a background of sclerosing cholangitis [2], or following radiotherapy. In the process of malignant transformation, hyperplasia of the biliary mucosa progresses to dysplasia and early carcinoma lesions [3], with subsequent changes in mucus production [4]. The changes are considered to be sufficiently widespread to have an influence on the tissue proteome (the entire complement of proteins expressed by the genome). The frequency of such lesions in patients without underlying pathology is less than 0.5 % of cholangiocarcinomas [4].

Only cholangiocytes, the epithelial cells lining the biliary tree, are considered to have the ability to differentiate into cholangiocarcinoma; although under severe injury or toxicity, they may develop a morphology suggestive of intestinal, pancreatic acinar, hepatocyte, or ductal cell origin. If the process continues to the development of cancer, the malignant cells continue to have a phenotype related to their metaplastic origin. Small hepatocellular carcinomas may imitate cholangiocarcinoma and produce similar mucins [5]. This great heterogeneity in the characteristics of cholangiocarcinoma and gallbladder carcinoma is often observed in the histology of gallbladders at the time of cholecystectomy where metaplasia similar to intestinal, gastric, or pancreatic epithelium is seen in association with dysplasia. It is therefore reasonable to expect that no one biomarker will exist which can distinguish cholangiocarcinoma from other chronic non-cancerous conditions of gastrointestinal ductal epithelium [6].

Knowledge about the biological processes involved in the initiation and progress of cancer has expanded with the ability to undertake cancer gene sequencing and to examine related epigenetic influences [7]. Srirakasa et al. [8] have reviewed hypermethylation of genes involving multiple important pathways related to tumor suppression, apoptosis, cell adhesion, and DNA repair, processes common to many malignant conditions. Interestingly, further investigation seeking to determine the influence of the epigenetic effect of

Table 1 Aims of biomarkers

1. Screening, diagnosis, and prognosis
(a) To discover candidate biomarkers
(b) To quantify sensitivity and specificity of biomarkers
(c) To monitor outcome of treatment
2. Therapy efficacy
(a) To evaluate biomarkers in clinical trials
(b) To determine the dose effect of a treatment
(c) To identify new therapeutic possibilities
3. Prediction of therapy response
(a) To identify novel targets and/or pathways
(b) To identify agents which predict clinical efficacy
(c) To develop markers which predict response to specific therapy

microRNA in the control of protein expression is underway [9, 10].

A number of benign strictures may be confused with cholangiocarcinoma, including primary sclerosing cholangitis, follicular cholangitis, and sclerosing cholangitis with granulocytic epithelial lesion [11]. An important new differential diagnosis is autoimmune IgG4-related sclerosing cholangitis, which is typified by histopathology demonstrating a characteristic lymphoplasmacytic infiltrate of CD4- or CD8-positive lymphocytes and IgG4-positive plasma cells, and exhibits interstitial fibrosis and acinar cell atrophy in later stages. Accurate diagnosis is significant because autoimmune IgG4-related sclerosing cholangitis can be treated with steroids, thus avoiding surgical intervention. It usually develops with diverse manifestations such as autoimmune pancreatitis, retroperitoneal fibrosis, and tubulointerstitial nephritis, and although there may be elevated blood levels of IgG4, this feature is not always present. Therefore, it is most important to make a definitive diagnosis which may require a biopsy including analysis for specific biomarkers.

Biomarkers can assume many functions (Table 1). The ideal of having a single blood test which could establish a specific diagnosis of cholangiocarcinoma is very difficult to achieve. This is particularly so in a rare condition because only a small false-negative rate would make the test impractical for screening purposes. In subgroups of patients at high risk of cholangiocarcinoma, such as primary sclerosing cholangitis (PSC) where there is a dominant stricture, the pretest probability of a malignant cause may be as high as 15 % (<http://www.gi.org/patients/gihealth/sclerosing.asp>). In these cases, a hypothetical test with a sensitivity and specificity of 90 % would result in a posttest probability of 65 %, i.e., a result of clinical value. Preliminary results using proteomic techniques are now approaching these values. Similar tests can be undertaken on bile, but

difficulties with the preparation of the sample make reliable results hard to achieve.

Biomarkers on tissue specimens (incision or excision biopsies for histology, fine needle aspiration biopsies, and brushings from bile duct strictures) may also be useful for improving the accuracy of diagnosis and prognostication. Identification of these biomarkers requires an understanding of the complex biology of cholangiocarcinoma.

1 Biological Considerations for Understanding of Biomarker Concepts

Cholangiocytes are arranged in a single layer and have important and diverse functions which affect bile flow and prevent the absorption of toxic substances in bile. They are also closely associated with dendritic cells as a protection from bacteria and other antigens. Cholangiocytes are strongly connected by cytokeratins, and they secrete bicarbonate and a number of specialized mucins to provide protection from the bile [12]. One area of importance when searching for new biomarkers is the rich mucin pool derived from cholangiocytes. The established serum biomarkers, carcino-embryonic antigen (CEA), and cancer antigen (CA) 19-9 are glycoproteins that are useful for monitoring the progress of treatment, but their sensitivity and specificity (60–80 %) make them poor diagnostic biomarkers, particularly because they are elevated in chronic inflammatory conditions which lead to the induction of cholangiocarcinoma. As they are also frequently elevated in other malignant conditions of the GI tract, they are poor discriminators between cancers of the GI tract.

Along with the production of mucin, cholangiocytes produce trefoil factor family (TFF) peptides which also protect cholangiocytes and act as receptors, inducing hyperplasia or apoptosis. These proteins have intense cross-linking with sulfur bridges. The synthesis and release of TFFs are regulated by a number of environmental and local agents, estrogens, and pro-inflammatory and anti-inflammatory cytokines [13]. It is worth noting that the majority of cholangiocarcinoma specimens demonstrate an increased production of spliced TFF2 and that when this occurs, it confers a better survival [14].

The significance of MUC mucins in the developing and the adult liver, various hepatobiliary diseases, and intrahepatic cholangiocarcinoma has recently been reviewed [15].

1.1 Importance of Glycoproteins for Cholangiocytes

When chronic inflammation induces metaplasia, this may take on an intestinal, gastric, or pancreatic appearance.

Inflammatory biliary conditions and tumors of the biliary tree are associated with altered expression of mucins. It is interesting that alteration of mucin production begins as early as the process of metaplasia leading to dysplasia and that this early switch is carried on through the malignant progression of cholangiocarcinomas [16]. Histological assessment of tissues may gain important diagnostic and prognostic information from the immunohistochemical study of the many mucins related to cholangiocarcinoma. When the metaplasia is of gastric cell type, it is likely to be associated with the production of *MUC1*, while metaplasia of intestinal cell arrangement is associated with *MUC2* over-production, implying slightly different malignant potential.

Hughes et al. [17] found that most cases of dysplastic biliary epithelium and cholangiocarcinoma display a Brunner or pyloric gland cell phenotype and a gastric foveolar cell phenotype. However, while aggressive invasive cholangiocarcinoma frequently is associated with *MUC1* over-expression, altered *MUC1* gene expression also occurs in inflammatory diseases and carcinomas of the GI tract and breast [18, 19], making *MUC1* a poor discriminator between tumors.

Cholangiocarcinomas with a better prognosis, particularly those of intraduct papillary type, produce large quantities of gelatinous mucin which is predominantly *MUC2*. Notably, there is a similar progression from preinvasive lesions in the pancreas with mucin production having a dichotomy in the dysplasia-CIS-invasive carcinoma sequence. In a study of 268 pancreatic tumors, 54 % of the intraductal papillary mucinous neoplasms expressed *MUC2*, whereas none of the pancreatic intraepithelial neoplasms (PanINs) did. In contrast, PanINs, especially higher-grade lesions, were often positive for *MUC1* (61 % of PanIN-3), whereas the expression of this glycoprotein was infrequent in intraductal papillary mucinous neoplasms (20 %). This dichotomy was further accentuated in the invasive carcinoma group [20]. The *MUC2* expression in the intrahepatic biliary system, including intestinal metaplasia, intraductal papillary tumors, and mucinous carcinoma, is dependent on the *CDX2* homeobox gene, which induces intestinal differentiation [21, 22].

Over-expression of mucins *MUC4* and *MUC5AC* has also been observed in the early phase of the development of hyperplasia and dysplasia in cholangiocarcinoma [15]. *MUC4* is a novel intramembrane ligand for receptor tyrosine kinase ErbB2 (HER-2) [23], which has been shown to be associated with a poorer prognosis in patients with mass-forming intrahepatic cholangiocarcinoma [24]. The expression of *MUC5AC* was associated with the dysplasia-carcinoma sequence.

In summary, tumors which predominantly express the gelatinous mucins *MUC2*, *MUC5AC*, *MUC5B*, and *MUC6*

are more likely to have a good prognosis, while those associated with the transmembrane mucins *MUC1*, *MUC3*, *MUC4*, *MUC12*, and *MUC17* have a poorer prognosis.

In a study of four cases of oncocytic biliary intraductal papillary neoplasms (IPNs), the IPNs were composed of distinctive oncocytic cells. The invasive carcinomas accompanying two of the cases were also composed of oncocytes. None of the cases showed aberrant expression of the Wnt/ β -catenin pathway proteins which frequently have a central role regulating cell fate decisions in neoplasia by integrating signals from many other pathways, including retinoic acid, FGF, TGF- β , and BMP. Despite this, cyclin D1 was markedly over-expressed in all four cases. Three of four cases had positive staining for *MUC3*, *MUC4*, *MUC5AC*, *MUC5B*, and *MUC6*. Thus, the Wnt pathway proteins (especially beta-catenin and E-cadherin) are expressed normally in oncocytic variants of IPNs of the biliary tree, and the mucin profile is similar to their counterparts in the pancreas [25].

Diagnosis of cholangiocarcinoma is further complicated by the presence of intrahepatic peribiliary glands, which, particularly when dysplastic, add to the complexity of the microscopic appearance of the biliary tree. These glands are present in the large intrahepatic bile ducts [26–28]. The lobules of branched tubuloalveolar seromucous glands communicate with bile ducts via conduits [29] with serous, mucous, and endocrine cells which stain positively for somatostatin, serotonin, and pancreatic polypeptide [30, 31], which adds to the variety of cell types which may become malignant. These glands have been shown to secrete a seromucin rich in amylase and lipase [32]. As well, the bile duct wall intramural glands have sparsely branching tubular mucus glands with tall columnar cells. These glands could be confused with invasive carcinoma.

At the ampulla of Vater, the distinction between tumors arising from biliary, intestinal, or pancreatic tissue may be helped by a study of the mucus subtypes. Ampullary tumors can be classified histologically as either intestinal type or pancreaticobiliary type and display different features according to tumor location, association with adenoma, and *MUC2* expression. Furthermore, KRAS mutation is supposed to be associated with tumors arising in the area from the ampulloduodenum to the ampullop pancreatic duct, with metaplastic mucus occurring in both intestinal and pancreaticobiliary types [33].

2 CA19-9 and CEA: Current Markers for Cholangiocarcinoma

CA19-9 and CEA are the established tumor markers with clinical utility in the management of cholangiocarcinoma and gallbladder carcinoma. Numerous studies show that the

mean values for these markers are elevated in patients with these carcinomas above those of patients presenting similarly who are found to have benign pathology [34]. However, there are numerous reasons for the limited value of these markers. Firstly, they can be extremely high [35, 36] in some patients with benign conditions, but this may be partly adjusted for by dividing the CA19-9 value by the serum CRP concentration, because the benign cases are frequently associated with inflammation [37]. Even with adjustment, however, the sensitivity, specificity, and positive predictive values remain low at 76.5, 68.6, and 70.9 %, respectively. Secondly, both CA19-9 and CEA are elevated in patients with other forms of gastrointestinal cancer and indeed cancers of the genitourinary system. Lastly, CA19-9 cannot be demonstrated in about 10 % of the population who have Lewis negative blood factors [38]. The use of these tumor markers for diagnosis of cholangiocarcinoma in patients with PSC is unfortunately not as valuable as previously reported [39]. The serum levels of CA19-9 frequently rise temporarily in association with a “biochemical relapse” of PSC (shown by increased values of serum alkaline phosphatase). However, although the marker product of CA19-9 and CEA has a low sensitivity, it has a relatively high specificity for the detection of cholangiocarcinoma in PSC patients [40]. Therefore, assessment of patients with elevated values needs to be made with the knowledge of these variations. These markers are of most value when used in conjunction with other tests, such as radiological findings.

An important drawback of CA19-9 as a tumor marker is that it does not detect early disease. A study of 208 patients with PSC who were followed longitudinally for 5 years with a cutoff of change in CA19-9 concentration of 63.2 U/ml gave 90 % sensitivity and 98 % specificity. However, only two of the 14 patients identified with cholangiocarcinoma were candidates for curative resection. Further, in a study of 866 patients with a presentation of general biliary symptoms, CA19-9 was investigated as a screening test for early pancreatic or biliary cancer. Of 117 subjects with an elevated level above the normal range, 115 did not develop a biliary or pancreatic malignancy after 2-years follow-up and therefore had a false-positive result [41]. Thus, a test with such a low specificity as CA19-9 is quite unacceptable as a screening test.

3 Use of CA15-3 and CA27.29 for Screening, Diagnosis, and Staging

Assays of the markers CA15-3 and CA27.29 are well characterized for the detection of circulating *MUC1* antigen in peripheral blood. This circulating marker has prognostic relevance in early-stage breast cancer [42]. The production

of *MUC1* in breast cancer is very limited compared to that in cholangiocytes, and yet, this topic has been more extensively studied in relation to breast cancer. Given the importance of mucin production by cholangiocytes, it is perhaps surprising that there is a dearth of publications studying the usefulness of such measures for the management of gallbladder carcinoma and cholangiocarcinoma. Two general types of assay measuring *MUC1* gene-derived glycoprotein are used: The assays for CA15-3 are sandwich assays, while those for CA27.29 are competitive assays. These types of assay measure slightly different parts of this tandem-repeat molecule. As long as the tests are calibrated carefully, CA15-3 and CA27.29 measurement of *MUC1* gives comparable results [43]. While it is likely that serum tumor markers CA15-3 and CA27.29 have prognostic value, their role in the management of early-stage breast cancer is unclear [44], and although they have value in detecting recurrence [45], there is no prospective randomized clinical trial to demonstrate survival benefit and so their role remains uncertain [46]. CA15-3 or CA27.29 can be used in conjunction with diagnostic imaging, history, and physical examination for the monitoring of patients with metastatic disease during active therapy, but they should not be used in isolation.

An interesting cross-sectional study evaluating two GI markers (CA19-9 and CEA) and four breast cancer markers (CA27.29, CA15-3, MCA and CEA) in 213 patients demonstrated sensitivity of 90 %, but specificity was 40.3 % for CEA and 32.3 % for CA19-9 when GI tumors were compared to benign GI disease. This was not as good as the result for breast cancer where a sensitivity of 90 % and specificity of 70 % was obtained for CA27.29, 67.5 % for CA15-3, 52.5 % for MCA, and 40 % for CEA. Comparison of breast cancer and GI malignancies with other malignancies leads to a marked shift of the receiver operating characteristic (ROC) curve to the right and loss of specificity. High serum antigen levels were found in late-stage tumors. Further, the presence of liver metastases in breast cancer was associated with abnormal levels of CA27.29 ($P = 0.028$). Pancreatic adenocarcinomas had a higher CA19-9 antigen level ($P < 0.001$) than other GI malignancies. None of the above markers retains its specificity for pancreaticobiliary cancer when compared with a control group consisting of other malignancies [47].

4 Markers of Proliferation

Markers of cellular proliferation can be obtained from tissue samples. For many tumors, such markers can be used as predictors of a poorer prognosis. In general, markers of elevated proliferative rate correlate with a worse prognosis

in untreated patients and may predict benefit from chemotherapy [48]. The implementation of DNA flow cytometry to measure proliferative rate is complicated by variation in methods of tissue preparation, differences in instrumentation, and methods for converting information on the histograms to the estimate of the cell cycle S-phase. In addition, interpretation of individual studies is difficult because many are too small to have statistical power, cutoffs have not been prospectively defined, and study populations have not been controlled for adjuvant systemic treatments.

A small number of studies have examined the value of measuring cellular proliferation in managing cholangiocarcinoma. The utility of identifying aneuploidy has been demonstrated in samples taken from paraffin blocks, indicating that this may also be a clinically useful approach in managing cholangiocarcinoma [49]. DNA flow cytometry determination of S-phase is one of the several markers of proliferative rate in tumor specimens, which is applicable to cytology specimens from the biopsy of masses or brush cytology at the time of endoscopic retrograde cholangiopancreatography (ERCP). In pancreatic cancer, aneuploidy has been shown to be predictive of a poorer outcome. Aneuploidy was associated with higher-than-normal levels of other biological markers of prognosis such as HER-2 [50]. Despite these findings, measures of proliferation rate in cholangiocarcinoma are not routinely used in clinical practice.

DNA analysis has been shown to add to the accuracy of CA19-9 and CEA for the diagnosis of cholangiocarcinoma in bile duct strictures. In 57 patients with a diagnosis of PSC undergoing ERCP, brush samples were taken from strictures for cytology and DNA analysis by flow cytometry to obtain measures of proliferation. The tumor markers CA19-9 and CEA were determined both in serum and bile fluid. Thirty-nine patients were found to have malignant strictures (seven with PSC), and a diagnostic sensitivity of 100 % and specificity of 85 % were reached when the results of brush cytology, DNA analysis, serum CA19-9, and serum CEA were combined. Analyses of CA19-9 and CEA in bile fluid yielded no diagnostic significance. The authors concluded that the combination of positive brush cytology at ERCP plus aneuploidy improves the results of serum CA19-9 and CEA. The results were valuable for distinguishing between malignant and benign biliary strictures, especially in PSC patients [51]. A recent review supports the use of fluorescent in situ hybridization (FISH) to identify cells with chromosomal abnormalities to improve sensitivity from that of routine cytology and digital image analysis to identify aneuploidy, but the sensitivity remains low at 40 % [52]. Examination of specific genetic changes in the biliary epithelium may give insights into these important mechanisms and improve our diagnostic ability.

Table 2 Studies of *P53* in cholangiocarcinoma

Reference	Number of cases	<i>P53</i> protein expression	
		Percent (%)	Effect on survival
Ahrendt et al. [95]	12	50	Reduced survival
Bergan et al. [96]	60 ductal type	25	Reduced survival: 0.76 vs. 1.4 years
	22 intestinal	50	
Cong et al. [97]	22	37	Reduced survival
Havlik et al. [98]	29		Reduced survival
Isa et al. [99]	23	21	No effect
Jarnagin et al. [65]	128	27	None, but effect of p27 and Mdm2 seen
Kim et al. [100]	25	37	No effect
Liu et al. [58]	36	51	Reduced survival
Kuroda et al. [66]	55	32	Reduced survival
Tannapfel et al. [101]	41	32	Reduced survival
Washington and Gottfried [102]	41	58	No effect
Shin et al. [59]	36	61	Reduced survival
Wang [107]	294		Meta-analysis reduced survival but not definitive

5 *P53* as a Marker for Cholangiocarcinoma

Inactivation of the tumor suppressor gene *p53*, either by mutation or by methylation, is the most common genetic abnormality in human cancer and has been implicated as a late event in the genesis of cholangiocarcinoma [53] and in gallbladder carcinogenesis [54]. Germline abnormalities appear to have a poor association with the onset of cholangiocarcinoma [55]. It is therefore assumed that the onset is caused by the exposure of cholangiocytes to toxic substances excreted in bile. *P53* (protein) may be measured in paraffin-fixed tissue by immunohistochemistry (IHC) and *p53* genetic changes by gene sequencing. *P53* is accumulated in the nucleus in up to 50 % of cholangiocarcinoma cases, reflecting a minor abnormality of the protein and an inhibition of its natural degradation. Notably, about 90 different mutations of *p53* have been recognized and there is little difference in the nature of these along the course of the biliary tree. The structure and function of *p53* and its role in linking cancer to specific carcinogens by way of mutational signatures have been reviewed [56], and recently, the ratio of two different isoforms, 133p53/Tap53, was shown to be a potential prognostic biomarker. In a study of 36 patients with cholangiocarcinoma [57], clinical outcome was compared for abnormalities of sequencing of *p53* gene in the region of exon 5–8 and for *P53* protein accumulation to find which measure is the better predictor of outcome. *p53* gene mutations were found in 22 of 36 (61.1 %) patients, and for *P53* protein, expression was positive in 19 of 36 (52.8 %) patients. There were significant differences in the extent of

differentiation and invasion between tumors with positive and negative expression of *P53* protein. However, there were no significant differences in pathologic parameters between the mutated and non-mutated tumors. The authors concluded that the identification of alterations of the *p53* gene evaluated by DNA sequence analysis is relatively accurate, but despite this, the over-expression of *P53* protein could not act as an independent index to estimate the prognosis of cholangiocarcinoma [58]. Fluke-associated cholangiocarcinoma appears more likely to over-express *p53* than sporadic cholangiocarcinoma. This may be because of the greater likelihood of an intestinal goblet cell phenotype which over-expresses *p53* arising in fluke-associated cholangiocarcinoma as with gallbladder cancer [54]. Differences in the aetiopathology of the cancers may reflect different pathways to the development of cholangiocarcinoma [17].

Several studies of patients with cholangiocarcinoma suggest that high tissue *P53* protein levels measured by IHC or mutations or deletions in the *p53* gene measured by single-strand conformational gel electrophoresis, manual sequencing, or allele-specific polymerase chain reaction (PCR) appear to predict poor outcome (Table 2). Results in studies showing no effect of *P53* accumulation on survival may have been affected by small study numbers. These studies indicate that about 36 % of cases accumulate *P53* in the nucleus and that in these cases, there is a poorer survival outcome. However, it seems unlikely that for IHC, *P53* will provide sufficient accurate results to be clinically useful, given that it detects both mutated *p53* and stabilized wild-type *p53*, and conversely will miss *p53* deletions. This is confirmed by a study where the patients with wild-type *P53* exhibited longer overall survival than those with defective *P53* [57]. Methods

to define genetic abnormalities in *p53* more precisely and conveniently might determine specific mutations of *p53* which strongly correlate with clinical outcomes and may be a predictor of benefit from systemic therapies. However, at present, methodologies to do so are cumbersome, expensive, and not widely available as routine clinical assays, limiting the utility of this marker in clinical practice. Furthermore, no prospective studies assessing clinical benefits using these new techniques have been published.

6 Markers of Epigenetic Influences on Gene Function

This is a rapidly progressing field with the advent of gene sequencing and the knowledge of the importance of epigenetic regulators of RNA function for many neoplasms. Recent development of epigenetic evaluation of cancer has demonstrated systematic aberrations where methylation silences specific genes. In particular, cholangiocarcinoma has consistent changes in *CDO1*, *DCLK1*, *SFRP1*, and *ZSCAN18* [7]. These genetic abnormalities were seen to occur in cell lines and fresh frozen samples of cholangiocarcinoma and were confirmed in paraffin blocks. When these potential biomarkers were combined as a panel of four, the sensitivity and specificity were 100 % in fresh frozen samples, but the sensitivity fell back to 87 % when tested in validation paraffin samples in this relatively small cohort. The advantage of this technology is that the DNA is stable in bile and so can be measured in bile collected at ERCP or other forms of biliary drainage. Unfortunately, the effect of dilution in bile collections reduces the sensitivity of the method. In many cases, brush cytology is available and this material has been a useful means of obtaining samples for epigenetic studies. Shin and colleagues used a five-marker panel of *CCND2*, *CDH13*, *GRIN2B*, *RUNX3*, and *TWIST1*, which improved the sensitivity of cytology from 43 to 83 % [59].

Another study reported *OPCML* methylation in 72 % of cholangiocarcinoma specimens which was not found in the uninvolved adjacent tissue. Previous studies have demonstrated that *OPCML* methylation in cholangiocarcinoma is associated with poorer differentiation and as such it should be a marker of poor outcome [60].

7 Urokinase Plasminogen Activator (uPA), Its Receptor uPAR, and Plasminogen Activator Inhibitor 2 (PAI-2) as Markers of Invasiveness in Cholangiocarcinoma

The uPA system has been shown to increase invasiveness, and increased expression of these factors has been associated with poor outcome in some cancers. This system

involves a cell surface receptor, uPAR, which becomes active when the uPA protein binds to it. Activation of the uPA/uPAR mechanism may be inhibited by the small proteins PAI-1 and PAI-2. Studies of pancreatobiliary cancers indicate that poor outcome is predicted by increased expression of uPA and uPAR and further that PAI-2 is an independent predictor of improved outcome by suppression of the uPAR mechanism. Several assay formats for these markers have been evaluated, including IHC, quantitative real-time reverse transcriptase polymerase chain reaction (qRT)-PCR, and enzyme-linked immunosorbent assays (ELISA) [61]. Both qRT-PCR and IHC have been shown to be predictive of survival [62] and to indicate the presence of lymph node metastasis in cholangiocarcinoma [63].

8 Expression of Cathepsin and Cyclin Proteins as Markers of Tumor Progression in Cholangiocarcinoma

Present data are insufficient to recommend use of cathepsin measurements for management of patients with cholangiocarcinoma although studies indicate that different cathepsins are involved in the mechanism of metastasis [64].

Similarly, the cyclin proteins which are expressed in the late G1 phase and promote the transition to the S-phase of the cell cycle are abnormally expressed in some cases of cholangiocarcinoma [25, 65, 66]. They can be measured by IHC in formalin-fixed paraffin-embedded (FFPE) tissue, and mRNA for cyclin E has been quantitated by RT-PCR in fresh frozen specimens [67]. Low molecular weight (LMW) forms of cyclin E have been measured by Western blot analysis of proteins in fresh frozen tissue [68]. Discordance between IHC and Western blot analysis in assessment of the prognostic value of cyclin E may be related to the antibodies used for each assay, given that the reagents that detect intact cyclin E may not react with the LMW fragments. Further work is required to demonstrate the role of these markers in the management of hepatobiliary tumors.

It is considered that the location of a cholangiocarcinoma may be related to the etiology of that tumor which may influence the pathways in the dysplasia-carcinoma sequence. In a study of cell cycle proteins, tissue arrays from tumors at different sites in the biliary tree have been examined by IHC. p27, Cyclin D1, and Bcl2 were more frequently over-expressed in proximal tumors, while *p53* and Mdm2 were more frequently over-expressed in distal tumors. While cholangiocarcinomas differentially express cell cycle regulatory proteins based on tumor location and morphology, these differences were not sufficiently distinct to be of diagnostic importance. Vascular invasion, lymph node metastases, absence of p27 expression, and Mdm2 over-expression independently predicted poor outcome on

Table 3 Proteomic techniques in use

Method	Description	Advantage
2-D gel electrophoresis (2-DE)	Uses isoelectric properties and SDS-PAGE gel electrophoresis to separate protein spots. Discovered proteins are biased toward abundant proteins [76]. 2-DE does not identify proteins which are small, very basic, very acidic, or hydrophobic. 2-DE is a slow process	MS can subsequently identify the proteins of interest, now aided by new software for analysis of protein spots [74]
MALDI-TOF	Matrix solution + sample are dried on glass slide. A laser directed at surface ionizes the complex. The ionized complex is accelerated through an electric potential along a flight tube to a detector. The time of flight is related to the mass-to-charge ratio (m/z) of the compound	Measures proteins up to 30 kDa. Can be helpful in sequencing of proteins and oligonucleids
SELDI-TOF MS	Similar principles to MALDI-TOF, but the glass chips have specific surfaces to select a subset of proteins. This is then covered by a matrix. The m/z of the proteins in the sample is measured by time-of-flight technique. The identification of unknown proteins requires further separation of the sample	Can identify patterns of proteins in low concentration. Not easy to collect individual proteins for identification
Multiplex ELISA	Multiple antibodies placed in different wells, measured by luminescence. Each antibody may require different test conditions	A multiplex phosphoarray study demonstrated cholangiocarcinoma survival was associated with increased tissue pAKT and pMTOR and reduced pTEN [103]
Phage display	Screens for protein–protein and protein–DNA interactions using genetic sequences from a DNA library of interactions. Many proteins can be tested at the same time by integrating their sequence into a suitable phage	Suitable for testing large sample sizes
SILAC	Stands for “stable isotope labeling by amino acids in cell culture.” Measures in vivo incorporation of specific amino acids into mammalian proteins [75]	Has identified proteins which are up-regulated in cholangiocarcinoma tissue [104]
Protein microarray	Different proteins are affixed in ordered fashion to a glass slide. Substrates, e.g., protein kinase, or biologically active small molecules, are identified when they bind, by luminescence or similar technique	Has been recently reviewed as an emerging technique to improve cancer diagnosis and prognosis [105]
Aptamer microarray	An aptamer is a nucleic acid (DNA or RNA) or a peptide macromolecule that binds tightly to a specific molecular target. They can be attached to nanoparticles and therefore help target diagnostic or therapeutic agents	Binds 1,000-fold more tightly than many factors. This technology is progressing rapidly and has been recently reviewed [106]

multivariate analysis, and there may be prognostic roles for the proteins Mdm2 and p27. However, these measures did not provide a strong guide for prognosis [65].

9 Proteomic Analysis of Biliary Carcinoma

New technology is revealing a complex array of proteins and peptides in tissue and blood samples and that the pattern of these is distinct for different conditions. Various mixtures of truncated peptide fragments, or of modifications of proteins or peptides, such as glycosylation, cysteinylolation, lipidation, and glutathionylation, require careful evaluation to determine their biological role and the value of this new knowledge for improved diagnosis and therapeutic possibilities. It is expected that these differences, either in tissue, in the circulation, or in secreted fluids, will be sufficiently specific to evaluate many different clinical questions. For proteomic pattern analysis, computer-based algorithms have

been developed to distinguish bile duct cancer from benign diseases [34]. More work is required on larger numbers of samples from patients to answer specific questions such as identifying the proteins which distinguish patients with PSC from those with cholangiocarcinoma.

Protein expression in tumors reflects the activation of biological pathways, and the degree of activation of these pathways is predictive of patient outcome [69]. Furthermore, tissue may be available for proteomic assessment from samples taken at surgery and through needle biopsy and from FNA or ERCP with cytology. Although cancer mechanisms are best studied in the cancer cells taken with laser dissection, many of the samples acquired include stroma. However, stroma may also hold important messages about cancer biology because the migration of tumor cells relies on an interaction with the stroma and the immune system through dendritic cells. Therefore, many opportunities exist for the discovery of new markers in the holistic cancer biology mechanism. These may be of necessity in low concentrations.

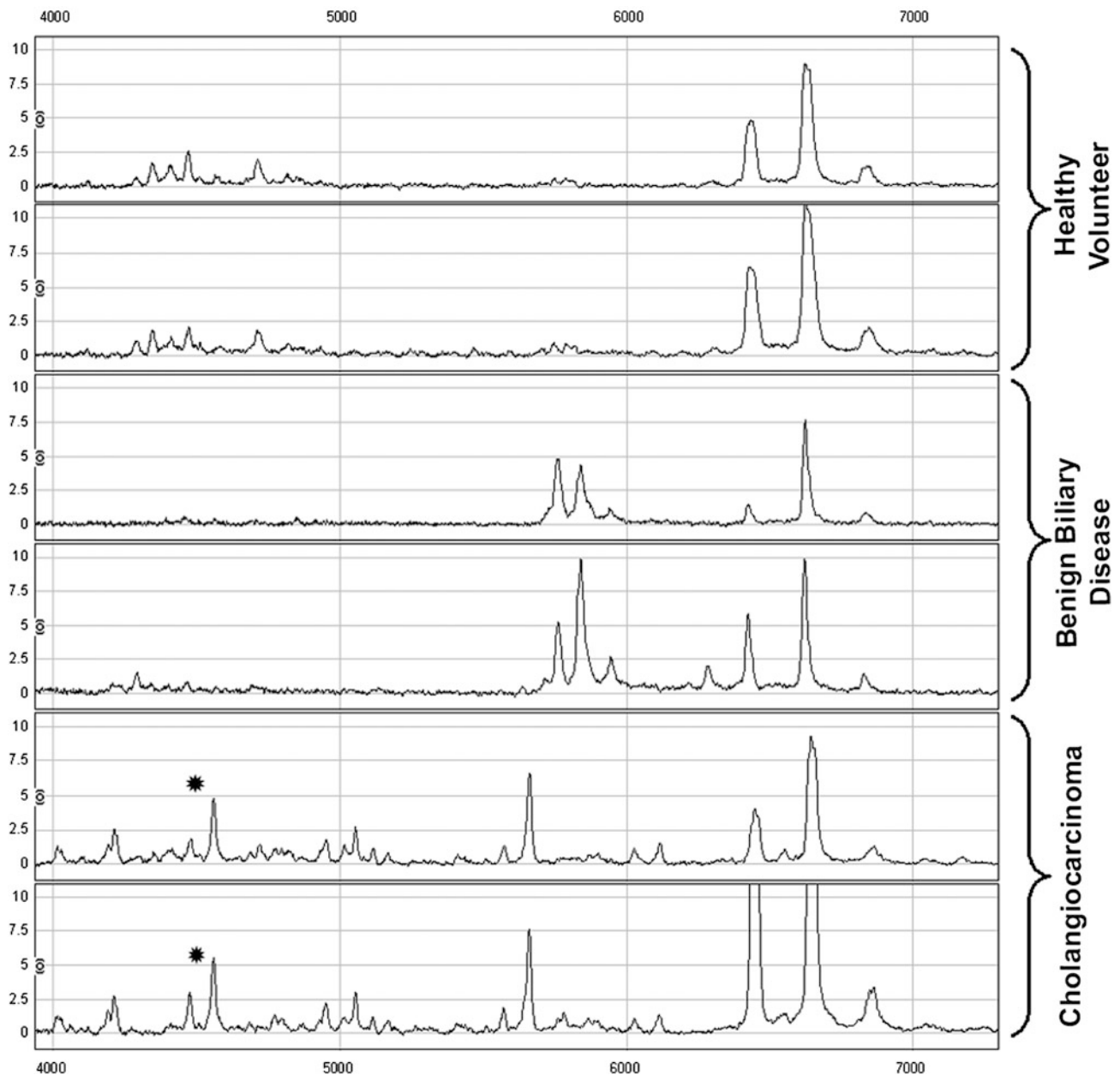


Fig. 1 The printout of the protein mass profile for a small segment of the SELDI MS curve. Results are from the spectrum of two subjects from each of the groups: healthy controls, benign biliary disease, and

cholangiocarcinoma. The *asterisk* marks the m/z 4462 peak. Modified from Scarlett et al. [34]

Many technological advances allow the assessment of numerous proteins at very low concentrations, which is useful in analysis of serum. The majority of serum proteins which differentiate patients with cancer from those without are actually not derived from the neoplastic cells, but are host-specific proteins originating in tissues such as stroma, liver, or immunological material [70]. New methods that allow isolation of low abundance serum proteins which are more likely to represent tumor markers are in development

[71, 72]. Once a number of candidate proteins have been identified and a limited panel is shown to be discriminatory for the tumor of interest, they may be measured by IHC or serum-based immunoassays. Markers can then be validated individually or in combination as a profile or signature. The development of a clinically valuable panel will require validation on a large independent sample set. Although many teams are working to this end, many large collaborative studies are still needed to validate these results.

9.1 Proteomic Pattern Analysis

Analysis of multiple proteins or peptide fragments simultaneously can be approached in several ways, and each has its positive and negative features [73]. Some of these methods include multiplex ELISA, phage display, and aptamer arrays and are summarized in Table 3 [74–76]. However, the most widely studied methods involve identification of proteomic profiles as peaks on mass spectrometric (MS) analysis with precise charge-to-mass ratios. In some cases, proteins have been designated by their apparent molecular weight and isoelectric point within two-dimensional (2-D) gel analysis [76]. Specific peptides can be identified from their amino acid sequence identity or homology to known proteins or their fragments. Some studies have used whole tumor specimens that include both epithelial cells and stroma, whereas others have used microdissected epithelial cells. If isolation of epithelial cells is not required, a fine-needle aspirate can provide adequate material [76]. Before mass spectroscopic analysis, preliminary separation of proteins can be performed with 2-D gel analysis or by binding of proteins to chips or specific surfaces to attract subsets of proteins, called surface-enhanced laser desorption and ionization (SELDI) [77] and matrix-associated laser desorption and ionization (MALDI) [78, 79], respectively. After desorption and ionization, the pattern of charged peptides generally has been analyzed by time-of-flight (TOF) mass spectroscopy. While these methods are excellent for measuring many proteins of low abundance, it may not be necessary to identify the protein in each peak, which may be very difficult when they are of low abundance [80]. Demonstrating a pattern of proteins associated with a cancer type may be sufficient to help make a diagnosis. The multiplex ELISA method can also be used to detect several different proteins simultaneously [81]. In addition, multiple peptides can be measured by phage displays or aptamers [82, 83]. Indeed, screening protein arrays with sera from patients with cancer would facilitate the identification of autoantibody signatures that can be used for diagnosis and/or prognosis of patients. The usefulness of multiplexed measurements lies not only in the ability to screen many individual marker candidates but also in evaluating the use of multiple markers in combination. The advantage of protein and serum screening of peptides and cDNA repertoires displayed on phages as well as the fabrication of protein microarrays for probing immune responses in patients has been recently reviewed [82].

9.2 Proteomic Pattern Analysis

Proteomic pattern analysis was a new field in 2007, when there were 362 articles listed in PubMed containing the key

words “proteomic analysis” and “neoplasms.” By 2012, these had increased to 2,069. SELDI-TOF was originally used to profile proteins in serum and tissue from cholangiocarcinoma subjects because it was able to screen samples from a large cohort of patients in a short time. A study demonstrated the potential of SELDI to authenticate serum biomarkers which differentiated cholangiocarcinoma from benign disease and/or healthy individuals [34].

In this preliminary study, SELDI-TOF MS proteomic profiling differentiated tissue and sera of cholangiocarcinoma from non-malignant subjects. Previous studies involving different cancer types [84–86] showed similar findings, but the pattern of biomarkers varied between the cancer types. The most interesting discovery of the study of the cholangiocarcinoma patients was the finding of a SELDI-derived peak (m/z 4462) which is demonstrated in Fig. 1. This peak was as effective as the tumor markers CEA or CA19-9 at discriminating between sera from cancer patients and disease controls. The relevant ROC curves are demonstrated in Fig. 2a, b. Diagnostic accuracy was improved when these three serum markers were combined in a panel. The diagnosis could be further enhanced using data generated from a panel of other proteins, suggesting that analysis of proteomic profiles, rather than individual proteins, may yield improved diagnostic ability. The value of this technology is in its capacity to analyze large numbers of proteins rapidly to determine which may become potential biomarkers. The LMW portion of the proteome, previously undetectable by the limited resolution of 2-D gel electrophoresis, appears to carry an abundance of tumor-specific information with the potential to improve diagnosis and the understanding of tumor pathogenesis.

A remarkable finding in that paper was that 14 peaks were common to both the tissue and serum of cancer patients. Of these, one peak was significantly up-regulated in both cancer subgroups: m/z 11664 ($P = 0.001$ for tissue, $P < 0.001$ for serum). Interestingly, the tenfold cross-validation/multivariate logistic regression models did not select either of these proteins for any of the putative biomarker panels used above. Nonetheless, these peaks are of significant interest for future investigation.

Alterations in the *serum* protein profile would also seem likely as a result of both the malignant process itself and as secondary to the inflammatory response, and would include release of cytokines and acute-phase proteins from the liver. It was therefore crucial to have a control group of patients who did not have cancer but who had a variety of biliary inflammatory processes with matched liver dysfunction measures.

Discrimination between patients with PSC and those with the added complication of cholangiocarcinoma is perhaps one of the most difficult clinical challenges, because transplantation for malignancy can lead to early recurrence. In a

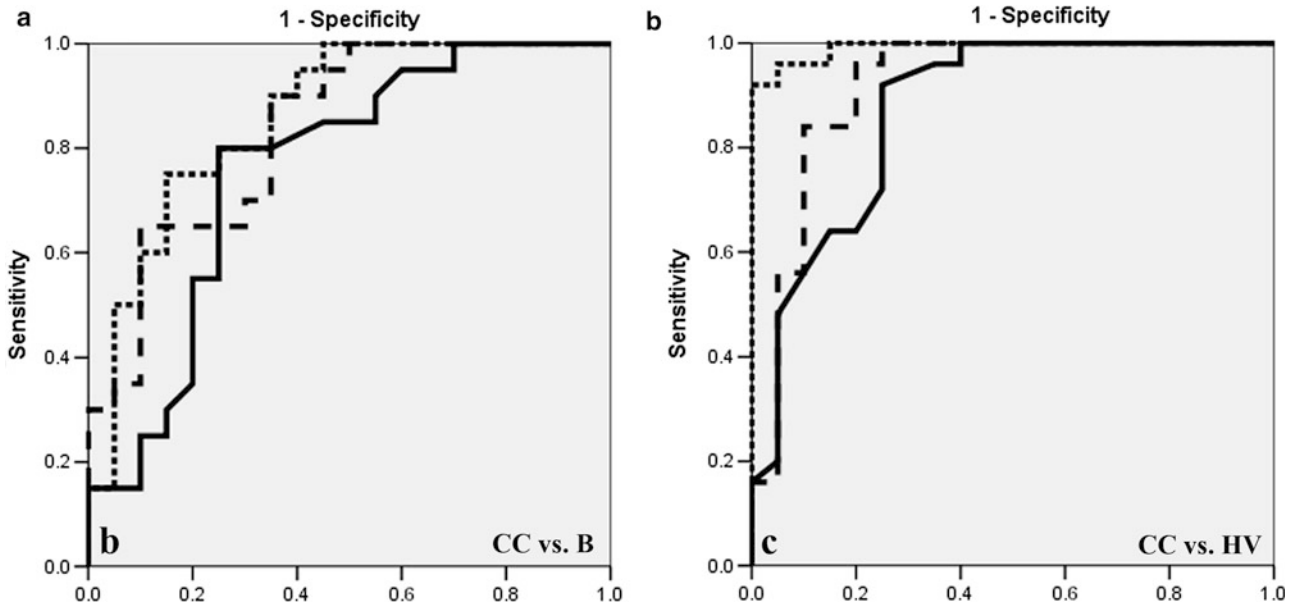


Fig. 2 ROC curves for the serum results from the following: **a** cholangiocarcinoma versus benign disease. *Solid line* marker m/z 4462, *dashed line* 2-marker panel, and *dotted line* CEA added to the

panel and **b** cholangiocarcinoma versus healthy volunteers. *Solid line* marker m/z 11535, *dashed line* 3-marker panel, *dotted line* CEA, and CA19-9 added to the panel. Modified from Scarlett et al. [34]

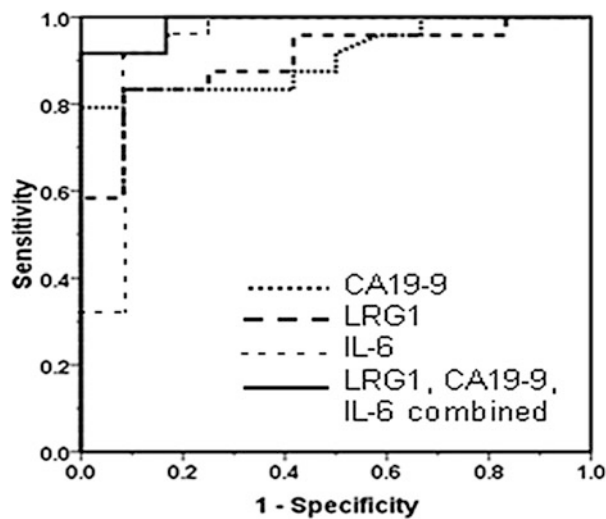


Fig. 3 ROC curves for CA19-9, LRG1, and IL-6 and for CA19-9, LRG1, and IL-6 combined (AUC 0.98) in discriminating cholangiocarcinoma from benign biliary disease

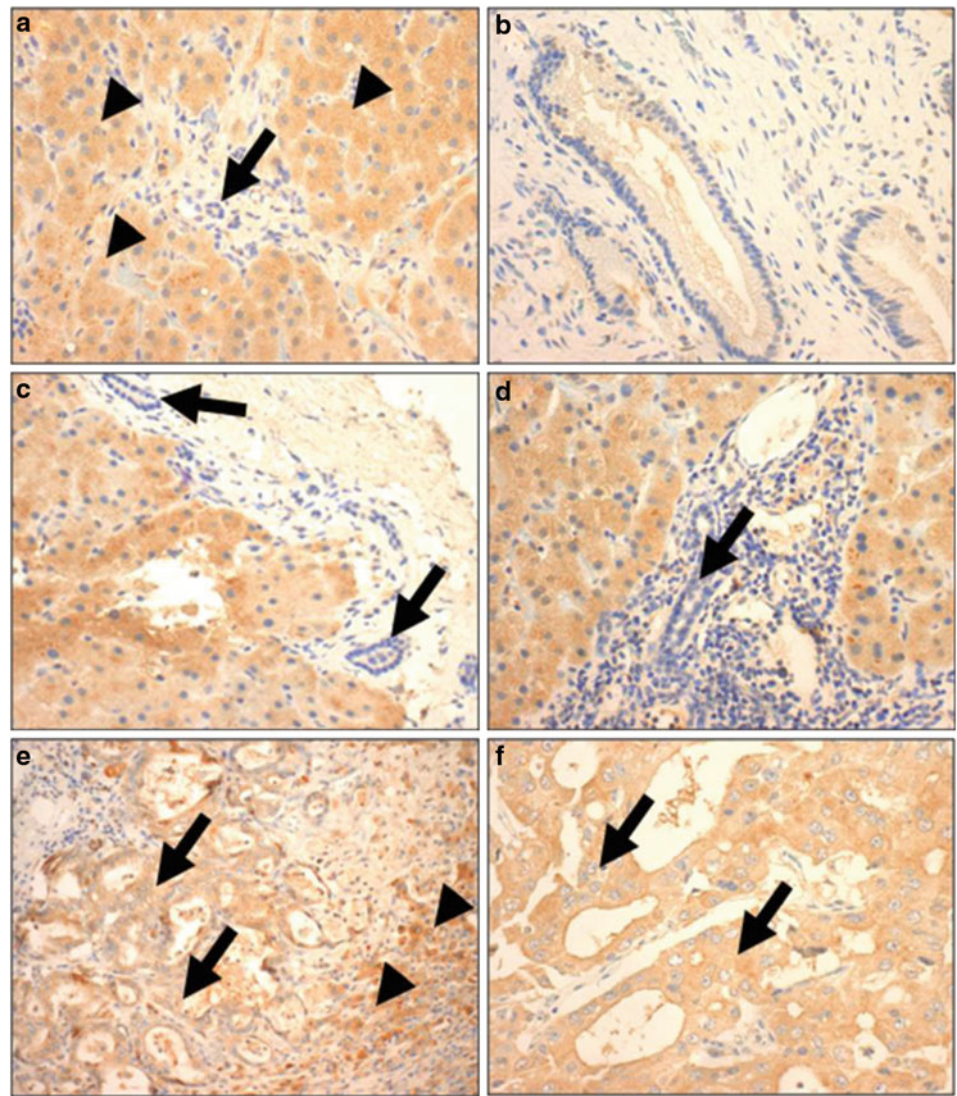
prospective study [87] involving 84 subjects, the novel tumor markers trypsinogen-1, trypsinogen-2, tumor-associated trypsin inhibitor, human chorionic gonadotropin-beta, and trypsin-2-alpha-antitrypsin were evaluated. 46 subjects had undergone transplantation for PSC, and three of these were found to have an unsuspected cholangiocarcinoma. Five of the patients with cholangiocarcinoma had PSC. These markers were measured by the immunofluorescence technique. Serum trypsinogen-2 showed the highest accuracy in differentiating between cholangiocarcinoma and PSC, with

an area under the curve (AUC) of 0.804, while for CA19-9, this was 0.613. For patients with simultaneous cholangiocarcinoma, serum trypsinogen-2 also showed the highest accuracy for differentiation between PSC and cholangiocarcinoma, with an AUC of 0.759. This finding needs to be considered within a multimarker platform using a method such as advanced protein microarray.

Studies of the discriminatory power of low abundance proteins can be greatly enhanced using immunoaffinity depletion, whereby large proteins are removed from the sample. It is then concentrated to increase the amplitude of the peaks measuring the smaller proteins of interest. This was undertaken in pooled samples of serum from patients with bile duct cancer. The results were compared with controls from patients with benign strictures and from healthy people. The remaining proteins were compared on a 2-dimensional difference gel electrophoresis (2D-DIGE), which demonstrated an over-expressed protein in the cholangiocarcinoma sample. This protein was able to be identified by nanoflow liquid chromatography electrospray ionization tandem mass spectroscopy and found to be leucine-rich alpha-2-glycoprotein 1 (LRG1). Subsequently, serum LRG1 was shown to have predictive diagnostic ability, both independently and combined in a panel with CA19-9 and IL-6 [88] (Fig. 3). The AUC of 0.98 for the panel indicates the value of utilizing a panel rather than a single marker.

Although this was an early study requiring confirmation, it indicates different influences on the development of cholangiocarcinoma. Importantly, LRG1 was found to be in

Fig. 4 LRG1 immunohistochemistry analysis demonstrating **a** moderate expression of LRG1 in normal liver (*arrowheads*) with absent expression in biliary epithelium (*arrow*) (original magnification 400×); **b** absent expression of LRG1 in normal biliary epithelium from gallbladder (original magnification 400×); **c** PSC showing positive staining of hepatocytes with absent staining of the biliary epithelium (*arrows*) (original magnification 400×); **d** PBC showing positive staining of hepatocytes with absent staining of the biliary epithelium (*arrow*) (original magnification 400×); **e** cholangiocarcinoma showing positive expression in malignant cells (*arrows*) and in adjacent non-neoplastic hepatocytes of the liver (*arrowheads*) (original magnification 200×); and **f** cholangiocarcinoma showing positive expression of LRG1 (*arrows*) (original magnification 400×). Adapted from Sandanayake et al. [88]



high concentrations in cholangiocarcinoma cells and less strongly in hepatocytes, but was not expressed in normal biliary epithelium (Fig. 4). The finding of elevated LRG1 in patients' serum can indicate the expression of this protein in the cancer cells' cytoplasm. This signifies a change in cellular metabolism from that of normal cholangiocytes. CA19-9 may be a response to biliary obstruction, and the altered expression of LRG1 in the biliary epithelium indicates a significant change in the biliary mucosa, while IL-6 implies the influence of inflammation on the etiology of cholangiocarcinoma.

10 Proteomic Analysis of Bile

Bile is a rich source of proteins, but the complexity of bile with its ample array of mucins and lipids, its high pH, concentrated inorganic ions, and active bile salts creates

problems with analysis. Bile is freely accessible through ERCP, and it is clear that there will be important biomarkers present if some of these difficulties can be overcome. Although it is early in the discovery of the complex map of proteins in bile, recent papers demonstrate that current methods are reproducible and that specific proteins can be recognized [89, 90]. Delipidation, desalination, and nucleic acid removal are necessary before the bile proteome can be examined by the widely accepted 2-DE technique or by tryptic digestion [91]. A 2-DE methodological study undertook a variety of sample preparation options to remove bile contaminants. A large number of protein spots were separated in 2-D maps from the experimental and control groups, with means of 250 and 216 spots on pH 3–10 IPG strips, and 182 and 176 spots on pH 4–7 strips, respectively. When the authors compared bile from a patient with malignancy with bile from a patient with benign disease, approximately 16 and 23 spots,

respectively, were differentially expressed. This study established a reliable sample preparation process suitable for 2-DE examination of bile fluid. The differentially displayed proteomes in the 2-D biliary maps from the experimental and control groups indicated the potential application for bile fluid analysis to identify disease-associated biomarkers, especially for biliary tract tumors [89]. A further paper has identified 97 proteins which are differentially expressed, of which 38 were up-regulated [92]. The authors found that phosphoglycerate mutase 1 (PGAM-1), protein disulfide isomerase family A, member 3 (PDIA3), heat shock 60-kDa protein 1 (chaperonin) (HSPD1) and SSP411 protein were confirmed to be up-regulated by Western blot analysis. Further, SSP411 displayed value as a potential serum diagnostic biomarker with a sensitivity of 90 % and specificity of 83 % at a cutoff value of 0.63.

One novel marker, Mac-2BP, found in bile using tandem mass spectrometry, was demonstrated to be as frequently elevated as CA19-9 in cholangiocarcinoma patients. Further analysis with ELISA indicated that Mac-2BP could discriminate cholangiocarcinoma specimens from patients with PSC, with a ROC AUC of 0.70. When both bile markers were combined, the AUC of the ROC curve increased to 0.75 [90]. Further markers have been sought using cell culture techniques which suggest that CK7, CK19, U2/2, and galectin-3 may be useful markers to differentiate cholangiocarcinoma from hepatocellular carcinoma [93].

Thus, there is a rich pool of proteins to study, but the methodology needs to be developed before its clinical utility can be realized. The protein patterns of biomarkers of cholangiocarcinoma will become apparent as we become familiar with the biliary proteome. Such markers could then add diagnostic value to bile cytology.

11 Multiparameter Markers

This chapter describes a number of emerging technologies which hold promise for the future, despite the heterogeneous nature of the neoplastic process. Within the development of each technology, there has already been an expansion of the number of potential novel biomarkers identified. The new technologies are exploring measures of DNA, microRNA, proteins, aptomers, and epigenetic factors, among others. They have found biomarkers, all correlating with neoplasia, some specifically with cholangiocarcinoma. A diagnosis, prognosis, and even potential therapies can be derived from these. Any one individual discovery, however, does not appear to have sufficient specificity and sensitivity to make it an ideal biomarker. Therefore, a process which allows the measures of independent markers to be brought together in a multiparameter panel will improve diagnostic accuracy to a

level which has widespread utility. The biomarkers discussed here will not be used in isolation in the clinical setting, but integrated with the results of clinical, standard blood pathology, and cytology tests, along with FISH and DNA morphology and in conjunction with different radiological findings to increase diagnostic accuracy [94].

Furthermore, correlation of protein levels with altered pathways within the cancer cells should give new insights into the mechanisms underlying the differences in proteins associated with cholangiocarcinoma. Biomarkers will provide an improved understanding of cholangiocarcinoma and the host response to it.

References

1. Cullen SN, Chapman RW (2005) Review article: current management of primary sclerosing cholangitis. *Aliment Pharmacol Ther* 21(8):933–948
2. Zen Y, Aishima S, Ajioka Y, Haratake J, Kage M, Kondo F et al (2005) Proposal of histological criteria for intraepithelial atypical/proliferative biliary epithelial lesions of the bile duct in hepatolithiasis with respect to cholangiocarcinoma: preliminary report based on interobserver agreement. *Pathol Int* 55(4):180–188
3. Terada T, Nakanuma Y (1990) Pathological observations of intrahepatic peribiliary glands in 1,000 consecutive autopsy livers. III. Survey of necroinflammation and cystic dilatation. *Hepatology* 12(5):1229–1233
4. Terada T, Nakanuma Y (1994) Expression of tenascin, type IV collagen and laminin during human intrahepatic bile duct development and in intrahepatic cholangiocarcinoma. *Histopathology* 25(2):143–150
5. Aishima S, Nishihara Y, Kuroda Y, Taguchi K, Iguchi T, Taketomi A et al (2007) Histologic characteristics and prognostic significance in small hepatocellular carcinoma with biliary differentiation: subdivision and comparison with ordinary hepatocellular carcinoma. *Am J Surg Pathol* 31(5):783–791
6. Van Eyken P, Desmet V (2008) Ductular metaplasia of hepatocytes. In: Sirica AE, Longnecker DS (eds) *Biliary and pancreatic ductal epithelia*, 1st edn. Maecel Dekker Inc., New York, pp 201–228
7. Andresen K, Boberg KM, Vedeld HM, Honne H, Hektoen M, Wadsworth CA et al (2012) Novel target genes and a valid biomarker panel identified for cholangiocarcinoma. *Epigenetics* 7(11):1249–1257
8. Sriraksa R, Zeller C, El-Bahrawy MA, Dai W, Daduang J, Jearanaikoon P et al (2011) CpG-island methylation study of liver fluke-related cholangiocarcinoma. *Br J Cancer* 104(8):1313–1318
9. An F, Yamanaka S, Allen S, Roberts LR, Gores GJ, Pawlik TM et al (2012) Silencing of miR-370 in human cholangiocarcinoma by allelic loss and interleukin-6 induced maternal to paternal epigenotype switch. *PLoS ONE* 7(10):e45606
10. Isomoto H (2009) Epigenetic alterations associated with cholangiocarcinoma (review). *Oncol Rep* 22(2):227–232
11. Zen Y, Nakanuma Y, Portmann B (2012) Immunoglobulin G4-related sclerosing cholangitis: pathologic features and histologic mimics. *Semin Diagn Pathol* 29(4):205–211
12. Lazaridis KN, Gores GJ (2006) Primary sclerosing cholangitis and cholangiocarcinoma. *Semin Liver Dis* 26(1):42–51

13. Baus-Loncar M, Giraud AS (2005) Multiple regulatory pathways for trefoil factor (TFF) genes. *Cell Mol Life Sci* 62(24):2921–2931
14. Kamlua S, Patrakitkomjorn S, Jearanaikoon P, Menheniott TR, Giraud AS, Limpaboon T (2012) A novel TFF2 splice variant (EX2TFF2) correlates with longer overall survival time in cholangiocarcinoma. *Oncol Rep* 27(4):1207–1212
15. Sasaki M, Ikeda H, Nakanuma Y (2007) Expression profiles of MUC mucins and trefoil factor family (TFF) peptides in the intrahepatic biliary system: physiological distribution and pathological significance. *Prog Histochem Cytochem* 42(2):61–110
16. Zen Y, Sasaki M, Fujii T, Chen TC, Chen MF, Yeh TS et al (2006) Different expression patterns of mucin core proteins and cytokeratins during intrahepatic cholangiocarcinogenesis from biliary intraepithelial neoplasia and intraductal papillary neoplasm of the bile duct—an immunohistochemical study of 110 cases of hepatolithiasis. *J Hepatol* 44(2):350–358
17. Hughes NR, Pairajkul C, Royce SG, Clouston A, Bhathal PS (2006) Liver fluke-associated and sporadic cholangiocarcinoma: an immunohistochemical study of bile duct, peribiliary gland and tumour cell phenotypes. *J Clin Pathol* 59(10):1073–1078
18. Kim YS, Gum JR Jr, Crawley SC, Deng G, Ho JJ (1999) Mucin gene and antigen expression in biliopancreatic carcinogenesis. *Ann Oncol* 10(Suppl 4):51–55
19. Kim YS, Gum JR Jr (1995) Diversity of mucin genes, structure, function, and expression. *Gastroenterology* 109(3):999–1001
20. Adsay NV, Merati K, Andea A, Sarkar F, Hruban RH, Wilentz RE et al (2002) The dichotomy in the preinvasive neoplasia to invasive carcinoma sequence in the pancreas: differential expression of MUC1 and MUC2 supports the existence of two separate pathways of carcinogenesis. *Mod Pathol* 15(10):1087–1095
21. Ishikawa A, Sasaki M, Ohira S, Ohta T, Oda K, Nimura Y et al (2004) Aberrant expression of CDX2 is closely related to the intestinal metaplasia and MUC2 expression in intraductal papillary neoplasm of the liver in hepatolithiasis. *Lab Invest* 84(5):629–638
22. Seno H, Oshima M, Taniguchi MA, Usami K, Ishikawa TO, Chiba T et al (2002) CDX2 expression in the stomach with intestinal metaplasia and intestinal-type cancer: prognostic implications. *Int J Oncol* 21(4):769–774
23. Komatsu M, Jepson S, Arango ME (2001) Carothers carraway CA, carraway KL. Muc4/sialomucin complex, an intramembrane modulator of ErbB2/HER2/Neu, potentiates primary tumor growth and suppresses apoptosis in a xenotransplanted tumor. *Oncogene* 20(4):461–470
24. Shibahara H, Tamada S, Goto M, Oda K, Nagino M, Nagasaka T et al (2004) Pathologic features of mucin-producing bile duct tumors: two histopathologic categories as counterparts of pancreatic intraductal papillary-mucinous neoplasms. *Am J Surg Pathol* 28(3):327–338
25. Rouzbahman M, Serra S, Adsay NV, Bejarano PA, Nakanuma Y, Chetty R (2007) Oncocytic papillary neoplasms of the biliary tract: a clinicopathological, mucin core and Wnt pathway protein analysis of four cases. *Pathology* 39(4):413–418
26. Nakanuma Y, Sasaki M, Terada T, Harada K (1994) Intrahepatic peribiliary glands of humans II. Pathological spectrum. *J Gastroenterol Hepatol* 9(1):80–86
27. Nakanuma Y, Katayanagi K, Terada T, Saito K (1994) Intrahepatic peribiliary glands of humans. I. Anatomy, development and presumed functions. *J Gastroenterol Hepatol* 9(1):75–79
28. Terada T, Nakanuma Y (1988) Morphological examination of intrahepatic bile ducts in hepatolithiasis. *Virchows Arch A Pathol Anat Histopathol* 413(2):167–176
29. Terada T, Nakanuma Y (1987) Solitary cystic dilation of the intrahepatic bile duct: morphology of two autopsy cases and a review of the literature. *Am J Gastroenterol* 82(12):1301–1305
30. Kurumaya H, Ohta G, Nakanuma Y (1989) Endocrine cells in the intrahepatic biliary tree in normal livers and hepatolithiasis. *Arch Pathol Lab Med* 113(2):143–147
31. Nakanuma Y, Kurumaya H, Ohta G (1984) Multiple cysts in the hepatic hilum and their pathogenesis. A suggestion of periductal gland origin. *Virchows Arch A Pathol Anat Histopathol* 404(4):341–350
32. Terada T, Kida T, Nakanuma Y (1993) Extrahepatic peribiliary glands express alpha-amylase isozymes, trypsin and pancreatic lipase: an immunohistochemical analysis. *Hepatology* 18(4):803–808
33. Matsubayashi H, Watanabe H, Yamaguchi T, Ajioka Y, Nishikura K, Kijima H et al (1999) Differences in mucus and K-ras mutation in relation to phenotypes of tumors of the papilla of Vater. *Cancer* 86(4):596–607
34. Scarlett CJ, Saxby AJ, Nielsen A, Bell C, Samra JS, Hugh T et al (2006) Proteomic profiling of cholangiocarcinoma: diagnostic potential of SELDI-TOF MS in malignant bile duct stricture. *Hepatology* 44(3):658–666
35. Murray MD, Burton FR, Di Bisceglie AM (2007) Markedly elevated serum CA 19-9 levels in association with a benign biliary stricture due to primary sclerosing cholangitis. *J Clin Gastroenterol* 41(1):115–117
36. Sanchez M, Gomes H, Marcus EN (2006) Elevated CA 19-9 levels in a patient with Mirizzi syndrome: case report. *South Med J* 99(2):160–163
37. La GG, Sofia M, Lombardo R, Latteri S, Ricotta A, Puleo S et al (2012) Adjusting CA19-9 values to predict malignancy in obstructive jaundice: influence of bilirubin and C-reactive protein. *World J Gastroenterol* 18(31):4150–4155
38. Vestergaard EM, Hein HO, Meyer H, Grunnet N, Jorgensen J, Wolf H et al (1999) Reference values and biological variation for tumor marker CA 19-9 in serum for different Lewis and secretor genotypes and evaluation of secretor and Lewis genotyping in a Caucasian population. *Clin Chem* 45(1):54–61
39. Levy C, Lymp J, Angulo P, Gores GJ, Larusso N, Lindor KD (2005) The value of serum CA 19-9 in predicting cholangiocarcinomas in patients with primary sclerosing cholangitis. *Dig Dis Sci* 50(9):1734–1740
40. Björnsson E, Kilander A, Olsson R (1999) CA 19-9 and CEA are unreliable markers for cholangiocarcinoma in patients with primary sclerosing cholangitis. *Liver* 19(6):501–508
41. Frebourg T, Bercoff E, Manchon N, Senant J, Basuyau JP, Breton P et al (1988) The evaluation of CA 19-9 antigen level in the early detection of pancreatic cancer. A prospective study of 866 patients. *Cancer* 62(11):2287–2290
42. Martin A, Corte MD, Alvarez AM, Rodriguez JC, Andicoechea A, Bongera M et al (2006) Prognostic value of pre-operative serum CA 15.3 levels in breast cancer. *Anticancer Res* 26(5B):3965–3971
43. Klee GG, Schreiber WE (2004) MUC1 gene-derived glycoprotein assays for monitoring breast cancer (CA 15-3, CA 27.29, BR): are they measuring the same antigen? *Arch Pathol Lab Med* 128(10):1131–1135
44. Molina R, Barak V, van Dalen A, Duffy MJ, Einarsson R, Gion M et al (2005) Tumor markers in breast cancer—European group on tumor markers recommendations. *Tumour Biol* 26(6):281–293

45. Kokko R, Holli K, Hakama M (2002) Ca 15-3 in the follow-up of localised breast cancer: a prospective study. *Eur J Cancer* 38(9):1189–1193
46. Khatcheressian JL, Wolff AC, Smith TJ, Grunfeld E, Muss HB, Vogel VG et al (2006) American society of clinical oncology 2006 update of the breast cancer follow-up and management guidelines in the adjuvant setting. *J Clin Oncol* 24(31):5091–5097
47. Frenette PS, Thirlwell MP, Trudeau M, Thomson DM, Joseph L, Shuster JS (1994) The diagnostic value of CA 27-29, CA 15-3, mucin-like carcinoma antigen, carcinoembryonic antigen and CA 19-9 in breast and gastrointestinal malignancies. *Tumour Biol* 15(5):247–254
48. Colozza M, Cardoso F, Sotiriou C, Larsimont D, Piccart MJ (2005) Bringing molecular prognosis and prediction to the clinic. *Clin Breast Cancer* 6(1):61–76
49. Bergquist A, Tribukait B, Glaumann H, Broome U (2000) Can DNA cytometry be used for evaluation of malignancy and premalignancy in bile duct strictures in primary sclerosing cholangitis? *J Hepatol* 33(6):873–877
50. Saxby AJ, Nielsen A, Scarlett CJ, Clarkson A, Morey A, Gill A et al (2005) Assessment of HER-2 status in pancreatic adenocarcinoma: correlation of immunohistochemistry, quantitative real-time RT-PCR, and FISH with aneuploidy and survival. *Am J Surg Pathol* 29(9):1125–1134
51. Lindberg B, Arnelo U, Bergquist A, Thorne A, Hjerpe A, Granqvist S et al (2002) Diagnosis of biliary strictures in conjunction with endoscopic retrograde cholangiopancreatography, with special reference to patients with primary sclerosing cholangitis. *Endoscopy* 34(11):909–916
52. Vasilieva LE, Papadimitriou SI, Dourakis SP (2012) Modern diagnostic approaches to cholangiocarcinoma. *Hepatobiliary Pancreat Dis Int* 11(4):349–359
53. Hsu M, Sasaki M, Igarashi S, Sato Y, Nakanuma Y (2013) KRAS and GNAS mutations and p53 overexpression in biliary intraepithelial neoplasia and intrahepatic cholangiocarcinomas. *Cancer* 119(9):1669–1674
54. Hughes NR, Bhathal PS (2013) Adenocarcinoma of gallbladder: an immunohistochemical profile and comparison with cholangiocarcinoma. *J Clin Pathol* 66(3):212–217
55. Zimmer V, Hoblinger A, Mihalache F, Assmann G, Acalovschi M, Lammert F (2012) Potential genotype-specific single nucleotide polymorphism interaction of common variation in p53 and its negative regulator mdm2 in cholangiocarcinoma susceptibility. *Oncol Lett* 4(1):101–106
56. Khan SA, Thomas HC, Toledano MB, Cox JJ, Taylor-Robinson SD (2005) p53 Mutations in human cholangiocarcinoma: a review. *Liver Int* 25(4):704–716
57. Nutthasirikul N, Limpaboon T, Leelayuwat C, Patrakitkomjorn S, Jearanaikoon P (2013) Ratio disruption of the 133p53 and TAp53 isoform equilibrium correlates with poor clinical outcome in intrahepatic cholangiocarcinoma. *Int J Oncol* 42(4):1181–1188
58. Liu XF, Zhang H, Zhu SG, Zhou XT, Su HL, Xu Z et al (2006) Correlation of p53 gene mutation and expression of P53 protein in cholangiocarcinoma. *World J Gastroenterol* 12(29):4706–4709
59. Shin SH, Lee K, Kim BH, Cho NY, Jang JY, Kim YT et al (2012) Bile-based detection of extrahepatic cholangiocarcinoma with quantitative DNA methylation markers and its high sensitivity. *J Mol Diagn* 14(3):256–263
60. Henson DE, Albores-Saavedra J, Corle D (1992) Carcinoma of the extrahepatic bile ducts. Histologic types, stage of disease, grade, and survival rates. *Cancer* 70(6):1498–1501
61. Duffy MJ (2002) Urokinase-type plasminogen activator: a potent marker of metastatic potential in human cancers. *Biochem Soc Trans* 30(2):207–210
62. Smith R, Xue A, Gill A, Scarlett C, Saxby A, Clarkson A et al (2007) High expression of plasminogen activator inhibitor-2 (PAI-2) is a predictor of improved survival in patients with pancreatic adenocarcinoma. *World J Surg* 31(3):493–502
63. Thummarati P, Wijitburaphat S, Prasopthum A, Menakongka A, Sripa B, Tohtong R et al (2012) High level of urokinase plasminogen activator contributes to cholangiocarcinoma invasion and metastasis. *World J Gastroenterol* 18(3):244–250
64. Terada T, Ohta T, Minato H, Nakanuma Y (1995) Expression of pancreatic trypsinogen/trypsin and cathepsin B in human cholangiocarcinomas and hepatocellular carcinomas. *Hum Pathol* 26(7):746–752
65. Jarnagin WR, Klimstra DS, Hezel M, Gonen M, Fong Y, Roggin K et al (2006) Differential cell cycle-regulatory protein expression in biliary tract adenocarcinoma: correlation with anatomic site, pathologic variables, and clinical outcome. *J Clin Oncol* 24(7):1152–1160
66. Kuroda Y, Aishima S, Taketomi A, Nishihara Y, Iguchi T, Taguchi K et al (2007) 14-3-3sigma negatively regulates the cell cycle, and its down-regulation is associated with poor outcome in intrahepatic cholangiocarcinoma. *Hum Pathol* 38(7):1014–1022
67. Sieuwerts AM, Look MP, Meijer-van Gelder ME, Timmermans M, Trapman AM, Garcia RR et al (2006) Which cyclin E prevails as prognostic marker for breast cancer? Results from a retrospective study involving 635 lymph node-negative breast cancer patients. *Clin Cancer Res* 12(11 Pt 1):3319–3328
68. Keyomarsi K, Tucker SL, Buchholz TA, Callister M, Ding Y, Hortobagyi GN et al (2002) Cyclin E and survival in patients with breast cancer. *N Engl J Med* 347(20):1566–1575
69. Romani AA, Crafa P, Desenzani S, Graiani G, Lagrasta C, Sianesi M et al (2007) The expression of HSP27 is associated with poor clinical outcome in intrahepatic cholangiocarcinoma. *BMC Cancer* 7(1):232
70. Bloomston M, Zhou JX, Rosemurgy AS, Frankel W, Muro-Cacho CA, Yeatman TJ (2006) Fibrinogen gamma overexpression in pancreatic cancer identified by large-scale proteomic analysis of serum samples. *Cancer Res* 66(5):2592–2599
71. Drake RR, Schwegler EE, Malik G, Diaz J, Block T, Mehta A et al (2006) Lectin capture strategies combined with mass spectrometry for the discovery of serum glycoprotein biomarkers. *Mol Cell Proteomics* 5(10):1957–1967
72. Yang Z, Harris LE, Palmer-Toy DE, Hancock WS (2006) Multilectin affinity chromatography for characterization of multiple glycoprotein biomarker candidates in serum from breast cancer patients. *Clin Chem* 52(10):1897–1905
73. Mirzaei H, McBee J, Watts J, Aebersold R (2007) Comparative evaluation of current peptide production platforms used in absolute quantification in proteomics. *Mol Cell Proteomics*
74. Elrick MM, Walgren JL, Mitchell MD, Thompson DC (2006) Proteomics: recent applications and new technologies. *Basic Clin Pharmacol Toxicol* 98(5):432–441
75. Orsburn BC (2013) SILAC in biomarker discovery. *Methods Mol Biol* 1002:123–131
76. Rabilloud T, Triboulet S (2013) Two-dimensional SDS-PAGE fractionation of biological samples for biomarker discovery. *Methods Mol Biol* 1002:151–165
77. Hu Y, Zhang S, Yu J, Liu J, Zheng S (2005) SELDI-TOF-MS: the proteomics and bioinformatics approaches in the diagnosis of breast cancer. *Breast* 14(4):250–255
78. Thanan R, Oikawa S, Yongvanit P, Hiraku Y, Ma N, Pinlaor S et al (2012) Inflammation-induced protein carbonylation contributes to poor prognosis for cholangiocarcinoma. *Free Radic Biol Med* 52(8):1465–1472

79. Sawanyawisuth K, Wongkham C, Riggins GJ, Wongkham S, Araki N (2012) Possible involvement of cyclophilin A in processing in fumagillin- induced suppression of cholangiocarcinoma cell proliferation. *Asian Pac J Cancer Prev* 13(Suppl):137–141
80. Xue A, Chang JW, Chung L, Samra J, Hugh T, Gill A et al (2012) Serum apolipoprotein C-II is prognostic for survival after pancreatic resection for adenocarcinoma. *Br J Cancer* 107(11):1883–1891
81. Wang X, Yu J, Sreekumar A, Varambally S, Shen R, Giacherio D et al (2005) Autoantibody signatures in prostate cancer. *N Engl J Med* 353(12):1224–1235
82. Cekaite L, Hovig E, Sioud M (2007) Protein arrays: a versatile toolbox for target identification and monitoring of patient immune responses. *Methods Mol Biol* 360:335–348
83. Fung ET, Yip TT, Lomas L, Wang Z, Yip C, Meng XY et al (2005) Classification of cancer types by measuring variants of host response proteins using SELDI serum assays. *Int J Cancer* 115(5):783–789
84. Rosty C, Christa L, Kuzdzal S, Baldwin WM, Zahurak ML, Carnot F et al (2002) Identification of hepatocarcinoma-intestine-pancreas/pancreatitis-associated protein I as a biomarker for pancreatic ductal adenocarcinoma by protein biochip technology. *Cancer Res* 62(6):1868–1875
85. Rai AJ, Zhang Z, Rosenzweig J, Shih I, Pham T, Fung ET et al (2002) Proteomic approaches to tumor marker discovery. *Arch Pathol Lab Med* 126(12):1518–1526
86. Kozak KR, Su F, Whitelegge JP, Faull K, Reddy S, Farias-Eisner R (2005) Characterization of serum biomarkers for detection of early stage ovarian cancer. *Proteomics* 5(17):4589–4596
87. Lempinen M, Isoniemi H, Makisalo H, Nordin A, Halme L, Arola J et al (2007) Enhanced detection of cholangiocarcinoma with serum trypsinogen-2 in patients with severe bile duct strictures. *J Hepatol* 47(5):677–683
88. Sandanayake NS, Sinclair J, Andreola F, Chapman MH, Xue A, Webster GJ et al (2011) A combination of serum leucine-rich alpha-2-glycoprotein 1, CA19-9 and interleukin-6 differentiate biliary tract cancer from benign biliary strictures. *Br J Cancer* 105(9):1370–1378
89. Chen B, Dong JQ, Chen YJ, Wang JM, Tian J, Wang CB et al (2007) Two-dimensional electrophoresis for comparative proteomic analysis of human bile. *Hepatobiliary Pancreat Dis Int* 6(4):402–406
90. Koopmann J, Thuluvath PJ, Zahurak ML, Kristiansen TZ, Pandey A, Schulick R et al (2004) Mac-2-binding protein is a diagnostic marker for biliary tract carcinoma. *Cancer* 101(7):1609–1615
91. Zhou L, Lu Z, Yang A, Deng R, Mai C, Sang X et al (2007) Comparative proteomic analysis of human pancreatic juice: methodological study. *Proteomics* 7(8):1345–1355
92. Shen J, Wang W, Wu J, Feng B, Chen W, Wang M et al (2012) Comparative proteomic profiling of human bile reveals SSP411 as a novel biomarker of cholangiocarcinoma. *PLoS ONE* 7(10):e47476
93. Srisomsap C, Sawangareetrakul P, Subhasitanont P, Panichakul T, Keeratichamroen S, Lirdprapamongkol K et al (2004) Proteomic analysis of cholangiocarcinoma cell line. *Proteomics* 4(4):1135–1144
94. Charatcharoenwithaya P, Enders FB, Halling KC, Lindor KD (2008) Utility of serum tumor markers, imaging, and biliary cytology for detecting cholangiocarcinoma in primary sclerosing cholangitis. *Hepatology* 48(4):1106–1117
95. Ahrendt SA, Rashid A, Chow JT, Eisenberger CF, Pitt HA, Sidransky D (2000) p53 overexpression and K-ras gene mutations in primary sclerosing cholangitis-associated biliary tract cancer. *J Hepatobiliary Pancreat Surg* 7(4):426–431
96. Bergan A, Gladhaug IP, Schjolberg A, Bergan AB, Clausen OP (2000) p53 accumulation confers prognostic information in resectable adenocarcinomas with ductal but not with intestinal differentiation in the pancreatic head. *Int J Oncol* 17(5):921–926
97. Cong WM, Bakker A, Swalsky PA, Raja S, Woods J, Thomas S et al (2001) Multiple genetic alterations involved in the tumorigenesis of human cholangiocarcinoma: a molecular genetic and clinicopathological study. *J Cancer Res Clin Oncol* 127(3):187–192
98. Havlik R, Sbisá E, Tullo A, Kelly MD, Mitry RR, Jiao LR et al (2000) Results of resection for hilar cholangiocarcinoma with analysis of prognostic factors. *Hepatogastroenterology* 47(34):927–931
99. Isa T, Tomita S, Nakachi A, Miyazato H, Shimoji H, Kusano T et al (2002) Analysis of microsatellite instability, K-ras gene mutation and p53 protein overexpression in intrahepatic cholangiocarcinoma. *Hepatogastroenterology* 49(45):604–608
100. Kim HJ, Yun SS, Jung KH, Kwun WH, Choi JH (1999) Intrahepatic cholangiocarcinoma in Korea. *J Hepatobiliary Pancreat Surg* 6(2):142–148
101. Tannapfel A, Engeland K, Weinans L, Katalinic A, Hauss J, Mossner J et al (1999) Expression of p73, a novel protein related to the p53 tumour suppressor p53, and apoptosis in cholangiocellular carcinoma of the liver. *Br J Cancer* 80(7):1069–1074
102. Washington K, Gottfried MR (1996) Expression of p53 in adenocarcinoma of the gallbladder and bile ducts. *Liver* 16(2):99–104
103. Chung JY, Hong SM, Choi BY, Cho H, Yu E, Hewitt SM (2009) The expression of phospho-AKT, phospho-mTOR, and PTEN in extrahepatic cholangiocarcinoma. *Clin Cancer Res* 15(2):660–667
104. Kawase H, Fujii K, Miyamoto M, Kubota KC, Hirano S, Kondo S et al (2009) Differential LC-MS-based proteomics of surgical human cholangiocarcinoma tissues. *J Proteome Res* 8(8):4092–4103
105. Casal JJ, Barderas R (2010) Identification of cancer autoantigens in serum: toward diagnostic/prognostic testing? *Mol Diagn Ther* 14(3):149–154
106. Liu K, Lin B, Lan X (2013) Aptamers: a promising tool for cancer imaging, diagnosis, and therapy. *J Cell Biochem* 114(2):250–255
107. Wang J (2011) Status and its prognostic role in extrahepatic bile duct cancer: a meta-analysis of published studies. p 53

Pathologic Staging

Taofic Mounajjed and Tsung-Teh Wu

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Abstract

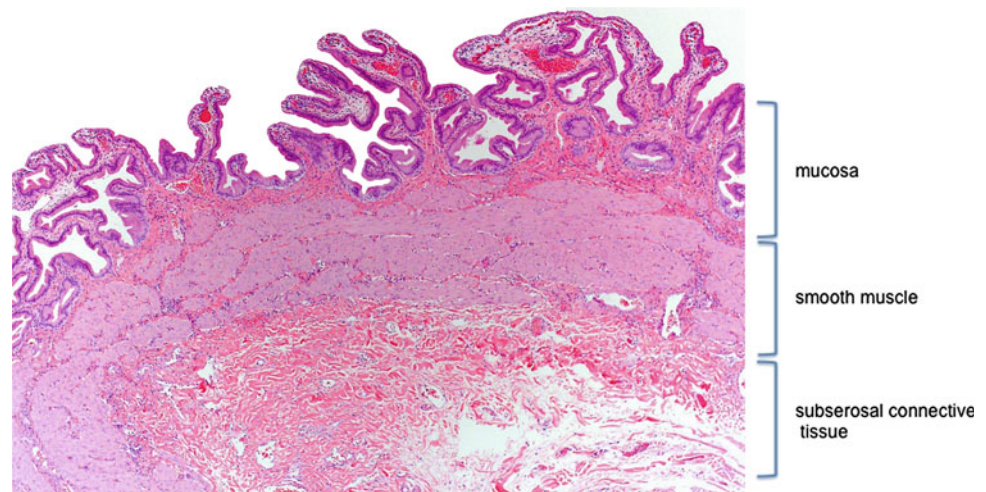
Pathologic staging of resected cancer specimens is critical in predicting patient outcome and guiding treatment decisions. This chapter will outline the current criteria for pathologic staging of carcinomas of the gallbladder, extrahepatic bile ducts, and ampulla of Vater based on the TNM staging scheme published by the American Joint Committee on Cancer (AJCC). The chapter will also discuss recent changes in the TNM and overall staging criteria based on the seventh edition of the AJCC staging manual. In order to understand pathologic staging, a brief description of normal anatomy and background cancer predisposing conditions will be provided. Finally, important pathologic features other than the TNM stage, which can be valuable predictors of cancer outcome, including tumor macroscopic features, histologic subtype, histologic grade, perineural invasion, angiolymphatic invasion, and resection margin status, will also be discussed.

1 Introduction

Pathologic staging of cancer plays a major role in predicting tumor behavior and patient outcome, guiding cancer management, and facilitating scholarly exchange of information. Therefore, pathologic staging of all resected cancer specimens is now a requirement mandated by the National Cancer Institute (NCI). Although several staging schemes exist [75, 96], the TNM staging scheme, a system based on regularly published criteria by the American Joint Committee on Cancer (AJCC) [30], is the most widely used scheme in the United States. The AJCC staging system is based on the TNM paradigm. T stage describes the relative extent of tumor invasion, N stage describes the status of lymph nodes and sometimes the location of lymph node metastases, and the M stage describes the presence or absence of distant metastases. Based on the combined TNM

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Fig. 1 Normal gallbladder histology. The mucosa consists of a single layer of tall columnar epithelial cells and underlying lamina propria. Underneath the mucosa are a smooth muscle layer and a perimuscular connective tissue layer. The serosa is not shown in this figure



findings, an overall tumor stage can be extrapolated. While TNM stages are expressed in digits (or Tis for in situ carcinoma), the overall stage grouping is denoted by roman numerals (I–IV). Stage I indicates localized (and usually curable) carcinoma, stages II–III indicate locally advanced carcinoma or regional lymph node involvement, and stage IV indicates inoperable tumors or distant metastases.

In this chapter, we will discuss the AJCC criteria (according to the AJCC cancer staging manual, seventh edition) for pathologic staging of carcinomas of the gallbladder, extrahepatic bile ducts, and the ampulla of Vater. We will also outline other important pathologic features of these carcinomas, which can affect patient prognosis and management, and should therefore be included in the pathology report. The latter features are usually reported along the TNM pathologic staging in a synoptic report format, based on protocols published by the College of American Pathologists (CAP). Tumors of the gallbladder, extrahepatic bile ducts, and ampulla of Vater will be discussed separately. Each section will include a summary of normal anatomy and cancer predisposing conditions, a brief outline of the key macroscopic and microscopic features of carcinoma, an outline of the TNM staging system, and a discussion of other important pathologic features. Of note, AJCC cancer staging guidelines for the gallbladder, extrahepatic bile ducts, and the ampulla of Vater are designed to stage carcinomas; carcinoid tumors and sarcomas are not included and will not be discussed in this chapter.

2 Gallbladder

2.1 Normal Anatomy and Predisposing Conditions

In order to understand pathologic staging, a brief description of normal anatomy is necessary [78]. The gallbladder is

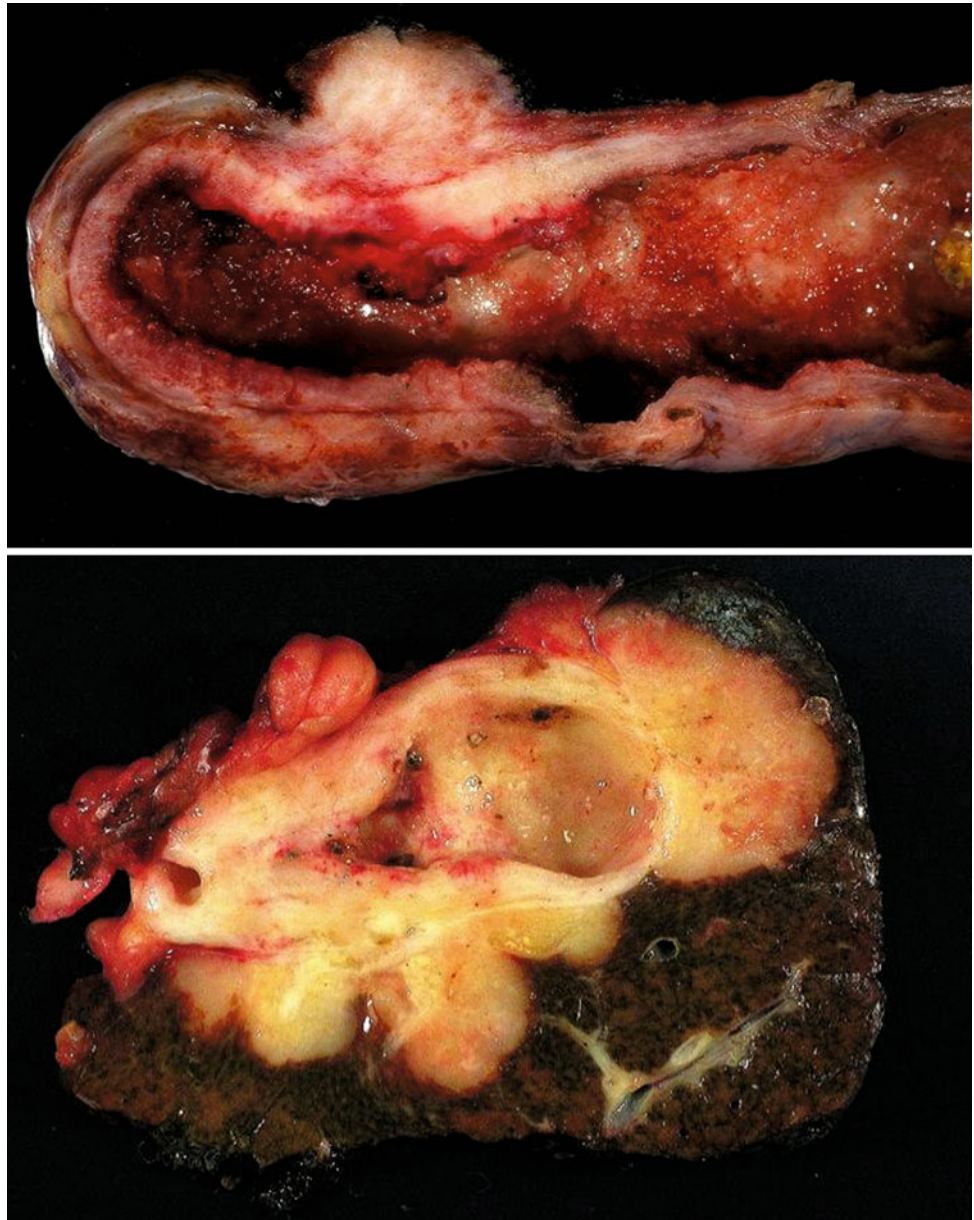
normally situated near the quadrate lobe of the liver, under the right hepatic lobe. Its upper surface is adherent to the liver, whereas the remainder of its surface is covered by peritoneum. Anatomic variants include gallbladder freely suspended from the liver by mesentery (floating gallbladder) or completely buried in the hepatic substance. The gallbladder consists of 3 parts: the fundus, the body, and the neck. Histologically, the gallbladder has the following four layers (Fig. 1):

1. The mucosa, which consists of a single layer of tall columnar epithelial cells basally anchored to a basement membrane, and underlying lamina propria. In the region of the neck, mucin-secreting glands surround the epithelium.
2. A smooth muscle layer, most prominent in the region of the neck.
3. Perimuscular connective tissue.
4. Serosa.

It is important to note that the gallbladder has no true muscularis propria or muscularis mucosa. It is also important to note that serosa is not present along the hepatic side. Instead, the perimuscular connective tissue is rather continuous with the interlobular hepatic connective tissue in this region; this facilitates tumor extension into the liver [66].

Gallbladder carcinoma has known association with gallstones, which are found in 80 % of carcinomas and are considered a risk factor for gallbladder carcinoma [24]. Nevertheless, the overall incidence of gallbladder carcinoma in patients with cholelithiasis is still low (<0.2 %) [65]. There is also an association between gallbladder carcinoma and an abnormal choledochopancreatic junction. Gallbladder carcinoma in this setting develops in younger (10 years younger) patients in the absence of gallstones [56, 108]. Therefore, when gallbladder carcinoma occurs in the absence of gallstones, the pathologist should recommend evaluation of the choledochopancreatic junction. Primary sclerosing cholangitis (PSC) is another risk factor for

Fig. 2 Gross features of gallbladder carcinoma. *Upper* carcinoma involving the gallbladder fundus and body and forming a transmural mass with diffuse wall infiltration. *Lower* gallbladder carcinoma infiltrating surrounding liver



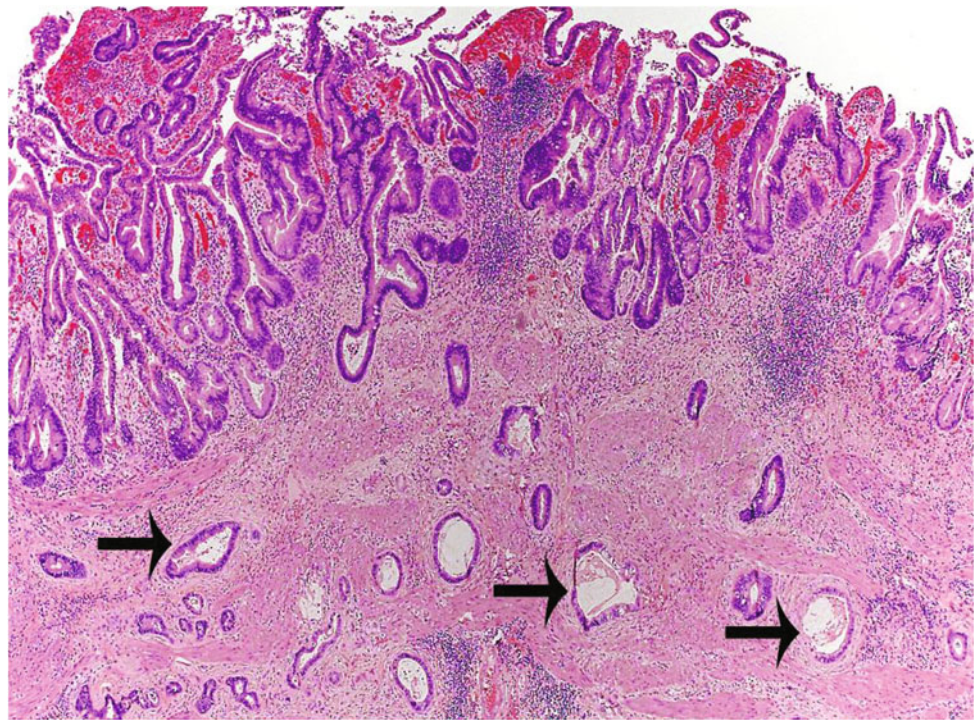
gallbladder carcinoma. In PSC, gallbladder adenocarcinoma often arises in a background of intestinal metaplasia and dysplasia. In fact, data support a metaplasia–dysplasia–carcinoma sequence in gallbladder carcinogenesis in PSC patients [62]. There is conflicting data on the association between calcified (porcelain) gallbladder and gallbladder carcinoma [2, 82, 100]. The location of calcifications may be an important determinant of carcinoma risk, whereas no carcinoma was observed in gallbladders with diffuse intramural calcification, and cancer incidence was significantly increased in gallbladders with selective mucosal calcification [93]. In any case, it is advisable to carefully evaluate calcified gallbladder specimens and adequately sample them to exclude the possibility of carcinoma. Other risk factors for gallbladder carcinoma include ulcerative colitis,

familial adenomatous polyposis (FAP), and certain chronic infections [2].

2.2 Gross and Microscopic Features

Gallbladder carcinomas can involve the fundus (60 %), body (30 %), and neck (10 %) [2]. Although early stage tumors can be difficult to recognize grossly, most gallbladder carcinomas produce a grossly recognizable mass. They can appear as localized or diffuse wall thickening, a raised plaque, a submucosal nodule, a polypoid growth, or a combination of all these patterns (Fig. 2). Infiltrative growth is often present and is considered an independent predictor of recurrence in gallbladder carcinoma [81]. Certain tumor

Fig. 3 Histologic features of gallbladder carcinoma. Invasive adenocarcinoma is characterized by infiltrative growth of variably sized and irregularly shaped glands (arrows), with desmoplastic response



subtypes have a characteristic gross appearance. For instance, papillary carcinomas have a sessile polypoid or cauliflower-like gross appearance, and mucinous tumors have a mucoid/gelatinous cut surface.

Microscopically, carcinoma in situ (CIS) is defined as carcinoma that is confined to the epithelium, usually growing upward or laterally, without evidence of stromal invasion. Histologic subtypes of CIS include intestinal type, signet ring cell type, and squamous cell CIS. When CIS is found in the gallbladder, multiple sections should be taken to exclude invasion. If no invasion is found and the margins are free, the survival is excellent (virtually 100 % at 5 years) and no further treatment is necessary [2].

More than 98 % of malignant gallbladder tumors are carcinomas [2], and adenocarcinoma is the most common type (87 %) [28] of invasive carcinoma. Adenocarcinoma is characterized by infiltrative growth of variably sized and irregularly shaped glands, usually with desmoplastic response (Fig. 3). It is important to differentiate adenocarcinoma from other benign entities such as adenomyomatous hyperplasia [3]. Variants of adenocarcinoma include the following:

1. Papillary adenocarcinoma is considered a variant of well-differentiated adenocarcinoma, showing a predominant polypoid/intraluminal rather than invasive growth, which results in a relatively improved prognosis (best survival rates of gallbladder carcinoma). Papillary adenocarcinoma can be invasive or noninvasive. Because noninvasive papillary carcinoma has such an excellent

prognosis [40, 41, 75], extensive sampling to exclude invasion is recommended.

2. Intestinal type adenocarcinoma is also a variant of well-differentiated adenocarcinoma, demonstrating either similar morphology to colon adenocarcinoma or predominant goblet cells lining [2, 5].
3. Clear cell adenocarcinoma is very rare [101]. It usually contains foci of conventional adenocarcinoma [2]. The latter feature can help differentiate primary clear cell carcinoma of the gallbladder from metastatic renal cell carcinoma and clear cell carcinoma of Mullerian origin.
4. Mucinous adenocarcinoma constitutes approximately 4 % of gallbladder carcinomas [28]. Because this tumor is uncommon, its behavior is uncertain, but it seems to be more likely to spread to the peritoneum than other types [2].
5. Signet ring cell adenocarcinoma constitutes approximately 3 % of gallbladder carcinomas.

Other carcinomas known to involve the gallbladder include adenosquamous carcinoma: (5–9 % of gallbladder carcinoma) [2, 28, 76], which consists of a mixture of squamous cell carcinoma and adenocarcinoma, squamous cell carcinoma (1–7 % of gallbladder carcinoma) [28], small cell carcinoma (4 % of gallbladder carcinoma), and undifferentiated carcinoma. Small cell carcinoma is morphologically similar to pulmonary small cell carcinoma and has a highly aggressive clinical behavior [2, 4, 84]. This histologic type should be reported even if it is a minor component of a gallbladder carcinoma, as such combined

tumors still behave aggressively. Undifferentiated carcinoma has four variants, the commonest of which are spindle/giant cell type [2, 16] and small cell type [2]. In undifferentiated carcinoma, good sampling can unveil a better-differentiated invasive carcinoma component or CIS in most cases. Recording the histologic type is important, since some types (e.g., papillary carcinoma) have a better survival rate, whereas others (e.g., undifferentiated carcinoma and small cell carcinoma) have the worst prognosis (patients usually survive less than 1 year) [2]. This is also important for treatment purposes; for instance, chemotherapeutic choice for small cell carcinoma is different from that of adenocarcinoma. When there is more than one histologic pattern, it is recommended to record all patterns in the pathologic diagnosis. It is also recommended to record the tumor's histologic grade because it is a significant predictor of patient outcome [11, 28] and an independent predictor of recurrence in gallbladder carcinoma [81]. Grading adenocarcinoma can be evaluated by assessing the glandular versus solid component of the tumor histologically (grade 1: >95 % glands, grade 2: 50–95 % glands, grade 3: <49 % or less glands, and grade 4: undifferentiated). Gallbladder adenocarcinoma is most commonly in grades 1 and 2 [28].

2.3 Staging

TNM stage is a significant predictor of patient outcome [11, 20, 28, 64, 105]. In fact, disease stage is the single most important factor in determining patient survival at the time of diagnosis. The depth/extent of tumor invasion has prognostic value; the incidence of lymph node and distant metastasis increases progressively with increased T stage [66, 88]. Invasion beyond one-third of the subserosal layer carries significantly increased risk of tumor spread and may warrant para-aortic lymph node sampling [88]. Tables 1 and 2 outline the current TNM and overall staging of gallbladder carcinoma. Of note, cystic duct carcinomas are now included in the gallbladder TNM classification scheme. Carcinomas of the gallbladder are staged according to their depth of invasion into the wall and extension into adjacent structures. Tumors confined to the gallbladder are classified as T1 or T2 depending on the depth of tumor invasion. Tumors extending beyond the gallbladder wall, through the serosa, and/or involving adjacent structures are designated T3. Because invasion of hilar structures usually renders the cancer unresectable, such tumors are designated T4.

Because the gallbladder has a thin wall, tumors extend quickly into the perimuscular connective tissue, gaining access to a rich vascular and lymphatic network, and facilitating tumor spread. Hence, gallbladder carcinoma often metastasizes early, even before the diagnosis is made.

Table 1 TNM staging of gallbladder carcinoma [30]

Primary tumor (T)	
TX	Cannot be assessed
TO	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor invades lamina propria or muscular layer
T1a	Tumor invades lamina propria
T1b	Tumor invades muscle layer
T2	Tumor invades perimuscular connective tissue; no extension beyond serosa or into liver
T3	Tumor perforates 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
T4	Tumor invades main portal vein or hepatic artery or invades 2 or more extrahepatic organs or structures
Regional lymph nodes (N)	
NX	Cannot be assessed
N0	No regional lymph node metastasis
N1	Metastases to nodes along the cystic duct, common bile duct, hepatic artery, and/or portal vein
N2	Metastases to periaortic, periaortic, superior mesentery artery, and/or celiac artery lymph nodes
Distant metastasis (M)	
M0	No distant metastasis
M1	Distant metastasis

Table 2 Overall stages of gallbladder carcinoma [30]

Overall pathologic stage			
Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage IIIA	T3	N0	M0
Stage IIIB	T1–3	N1	M0
Stage IVA	T4	N0–1	M0
Stage IVB	Any T	N2	M0
	Any T	Any N	M1

Gallbladder tumors can involve the liver, stomach, duodenum, or colon by direct extension, can implant on peritoneal surfaces, or metastasize to regional lymph nodes and distant organs. Regional lymph node metastasis is a significant independent predictor of patient survival [25, 86, 107]. It occurs frequently early in gallbladder cancer (found in 19–50 % of patients at the time of diagnosis) [25]. Regional lymph nodes are limited to the hilar (cystic, pericholedochal, hepatic artery, and portal vein) lymph nodes staged as N1 and other regional (para-aortic, pericaval, superior

mesenteric artery, and celiac artery) staged as N2 [51]. Hence, involvement of peripancreatic nodes along the body and tail of the pancreas is considered distant metastases (M1). In addition to topographic location, which determines the N stage, the number of involved lymph nodes is also important in predicting survival [31]. Therefore, the surgical pathologist should document the number of examined and number of positive lymph nodes. Accurate N staging requires sampling of a minimum of three regional lymph nodes for microscopic examination [66]. In addition to histologically evident metastases, one study showed that immunohistochemically detected micrometastases, regardless of size, are associated with poor survival and should be designated N1 [86]. Because these findings are limited to one study and remain unvalidated by larger series, the data are currently insufficient to recommend routine immunohistochemical evaluation of regional lymph nodes. Hence, routine assessment of regional lymph nodes is currently limited to H&E sections. Distant metastases are found in 57 % of patients with gallbladder carcinoma [28]. They most often involve the liver, peritoneum, and lung [66]. The liver is involved in 13–70 % of patients at the time of surgery, either by direct extension (most common) or by metastasis [25].

2.4 Other Important Pathologic Features

Additional important pathologic features in gallbladder carcinoma include perineural invasion, angiolymphatic invasion, and surgical margin status. Perineural invasion occurs in most (71 %) gallbladder carcinomas and is associated with significantly lower 5 year survival rate [20], but there is conflicting data in regard to its value as an independent predictor of survival [7, 20, 86, 105]. Perineural invasion is also significantly associated with extrahepatic bile duct invasion [107]. Likewise, angiolymphatic invasion is a significant prognostic factor in gallbladder carcinoma and its absence is significantly associated with long-term survival [7, 20, 105]. In fact, vascular invasion seen histologically has the effect of reducing outcome to the next disease stage. It is therefore important to report it. The surgical margin status is a significant prognostic factor in gallbladder carcinoma [105]; long-term survival is significantly associated with complete resection [11].

Gallbladder carcinoma is often identified in laparoscopically resected gallbladders. Laparoscopic cholecystectomy carries a risk of cancer implantation/dissemination; it should not be performed in gallbladders with high suspicion of malignancy [9, 34, 68, 92]. Nevertheless, invasive carcinoma, undetected clinically, is occasionally discovered first by the pathologist evaluating laparoscopic cholecystectomy specimens. These tumors are often low stage (T1 or T2) [23],

but specimen disruption can complicate pathologic staging. Port-site recurrence is also a serious complication of gallbladder cancers resected laparoscopically [80] usually treated by follow-up surgery, which includes port-sites excision if the cancer is resectable (T1 and T2) [35]. Because carcinoma can be found incidentally, careful gross examination and mandatory histologic evaluation (especially if the gallbladder wall is thickened) are essential in detecting such tumors. In addition, when a polyp or a distinct lesion is present, the entire lesion should be examined microscopically.

3 Extrahepatic Bile Ducts

3.1 Normal Anatomy and Predisposing Conditions

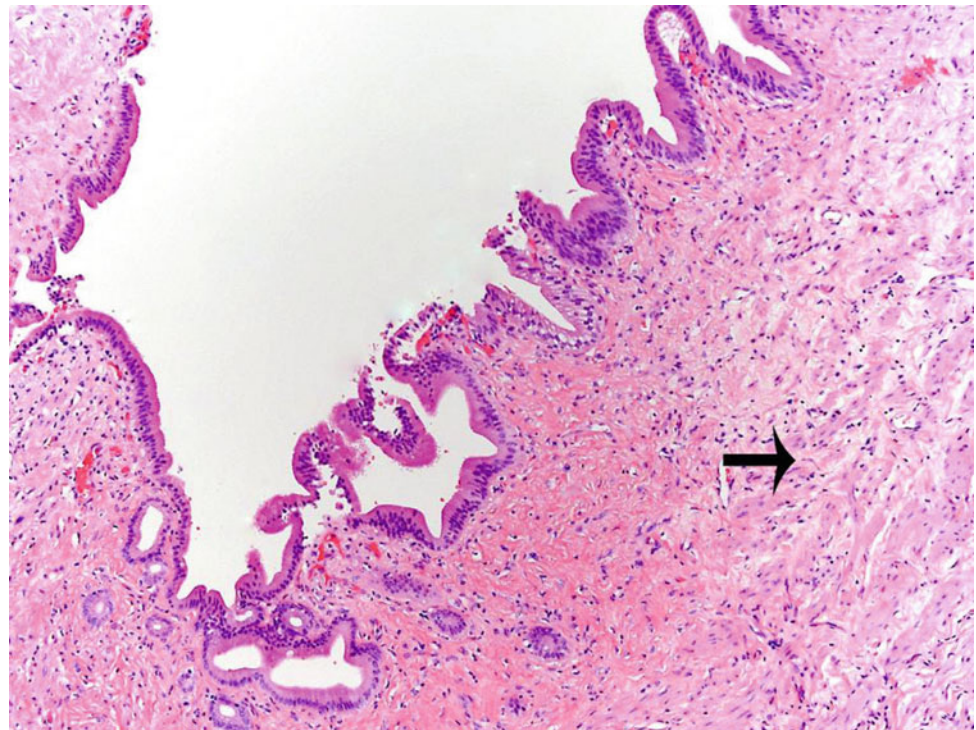
Bile flows from the liver through the hepatic ducts, which join to form the common hepatic duct (0.8–5.2 cm in length), which in turn descends along the lateral aspect of the hepatoduodenal ligament, and joins the cystic duct (0.4–6.5 cm in length) to form the common bile duct (1.5–9 cm in length). The common bile duct passes posterior to the duodenum, traverses the head of the pancreas, and finally opens into the second part of the duodenum through the major papilla (papilla of Vater). The common bile duct is divided into four parts: supraduodenal (longest and most accessible), retroduodenal, pancreatic, and intraduodenal. The walls of the extrahepatic bile ducts are very thin (<1.5 mm in thickness), predisposing to rapid invasion of malignant tumors into the periductal tissue, gaining access to a rich vascular and lymphatic network, which facilitates tumor spread. The microscopic anatomy of all extrahepatic bile ducts is essentially the same (Fig. 4). They consist of:

1. Mucosa, which consists of tall columnar epithelium with small longitudinal folds and a subepithelial layer containing elastic and collagen fibers.
2. Scattered small bundles of smooth muscle in the ductal wall parallel with the lumen. This layer becomes prominent in the common bile duct near the sphincter of Oddi.
3. Periductal layer of loose connective tissue.

Intramural mucous glands are present and increase toward the distal part; they open into the lumen in small pits (the sacculi of Beale).

Extrahepatic bile duct carcinoma can be associated with ulcerative colitis, PSC (carcinoma usually occurs at younger age), abnormal choledochopancreatic junction, choledochal cysts (carcinoma usually occurs at younger age), and certain longstanding biliary infections [2, 19, 32, 47, 52, 58, 60, 67, 69, 71, 97]. The dysplasia–carcinoma sequence appears to be the usual pathway for development of invasive

Fig. 4 Normal extrahepatic bile duct histology. The mucosa consists of columnar epithelium with small longitudinal folds and a subepithelial layer containing elastic and collagen fibers. Scattered small bundles of smooth muscle (*arrow*) underlie the mucosa



carcinoma of the extrahepatic bile ducts, especially in ulcerative colitis and PSC [2].

3.2 Gross and Microscopic Features

Approximately 54 % of extrahepatic bile duct carcinomas are perihilar and 42 % are distal (see Sect. 2.3) [26]. Distal bile duct carcinomas should be distinguished from pancreatic carcinomas because they have a better outcome [33, 53]. Grossly, extrahepatic bile duct carcinomas can be constrictive (sclerosing), nodular, polypoid/papillary, diffusely infiltrative, or mixed sclerosing/nodular [99, 102] (Fig. 5). Sclerosing tumors are the most common. They usually have a perihilar location and show diffuse infiltration and fibrosis of periductal tissue, producing a very firm and thickened duct. Papillary tumors are common in the distal extrahepatic bile ducts and are soft and friable; they produce little if any mural invasion and therefore have a favorable outcome [48]. Similar to the gallbladder, certain tumor subtypes have a characteristic gross appearance. For instance, papillary carcinomas have a polypoid appearance and mucinous tumors have a gelatinous cut surface.

Microscopically, the morphologic criteria for classification and grading of in situ and invasive carcinoma of the extrahepatic bile ducts are similar to those in the gallbladder. Like in the gallbladder, the vast majority (>90 %) of extrahepatic bile duct carcinomas are adenocarcinoma [48] (Fig. 6). Papillary and grade 1 adenocarcinomas are associated with polypoid and nodular macroscopic types and are

often located in the upper portion of the extrahepatic biliary tree, whereas grade 2–3 adenocarcinomas are associated with constrictive (sclerosing) macroscopic type and tend to involve the lower biliary tree. The latter carcinomas have higher rates of angiolymphatic invasion and lymph node metastasis [106]. Papillary carcinomas represent 3–23 % of extrahepatic bile duct tumors [27, 49]. They are larger, more well-differentiated and have an earlier stage and significantly improved survival (especially if noninvasive) compared to nodular sclerosing type adenocarcinoma [49]. In fact, noninvasive and minimally invasive papillary carcinomas have the best prognosis among extrahepatic bile duct carcinoma. In contrast, squamous cell carcinoma, undifferentiated carcinoma, signet ring cell carcinoma, and small cell carcinoma have the worst prognosis [39]. Mucinous, adenosquamous, and squamous cell carcinomas represent 4, 2, and 2 %, of extrahepatic carcinomas, respectively [27]. The frequency of small cell carcinoma and undifferentiated carcinoma is lower in the extrahepatic bile ducts than in the gallbladder. Similar to gallbladder carcinoma, histologic grade is a useful prognostic marker in extrahepatic bile duct carcinoma [26, 29, 39, 50].

3.3 Staging

Disease stage is an important prognostic indicator [37, 39, 45, 70]. For instance, increased depth of invasion (T stage) carries poor outcome [43]; the frequency of lymph node metastasis increases with increased tumor depth [42, 79]. In addition,

Fig. 5 Gross features of perihilar bile duct carcinoma. Perihilar carcinoma characterized by diffuse thickening of the extrahepatic bile ducts

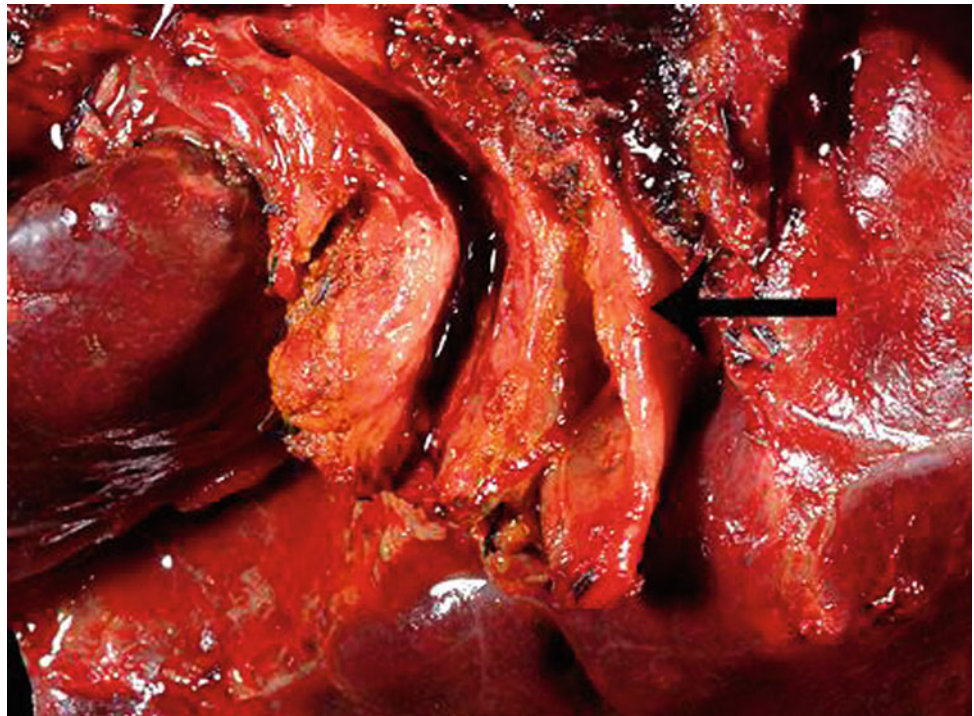
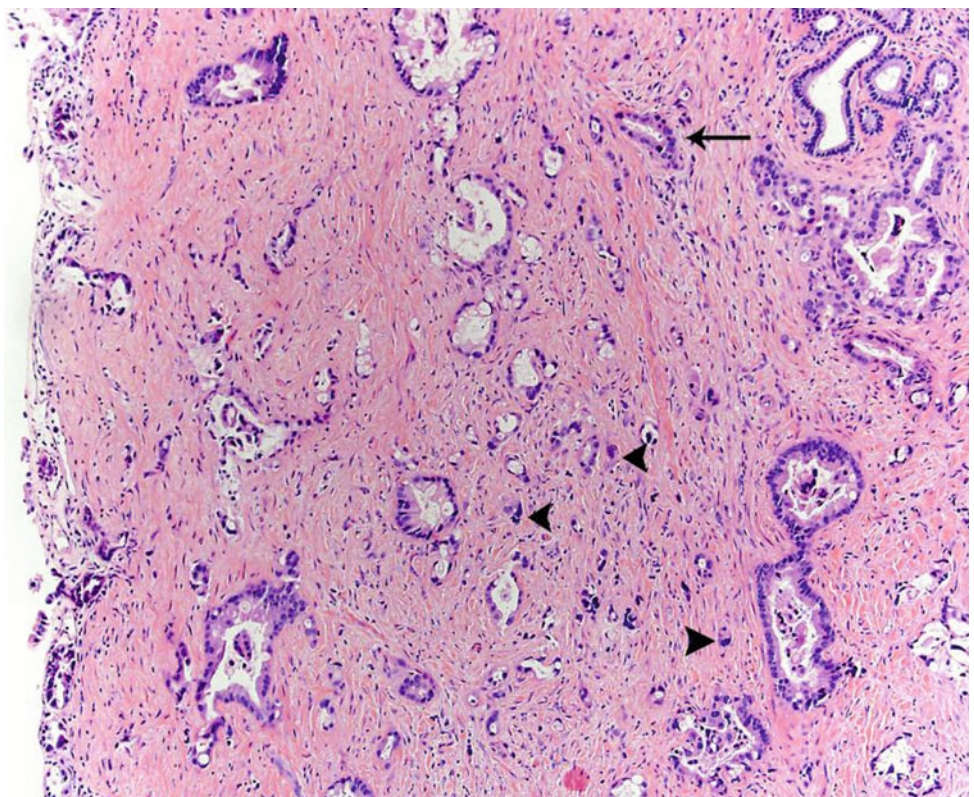


Fig. 6 Histologic features of bile duct carcinoma. Invasive adenocarcinoma is characterized by infiltrative growth of variably sized and irregularly shaped glands (arrow) as well as single cells (arrowheads), with desmoplastic response



gross (not microscopic) portal vein invasion by perihilar carcinoma is independently associated with poor prognosis [29]. Hence, invasion of the portal vein and regional lymph node metastasis should be weighted equally [77] (both stages

III–IV in perihilar bile duct carcinomas). Extrahepatic bile duct carcinomas invade through the thin duct wall early and hence spread early by direct extension into adjacent structures, including the portal vein, hepatic artery, perihilar liver

parenchyma, and pancreas. Distal tumors can also involve the colon, duodenum, and rarely the stomach.

It is important to note that different T and N criteria now exist for staging perihilar and distal extrahepatic bile ducts. Tables 3, 4, 5, and 6 outline the current TNM and overall staging of perihilar and distal extrahepatic bile ducts, respectively. Perihilar tumors are defined as those involving the hepatic duct bifurcation or extrahepatic ducts proximal to the origin of the cystic duct, whereas distal tumors are defined as those involving the biliary system between the cystic duct–common bile duct junction and the ampulla of Vater. The distal bile duct staging system also applies to choledochal cysts. In contrast to gallbladder cancer staging, only T1 lesions are confined to the bile duct, whereas T2 lesions invade beyond the wall of the bile duct and can involve the liver in the perihilar location. To differentiate between T1 and T2 tumors, definition of the boundaries of the bile duct wall is important. T1 lesions are best defined as tumors within the outermost part of the muscle layer or fibrous tissue, whereas T2 tumors involve the area of large clusters of adipose tissue and beyond [45]. Despite their relatively small size, most bile duct tumors have usually spread to adjacent organs and many have spread to regional lymph nodes by the time of diagnosis. Therefore, early (T1) extrahepatic bile duct carcinomas are uncommon (10 % of resected specimens). They are frequently asymptomatic and most show intraductal-growth, with approximately one-third representing papillary carcinoma [17]. In perihilar tumors, unilateral vascular (portal vein or hepatic artery) involvement is now designated T3, whereas bilateral involvement of vascular structures, bilateral tumor expansion into secondary biliary radicals, or extension into secondary biliary radicals with contralateral vascular invasion is designated T4. Invasion of adjacent structures besides the celiac axis or superior mesenteric artery is classified as T3 tumors in the distal extrahepatic bile ducts. In these T3 lesions, deep pancreatic invasion (more than 1 mm) is a significant and independent determinant of patient prognosis [44].

Lymph nodes metastasis, a frequent (30–70 % of tumors) event [2, 48, 87], is a significant indicator of poor survival [12, 26, 29, 33, 37, 42, 45, 54, 70, 90, 109] and predictor of liver metastases [87]. Topographic location of lymph node metastasis determines N stage (N1 vs. N2) in perihilar bile duct carcinoma (similar lymph node groups to gallbladder carcinoma), whereas any regional lymph node metastasis in distal bile duct cancer is designated N1 (N2 does not exist). In addition to the topographic location [12], the number of positive lymph nodes is also an important prognostic factor, which should be reported by the surgical pathologist [42, 70, 87, 109]. In pancreaticoduodenectomy specimens, a minimum of 12 lymph nodes submitted for histologic examination should be sought. Distant metastases occur in 30–70 % of extrahepatic bile duct carcinomas.

Table 3 TNM staging of perihilar bile duct carcinoma [30]

<i>Primary tumor (T)</i>	
TX	Cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor confined to the bile duct, with extension up to the muscle layer or fibrous tissue
T2a	Tumor invades beyond the wall of the bile duct to surroundings adipose tissue
T2b	Tumor invades adjacent hepatic parenchyma
T3	Tumor invades unilateral branches of the portal vein or hepatic artery
T4	Tumor invades main portal vein or its branches bilaterally; or the common hepatic artery; or the second-order biliary radicals bilaterally; or unilateral second-order biliary radicals with contralateral portal vein or hepatic artery involvement
<i>Regional lymph nodes (N)</i>	
NX	Cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis (including nodes along the cystic duct, common bile duct, hepatic artery, and portal vein)
N2	Metastasis to periaortic, pericaval, superior mesentery artery, and/or celiac artery lymph nodes
<i>Distant metastasis (M)</i>	
M0	No distant metastasis
M1	Distant metastasis

Table 4 Overall stages of perihilar bile duct carcinoma [30]

Overall pathologic stage			
Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage II	T2a-b	N0	M0
Stage IIIA	T3	N0	M0
Stage IIIB	T1-3	N1	M0
Stage IVA	T4	N0–1	M0
Stage IVB	Any T	N2	M0
	Any T	Any N	M1

Liver involvement by metastasis and direct invasion (in perihilar tumors) is common, but other distant organ involvement is uncommon [48].

3.4 Other Important Pathologic Features

Perineural invasion occurs in 75–80 % of extrahepatic bile duct carcinomas. This feature should be reported as it has a significant negative impact on patient outcome [37, 70, 90, 106]. Likewise, angiolymphatic invasion is a significant

Table 5 TNM staging of distal bile duct carcinoma [30]

Primary tumor (T)	
TX	Cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor confined to the bile duct histologically
T2	Tumor invades beyond the wall of the bile duct
T3	Tumor invades the gallbladder, pancreas, duodenum, or other adjacent organs without involvement of the celiac axis or the superior mesenteric artery
T4	Tumor involves the celiac axis or the superior mesenteric artery
Regional lymph nodes (N)	
NX	Cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis
Distant metastasis (M)	
M0	No distant metastasis
M1	Distant metastasis

Table 6 Overall stages of distal bile duct carcinoma [30]

Overall pathologic stage			
Stage 0	Tis	N0	M0
Stage IA	T1	N0	M0
Stage IB	T2	N0	M0
Stage IIA	T3	N0	M0
Stage IIB	T1	N1	M0
	T2	N1	M0
	T3	N1	M0
Stage III	T4	Any N	M0
Stage IV	Any T	Any N	M1

prognostic factor [106] and shows strong correlation with lymph node metastasis [37, 42]. Margin status is also a significant and independent prognostic factor in extrahepatic bile duct carcinoma [12, 26, 27, 33, 37, 38, 48–50, 70, 72, 74, 106]; a margin of 5 mm is associated with improved long-term survival [90].

4 Ampulla of Vater

4.1 Normal Anatomy and Predisposing Conditions

The Vaterian system is located in the wall of the duodenum, at the confluence of the common bile duct and the pancreatic duct, and also includes the primary duodenal papilla, the ampulla of Vater (when present), the sphincter of Oddi

(sphincter muscle surrounding the ducts and ampulla, and controlling bile flow), and fibrous coverings. The common bile duct and the pancreatic ducts usually open into the tip of the papilla, but variations in the connections between the ducts exist; they may open separately, form a common channel (most common variation) measuring 1–12 mm (the pancreaticobiliary duct), or have an intervening septum. When there is the pancreaticobiliary duct, the ampulla of Vater may form. Histologically, the epithelial lining of the pancreatic and common bile duct becomes papillary with long narrow fronds projecting into the lumen near the papilla and then abruptly transitions from the biliary type to small intestinal type epithelium, which covers the papilla (Fig. 7). Multiple mucin-secreting glands are present in the Vaterian fibrovascular connective tissue and empty into the terminal ducts and ampulla.

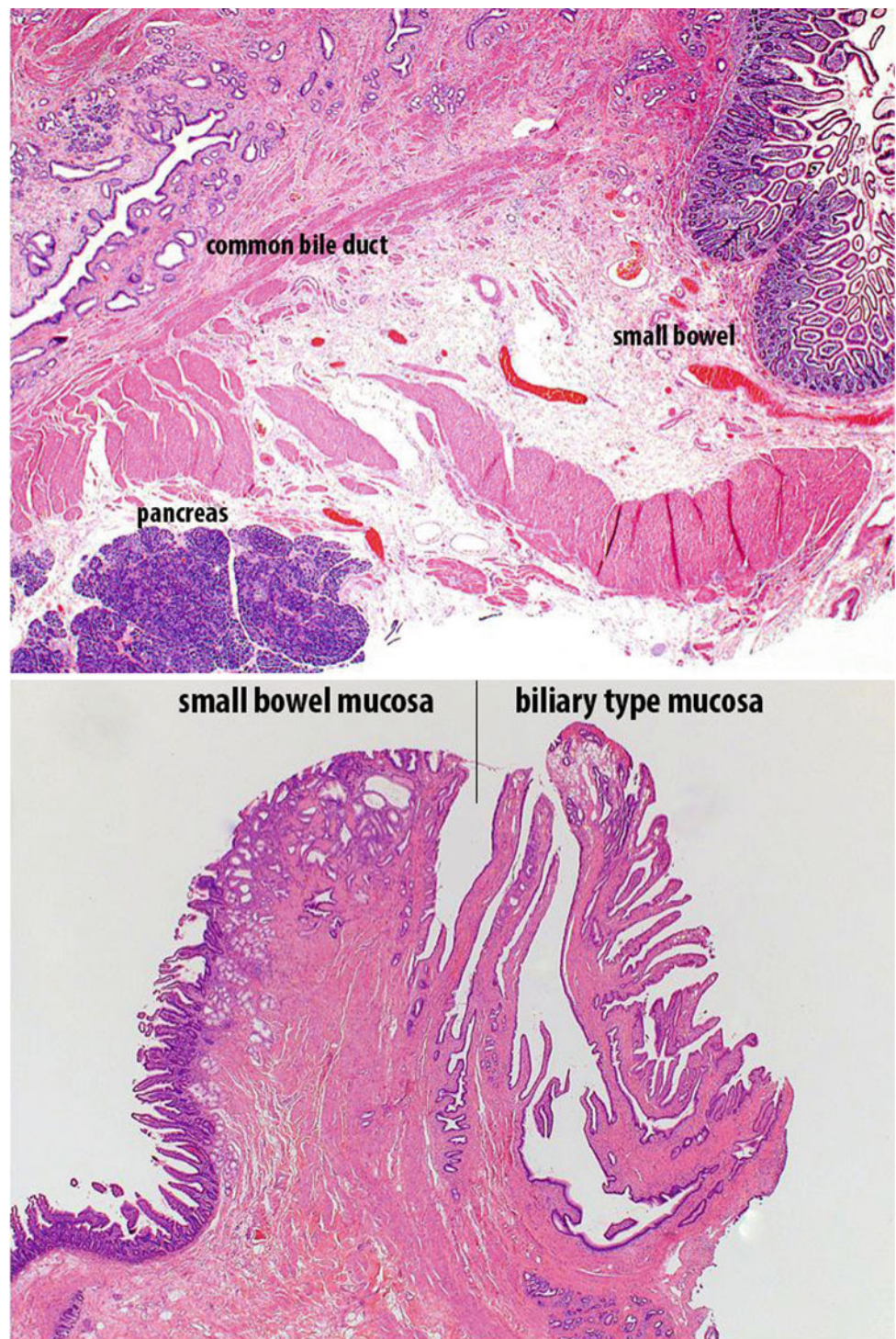
Most (35–90 %) ampullary carcinomas arise in an adenoma, a premalignant lesion that carries a 26 % risk of malignant transformation [2, 8, 13, 61]. Progression from adenoma to carcinoma usually appears to take many years [2]. Patients with FAP are at risk of ampullary carcinoma; 50 % have adenomatous polyps of the papilla. Therefore, screening these patients by upper GI endoscopy is advised [6]. Although neurofibromas and carcinoids are the two commonest ampullary tumors in patients with von Recklinghausen's disease, risk of ampullary adenocarcinoma is also increased in these patients [59].

4.2 Gross and Microscopic Features

Because the common bile duct and pancreatic duct come together in the peri-ampullary region, it is virtually impossible to localize the precise origin of tumors after they have invaded adjacent structures. Hence, the larger the tumor is, the more difficult it becomes to determine its precise site of origin. Nevertheless, it is important to separate primary duodenal and distal common bile duct carcinomas from ampullary carcinomas, though sometimes this can be difficult. This distinction can be best accomplished by a thorough gross evaluation, which can usually determine where the epicenter of the tumor is. Distinction between ampullary and pancreatic carcinoma is critical because ampullary carcinoma has significantly better survival rates than pancreatic carcinoma. Distinction between ampullary and duodenal carcinoma also has implications for staging; pancreatic involvement in duodenal carcinoma is considered T4, whereas pancreatic involvement in ampullary carcinoma is considered T3.

Because of their location, ampullary carcinomas often produce symptoms and are detected early and are therefore often small (median size is 2 cm and most smaller than 4 cm) [103]. Tumor size is a significant predictor of tumor

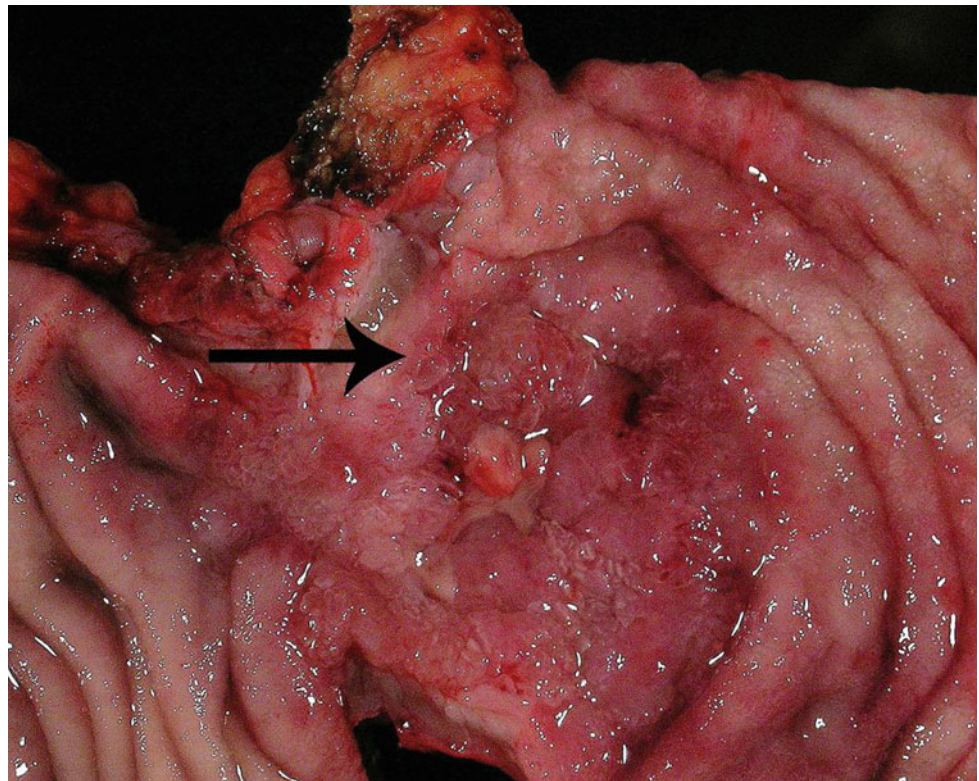
Fig. 7 Normal histology of the ampulla of Vater. A histologic section from the ampullary region shows confluence of small bowel mucosa and common bile duct wall; the pancreas is also present in this section (*top*). The bile duct epithelial lining becomes papillary with long narrow fronds projecting into the lumen near the papilla and then abruptly transitions from biliary type to small intestinal type epithelium (*bottom*)



recurrence and patient survival [1, 15, 46]. Both Cubilla and Fitzgerald [22] and Tasaka [95] have developed macroscopic classifications for ampullary carcinoma. The Armed Forces Institute of Pathology (AFIP), however, proposed a macroscopic classification that combines both schemes and separates ampullary carcinomas into four groups [2]:

1. Intra-ampullary (24 %): largely confined to the ampulla, and therefore usually small (less than 2 cm). This type carries the best prognosis [46].
2. Peri-ampullary duodenal (6 %): centered in the peri-ampullary duodenal mucosa and luminal surface of papilla.

Fig. 8 Gross features of ampullary carcinoma. Ampullary carcinoma (*arrow*) forming an ulcerated mass (mixed ulcerated type)



3. Mixed exophytic (31 %): large fungating polypoid masses that occupy the peri-ampullary area and compromise the mucosa of both the duodenum and papilla.
4. Mixed ulcerated (39 %): highly infiltrative tumors that carry the worst prognosis [103] (Fig. 8).

Microscopically, more than 90 % of ampullary carcinomas are adenocarcinomas (Fig. 9), and most (50–85 %) of these are intestinal type (often mixed exophytic) [8, 57, 94, 103]. Pancreaticobiliary-type (often intra-ampullary) adenocarcinomas constitute 22 % of ampullary adenocarcinomas [18, 103]. Intestinal type tumors have a somewhat better prognosis than pancreaticobiliary type and less often display perineural invasion [2]. Noninvasive papillary carcinomas are typically exophytic intra-ampullary. Microscopically, they resemble their non-intestinal pancreaticobiliary-type counterpart in the pancreas. A thorough search for invasion should be performed, as it can sometimes be very focal. If invasion is present, the invasive component should be designated separately (usually intestinal type) [2]. On the other hand, invasive papillary adenocarcinoma (less than 10 % of ampullary carcinoma) characterized by invasive growth of branching papillary structures does not carry the favorable outcome of noninvasive/minimally invasive papillary carcinoma [2]. Mucinous (colloid) carcinoma constitutes less than 10 % of ampullary adenocarcinomas and has a somewhat unfavorable prognosis [2, 89, 104]. Diffusely infiltrating signet ring cell adenocarcinoma is rare in the ampulla and is usually of mixed ulcerated type. Ampullary clear cell

carcinoma, squamous cell carcinomas, undifferentiated carcinoma, small cell carcinoma, and large cell neuroendocrine carcinoma are very rare [2]. The latter two are highly aggressive [2]. Carcinoid tumors can occur rarely in the ampullary region and are clinically and pathologically distinct from duodenal carcinoids [63]. The AJCC staging system does not apply to these tumors [36]. Grading of adenocarcinoma in the ampulla is similar to that in gallbladder, but there is conflicting data on the value of histologic grade as an independent prognostic factor [1, 2, 14, 15, 55, 73].

4.3 Staging

T stage and overall tumor stage show strong correlation with patient survival [1, 14, 15, 21, 55]. Tables 7 and 8 outline the current TNM and overall staging of ampullary carcinoma. Tumors limited to the ampulla are designated T1, but tumors invading the duodenal wall or pancreas are designated T2 and T3, respectively. Involvement of other adjacent structure or extension beyond the pancreas is considered T4. Regional lymph node metastasis is an established independent prognostic factor in ampullary carcinoma [1, 10, 13, 55, 83, 91] and is the sole predictor for liver metastases [46]. It is detected in 30–55 % of ampullary carcinomas at resection time [2]. The regional lymph nodes are subdivided into superior (superior to head

Fig. 9 Histologic features of ampullary carcinoma. Invasive adenocarcinoma characterized by complex infiltrating glandular growth invading the wall of the duodenum

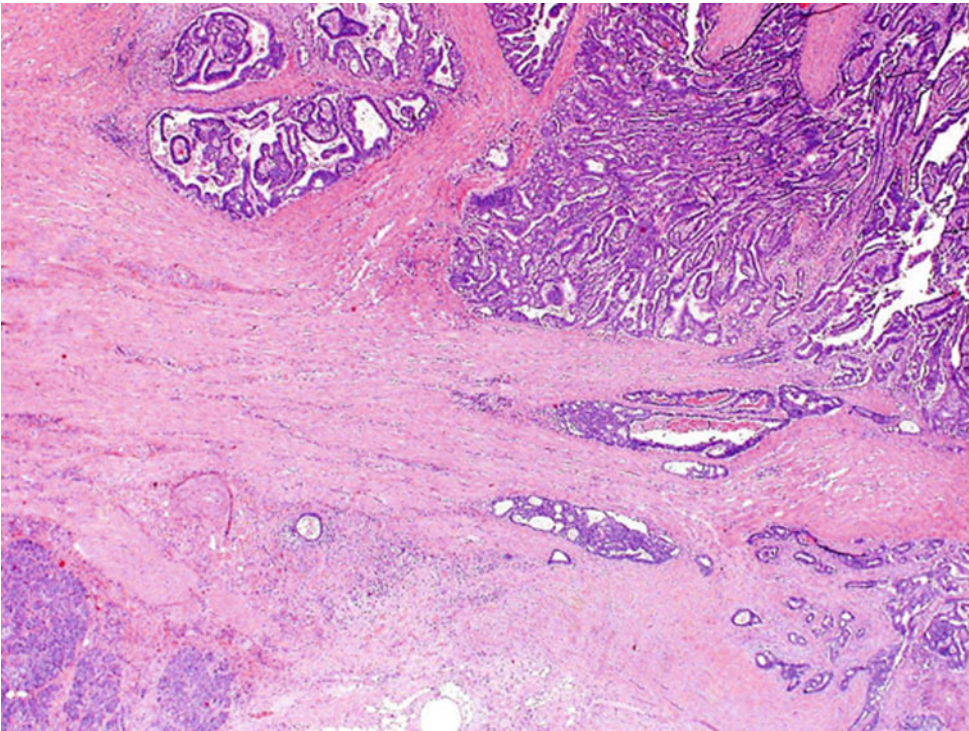


Table 7 TNM staging of ampullary carcinoma [30]

Primary tumor (T)	
TX	Cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor limited to ampulla of Vater or sphincter of Oddi
T2	Tumor invades duodenal wall
T3	Tumor invades pancreas
T4	Tumor invades peripancreatic soft tissues or other adjacent organs or structures
Regional lymph nodes (N)	
NX	Cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis
Distant metastasis (M)	
M0	No distant metastasis
M1	Distant metastasis

and body of pancreas), inferior (inferior to head and body of pancreas), anterior (anterior pancreaticoduodenal, pyloric, and proximal mesenteric), and posterior (posterior pancreaticoduodenal, pericholedochal, and proximal mesenteric). Hepatic artery, intrapyloric, subpyloric, celiac, superior mesenteric, retroperitoneal, and lateral aortic lymph nodes are also considered regional. Metastasis to lymph nodes outside this regional group is considered distant metastasis.

Table 8 Overall stages of ampullary carcinoma [30]

Overall pathologic stage			
Stage 0	Tis	N0	M0
Stage IA	T1	N0	M0
Stage IB	T2	N0	M0
Stage IIA	T3	N0	M0
Stage IIB	T1	N1	M0
	T2	N1	M0
	T3	N1	M0
Stage III	T4	Any N	M0
Stage IV	Any T	Any N	M1

In addition to the topographic location, the number of positive lymph nodes is an independent prognostic factor [85]. Although a minimum number of lymph node sampling has not been determined for optimal staging, it is recommended to obtain at least 10–12 lymph nodes for microscopic examination in pancreaticoduodenectomy specimens. Distant metastases most frequently involve the liver, followed by peritoneum, lungs, and pleura.

4.4 Other Important Pathologic Features

Perineural invasion is a significant but not an independent predictor of poor patient outcome [14, 15]. Angiolymphatic

invasion is seen in 35–80 % of carcinomas and is considered a significant poor prognostic indicator, but the data is conflicting on its value as an independent predictor of survival [15, 98]. A negative surgical margin is a significant but not independent predictor of good prognosis [1, 13, 15].

References

- Abrams RA, Yeo CJ (2004) Combined modality adjuvant therapy for resected periampullary pancreatic and nonpancreatic adenocarcinoma: a review of studies and experience at The Johns Hopkins Hospital, 1991–2003. *Surg Oncol Clin North Am* 13:621–638, ix
- Albores-Saavedra J, Henson DE, Armed Forces Institute of Pathology (U.S.) (2000) Tumors of the gallbladder, extrahepatic bile ducts and ampulla of Vater. Armed Forces Institute of Pathology: Supt. of Docs., U.S. G.P.O. For sale by the Armed Forces Institute of Pathology, Washington, D.C
- Albores-Saavedra J, Keenportz B, Bejarano PA et al (2007) Adenomatous hyperplasia of the gallbladder with perineural invasion: revisited. *Am J Surg Pathol* 31:1598–1604
- Albores-Saavedra J, Molberg K, Henson DE (1996) Unusual malignant epithelial tumors of the gallbladder. *Semin Diagn Pathol* 13:326–338
- Albores-Saavedra J, Nadji M, Henson DE (1986) Intestinal-type adenocarcinoma of the gallbladder. A clinicopathologic study of seven cases. *Am J Surg Pathol* 10:19–25
- Alexander JR, Andrews JM, Buchi KN et al (1989) High prevalence of adenomatous polyps of the duodenal papilla in familial adenomatous polyposis. *Dig Dis Sci* 34:167–170
- Aramaki M, Matsumoto T, Shibata K et al (2004) Factors influencing recurrence after surgical treatment for T2 gallbladder carcinoma. *Hepatogastroenterology* 51:1609–1611
- Baczako K, Buchler M, Beger HG et al (1985) Morphogenesis and possible precursor lesions of invasive carcinoma of the papilla of Vater: epithelial dysplasia and adenoma. *Hum Pathol* 16:305–310
- Baer HU, Metzger A, Glatzli A et al (1995) Subcutaneous periumbilical metastasis of a gallbladder carcinoma after laparoscopic cholecystectomy. *Surg Laparosc Endosc* 5:59–63
- Bakkevold KE, Kambestad B (1995) Staging of carcinoma of the pancreas and ampulla of Vater. Tumor (T), lymph node (N), and distant metastasis (M) as prognostic factors. *Int J Pancreatol: Official J Int Assoc Pancreatol* 17:249–259
- Balachandran P, Agarwal S, Krishnani N et al (2006) Predictors of long-term survival in patients with gallbladder cancer. *J Gastroint Surg: Official J Soc Surg Aliment Tract* 10:848–854
- Baton O, Azoulay D, Adam DV et al (2007) Major hepatectomy for hilar cholangiocarcinoma type 3 and 4: prognostic factors and longterm outcomes. *J Am Coll Surg* 204:250–260
- Beger HG, Treitschke F, Gansauge F et al (1999) Tumor of the ampulla of Vater: experience with local or radical resection in 171 consecutively treated patients. *Arch Surg* 134:526–532
- Bettschart V, Rahman MQ, Engelken FJ et al (2004) Presentation, treatment and outcome in patients with ampullary tumours. *Br J Surg* 91:1600–1607
- Bouvet M, Gamagami RA, Gilpin EA et al (2000) Factors influencing survival after resection for periampullary neoplasms. *Am J Surg* 180:13–17
- Caruso RA, Famulari C, Giuffre G et al (1991) Pleomorphic carcinoma of the gallbladder: report of a case. *Tumori* 77:523–526
- Cha JM, Kim MH, Lee SK et al (2006) Clinicopathological review of 61 patients with early bile duct cancer. *Clin Oncol (R Coll Radiol)* 18:669–677
- Chareton B, Coiffic J, Landen S et al (1996) Diagnosis and therapy for ampullary tumors: 63 cases. *World J Surg* 20:707–712
- Chen MF, Jan YY, Wang CS et al (1989) Intrahepatic stones associated with cholangiocarcinoma. *Am J Gastroenterol* 84:391–395
- Chijiwa K, Yamaguchi K, Tanaka M (1997) Clinicopathologic differences between long-term and short-term postoperative survivors with advanced gallbladder carcinoma. *World J Surg* 21:98–102
- Compton CC (1997) Protocol for the examination of specimens from patients with carcinoma of the ampulla of Vater: a basis for checklists. Cancer Committee, College of American Pathologists. *Arch Pathol Lab Med* 121:673–677
- Cubilla AL, Fitzgerald PJ (1980) Surgical pathology aspects of cancer of the ampulla-head-of-pancreas region. *Monogr Pathol* 21:67–81
- Darabos N, Stare R (2004) Gallbladder cancer: laparoscopic and classic cholecystectomy. *Surg Endosc* 18:144–147
- De Aretxabala X, Riedeman P, Burgos L et al (1995) Gallbladder cancer. Case-control study. *Rev Med Chil* 123:581–586
- De Aretxabala X, Roa I, Burgos L et al (2006) Gallbladder cancer: an analysis of a series of 139 patients with invasion restricted to the subserosal layer. *J Gastrointest Surg: Official J Soc Surg Aliment Tract* 10:186–192
- Deoliveira ML, Cunningham SC, Cameron JL et al (2007) Cholangiocarcinoma: thirty-one-year experience with 564 patients at a single institution. *Ann Surg* 245:755–762
- Dixon E, Vollmer CM Jr, Sahajpal A et al (2005) An aggressive surgical approach leads to improved survival in patients with gallbladder cancer: a 12-year study at a North American Center. *Ann Surg* 241:385–394
- Donohue JH, Nagorney DM, Grant CS et al (1990) Carcinoma of the gallbladder. Does radical resection improve outcome? *Arch Surg* 125:237–241
- Ebata T, Nagino M, Kamiya J et al (2003) Hepatectomy with portal vein resection for hilar cholangiocarcinoma: audit of 52 consecutive cases. *Ann Surg* 238:720–727
- Edge SB, American Joint Committee on Cancer (2010) *AJCC cancer staging manual*. Springer, New York
- Endo I, Shimada H, Tanabe M et al (2006) Prognostic significance of the number of positive lymph nodes in gallbladder cancer. *J Gastrointest Surg: Official J Soc Surg Aliment Tract* 10:999–1007
- Flanigan DP (1977) Biliary carcinoma associated with biliary cysts. *Cancer* 40:880–883
- Fong Y, Blumgart LH, Lin E et al (1996) Outcome of treatment for distal bile duct cancer. *Br J Surg* 83:1712–1715
- Fong Y, Brennan MF, Turnbull A et al (1993) Gallbladder cancer discovered during laparoscopic surgery. Potential for iatrogenic tumor dissemination. *Arch Surg* 128:1054–1056
- Giulante F, Ardito F, Vellone M et al (2006) Port-sites excision for gallbladder cancer incidentally found after laparoscopic cholecystectomy. *Am J Surg* 191:114–116
- Hartel M, Wente MN, Sido B et al (2005) Carcinoid of the ampulla of Vater. *J Gastroenterol Hepatol* 20:676–681
- He P, Shi JS, Chen WK et al (2002) Multivariate statistical analysis of clinicopathologic factors influencing survival of patients with bile duct carcinoma. *World J Gastroenterol: WJG* 8:943–946
- Hemming AW, Reed AI, Fujita S et al (2005) Surgical management of hilar cholangiocarcinoma. *Ann Surg* 241:693–699; discussion 699–702

39. Henson DE, Albores-Saavedra J, Corle D (1992) Carcinoma of the extrahepatic bile ducts. Histologic types, stage of disease, grade, and survival rates. *Cancer* 70:1498–1501
40. Henson DE, Albores-Saavedra J, Corle D (1992) Carcinoma of the gallbladder. Histologic types, stage of disease, grade, and survival rates. *Cancer* 70:1493–1497
41. Hisatomi K, Haratake J, Horie A et al (1990) Relation of histopathological features to prognosis of gallbladder cancer. *Am J Gastroenterol* 85:567–572
42. Hong SM, Cho H, Lee OJ et al (2005) The number of metastatic lymph nodes in extrahepatic bile duct carcinoma as a prognostic factor. *Am J Surg Pathol* 29:1177–1183
43. Hong SM, Cho H, Moskaluk CA et al (2007) Measurement of the invasion depth of extrahepatic bile duct carcinoma: an alternative method overcoming the current T classification problems of the AJCC staging system. *Am J Surg Pathol* 31:199–206
44. Hong SM, Kim MJ, Cho H et al (2005) Superficial vs deep pancreatic parenchymal invasion in the extrahepatic bile duct carcinomas: a significant prognostic factor. *Mod Pathol: Official J US Can Acad Pathol, Inc* 18:969–975
45. Hong SM, Kim MJ, Pi DY et al (2005) Analysis of extrahepatic bile duct carcinomas according to the New American Joint Committee on Cancer staging system focused on tumor classification problems in 222 patients. *Cancer* 104:802–810
46. Hsu HP, Yang TM, Hsieh YH et al (2007) Predictors for patterns of failure after pancreaticoduodenectomy in ampullary cancer. *Ann Surg Oncol* 14:50–60
47. Ikoma A, Nakamura N, Miyazaki T et al (1992) Double cancer of the gallbladder and common bile duct associated with anomalous junction of pancreaticobiliary ductal system. *Surgery* 111:595–600
48. Jarnagin WR (2000) Cholangiocarcinoma of the extrahepatic bile ducts. *Semin Surg Oncol* 19:156–176
49. Jarnagin WR, Bowne W, Klimstra DS et al (2005) Papillary phenotype confers improved survival after resection of hilar cholangiocarcinoma. *Ann Surg* 241:703–712; discussion 712–704
50. Jarnagin WR, Fong Y, Dematteo RP et al (2001) Staging, resectability, and outcome in 225 patients with hilar cholangiocarcinoma. *Ann Surg* 234:507–517; discussion 517–509
51. Kapoor VK, Sonawane RN, Haribhakti SP et al (1998) Gall bladder cancer: proposal for a modification of the TNM classification. *Eur J Surg Oncol: J Eur Soc Surg Oncol Brit Assoc Surg Oncol* 24:487–491
52. Katabi N, Albores-Saavedra J (2003) The extrahepatic bile duct lesions in end-stage primary sclerosing cholangitis. *Am J Surg Pathol* 27:349–355
53. Katz MH, Bouvet M, Al-Refaie W et al (2004) Non-pancreatic periampullary adenocarcinomas: an explanation for favorable prognosis. *Hepatogastroenterology* 51:842–846
54. Kayahara M, Nagakawa T, Ohta T et al (1999) Role of nodal involvement and the periductal soft-tissue margin in middle and distal bile duct cancer. *Ann Surg* 229:76–83
55. Kim RD, Kundhal PS, McGilvray ID et al (2006) Predictors of failure after pancreaticoduodenectomy for ampullary carcinoma. *J Am Coll Surg* 202:112–119
56. Kimura K, Ohto M, Saisho H et al (1985) Association of gallbladder carcinoma and anomalous pancreaticobiliary ductal union. *Gastroenterology* 89:1258–1265
57. Kimura W, Futakawa N, Yamagata S et al (1994) Different clinicopathologic findings in two histologic types of carcinoma of papilla of Vater. *Jpn J Cancer Res* 85:161–166
58. Kimura W, Shimada H, Kuroda A et al (1989) Carcinoma of the gallbladder and extrahepatic bile duct in autopsy cases of the aged, with special reference to its relationship to gallstones. *Am J Gastroenterol* 84:386–390
59. Klein A, Clemens J, Cameron J (1989) Periampullary neoplasms in von Recklinghausen's disease. *Surgery* 106:815–819
60. Koga A, Ichimiya H, Yamaguchi K et al (1985) Hepatolithiasis associated with cholangiocarcinoma. Possible etiologic significance. *Cancer* 55:2826–2829
61. Kozuka S, Tsubone M, Yamaguchi A et al (1981) Adenomatous residue in cancerous papilla of Vater. *Gut* 22:1031–1034
62. Lewis JT, Talwalkar JA, Rosen CB et al (2007) Prevalence and risk factors for gallbladder neoplasia in patients with primary sclerosing cholangitis: evidence for a metaplasia-dysplasia-carcinoma sequence. *Am J Surg Pathol* 31:907–913
63. Makhlof HR, Burke AP, Sobin LH (1999) Carcinoid tumors of the ampulla of Vater: a comparison with duodenal carcinoid tumors. *Cancer* 85:1241–1249
64. Manfredi S, Benhamiche AM, Isambert N et al (2000) Trends in incidence and management of gallbladder carcinoma: a population-based study in France. *Cancer* 89:757–762
65. Maringhini A, Moreau JA, Melton LJ 3rd et al (1987) Gallstones, gallbladder cancer, and other gastrointestinal malignancies. An epidemiologic study in Rochester, Minnesota. *Ann Intern Med* 107:30–35
66. Miller G, Jarnagin WR (2008) Gallbladder carcinoma. *Eur J Surg Oncol: J Eur Soc Surg Oncol Br Assoc Surg Oncol* 34:306–312
67. Mir-Madjlessi SH, Farmer RG, Sivak MV Jr (1987) Bile duct carcinoma in patients with ulcerative colitis. Relationship to sclerosing cholangitis: report of six cases and review of the literature. *Dig Dis Sci* 32:145–154
68. Misra MC, Guleria S (2006) Management of cancer gallbladder found as a surprise on a resected gallbladder specimen. *J Surg Oncol* 93:690–698
69. Morowitz DA, Glagov S, Dordal E et al (1971) Carcinoma of the biliary tract complicating chronic ulcerative colitis. *Cancer* 27:356–361
70. Murakami Y, Uemura K, Hayashidani Y et al (2007) Pancreatoduodenectomy for distal cholangiocarcinoma: prognostic impact of lymph node metastasis. *World J Surg* 31:337–342; discussion 343–334
71. Nakanuma Y, Terada T, Tanaka Y et al (1985) Are hepatolithiasis and cholangiocarcinoma aetiologically related? A morphological study of 12 cases of hepatolithiasis associated with cholangiocarcinoma. *Virchows Arch A, Pathol Anat Histopathol* 406:45–58
72. Nakeeb A, Pitt HA, Sohn TA et al (1996) Cholangiocarcinoma. A spectrum of intrahepatic, perihilar, and distal tumors. *Ann Surg* 224:463–473; discussion 473–465
73. Neoptolemos JP, Talbot IC, Carr-Locke DL et al (1987) Treatment and outcome in 52 consecutive cases of ampullary carcinoma. *Br J Surg* 74:957–961
74. Neuhaus P, Jonas S, Bechstein WO et al (1999) Extended resections for hilar cholangiocarcinoma. *Ann Surg* 230:808–818; discussion 819
75. Nevin JE, Moran TJ, Kay S et al (1976) Carcinoma of the gallbladder: staging, treatment, and prognosis. *Cancer* 37:141–148
76. Nishihara K, Nagai E, Izumi Y et al (1994) Adenosquamous carcinoma of the gallbladder: a clinicopathological, immunohistochemical and flow-cytometric study of twenty cases. *Jpn J Cancer Res: Gann* 85:389–399
77. Nishio H, Nagino M, Oda K et al (2005) TNM classification for perihilar cholangiocarcinoma: comparison between 5th and 6th editions of the AJCC/UICC staging system. *Langenbecks Arch Surg/Dtscher Ges fur Chir* 390:319–327

78. Odze RD, Goldblum JR (2009) *Surgical Pathology of the GI tract, liver, biliary tract and pancreas*. Saunders Elsevier, Philadelphia, PA
79. Ogura Y, Mizumoto R, Tabata M et al (1993) Surgical treatment of carcinoma of the hepatic duct confluence: analysis of 55 resected carcinomas. *World J Surg* 17:85–92; discussion 92–83
80. Paolucci V, Schaeff B, Schneider M et al (1999) Tumor seeding following laparoscopy: international survey. *World J Surg* 23:989–995; discussion 996–987
81. Park JS, Yoon DS, Kim KS et al (2007) Actual recurrence patterns and risk factors influencing recurrence after curative resection with stage II gallbladder carcinoma. *J Gastrointest Surg: Official J Soc Surg Aliment Tract* 11:631–637
82. Polk HC Jr (1966) Carcinoma and the calcified gall bladder. *Gastroenterology* 50:582–585
83. Roder JD, Schneider PM, Stein HJ et al (1995) Number of lymph node metastases is significantly associated with survival in patients with radically resected carcinoma of the ampulla of Vater. *Br J Surg* 82:1693–1696
84. Ron IG, Wigler N, Ilie B et al (1992) Small cell carcinoma of the gallbladder: clinical course and response to chemotherapy. *Tumori* 78:207–210
85. Sakata J, Shirai Y, Wakai T et al (2007) Number of positive lymph nodes independently affects long-term survival after resection in patients with ampullary carcinoma. *Eur J Surg Oncol: J Eur Soc Surg Oncol Br Assoc Surg Oncol* 33:346–351
86. Sasaki E, Nagino M, Ebata T et al (2006) Immunohistochemically demonstrated lymph node micrometastasis and prognosis in patients with gallbladder carcinoma. *Ann Surg* 244:99–105
87. Sasaki R, Takahashi M, Funato O et al (2001) Prognostic significance of lymph node involvement in middle and distal bile duct cancer. *Surgery* 129:677–683
88. Sasaki R, Uesugi N, Itabashi H et al (2005) Clinicopathological study of depth of subserosal invasion in patients with pT2 gallbladder carcinoma. *J Surg Oncol* 92:83–88
89. Seifert E, Schulte F, Stolte M (1992) Adenoma and carcinoma of the duodenum and papilla of Vater: a clinicopathologic study. *Am J Gastroenterol* 87:37–42
90. Seyama Y, Kubota K, Sano K et al (2003) Long-term outcome of extended hemihepatectomy for hilar bile duct cancer with no mortality and high survival rate. *Ann Surg* 238:73–83
91. Shutze WP, Sack J, Aldrete JS (1990) Long-term follow-up of 24 patients undergoing radical resection for ampullary carcinoma, 1953 to 1988. *Cancer* 66:1717–1720
92. Sikora SS, Singh RK (2006) Surgical strategies in patients with gallbladder cancer: nihilism to optimism. *J Surg Oncol* 93:670–681
93. Stephen AE, Berger DL (2001) Carcinoma in the porcelain gallbladder: a relationship revisited. *Surgery* 129:699–703
94. Talbot IC, Neoptolemos JP, Shaw DE et al (1988) The histopathology and staging of carcinoma of the ampulla of Vater. *Histopathology* 12:155–165
95. Tasaka K (1977) Carcinoma in the region of the duodenal papilla. A histopathologic study (author's transl). *Fukuoka igaku zasshi = Hukuoka acta medica* 68:20–44
96. Thierfelder S, Hirche H, Benndorf K (1994) Anoxia decreases the transient K⁺ outward current in isolated ventricular heart cells of the mouse. *Pflugers Arch* 427:547–549
97. Todani T, Tabuchi K, Watanabe Y et al (1979) Carcinoma arising in the wall of congenital bile duct cysts. *Cancer* 44:1134–1141
98. Todoroki T, Koike N, Morishita Y et al (2003) Patterns and predictors of failure after curative resections of carcinoma of the ampulla of Vater. *Ann Surg Oncol* 10:1176–1183
99. Todoroki T, Okamura T, Fukao K et al (1980) Gross appearance of carcinoma of the main hepatic duct and its prognosis. *Surg, Gynecol Obstet* 150:33–40
100. Towfigh S, Mcfadden DW, Cortina GR et al (2001) Porcelain gallbladder is not associated with gallbladder carcinoma. *Am Surg* 67:7–10
101. Vardaman C, Albores-Saavedra J (1995) Clear cell carcinomas of the gallbladder and extrahepatic bile ducts. *Am J Surg Pathol* 19:91–99
102. Weinbren K, Mutum SS (1983) Pathological aspects of cholangiocarcinoma. *J Pathol* 139:217–238
103. Williams JA, Cubilla A, Maclean BJ et al (1979) Twenty-two year experience with perampullary carcinoma at Memorial Sloan-Kettering Cancer Center. *Am J Surg* 138:662–665
104. Wise L, Pizzimbono C, Dehner LP (1976) Perampullary cancer. A clinicopathologic study of sixty-two patients. *Am J Surg* 131:141–148
105. Yamaguchi K, Chijiwa K, Saiki S et al (1997) Retrospective analysis of 70 operations for gallbladder carcinoma. *Br J Surg* 84:200–204
106. Yamaguchi K, Chijiwa K, Saiki S et al (1997) Carcinoma of the extrahepatic bile duct: mode of spread and its prognostic implications. *Hepatogastroenterology* 44:1256–1261
107. Yamaguchi R, Nagino M, Oda K et al (2002) Perineural invasion has a negative impact on survival of patients with gallbladder carcinoma. *Br J Surg* 89:1130–1136
108. Yamauchi S, Koga A, Matsumoto S et al (1987) Anomalous junction of pancreaticobiliary duct without congenital choledochal cyst: a possible risk factor for gallbladder cancer. *Am J Gastroenterol* 82:20–24
109. Yoshida T, Matsumoto T, Sasaki A et al (2002) Prognostic factors after pancreaticoduodenectomy with extended lymphadenectomy for distal bile duct cancer. *Arch Surg* 137:69–73

Molecular Profiling

Domenico Alvaro and Vincenzo Cardinale

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Abstract

Molecular studies concerning cholangiocarcinoma (CCA) or gallbladder cancer are only at the beginning, and the epidemiologic, biologic, and pathological heterogeneity of these cancers constitutes a challenge for the future. Recent studies, in fact, highlighted how CCA is composed of different clinical–pathological subtypes with different cells of origin, pathogenesis, and risk factors. In this chapter, we discuss recent studies regarding the molecular profiling of CCA and gallbladder cancer, which aimed to clarify tumor etiopathogenesis, support diagnosis, and target treatments. Published studies have been critically analyzed taking into consideration the geographic and racial variability, and the pathologic features of the CCA.

1 Introduction

Cholangiocarcinoma (CCA) is a malignant tumor that arises in the biliary tree from the neoplastic proliferation of cholangiocytes, the epithelial cells lining bile ducts. According to current classifications, CCA is divided into intrahepatic (IH-CCA) and extrahepatic (EH-CCA), the latter comprising the perihilar and distal forms [1–3]. Neither gallbladder cancer nor ampullary cancer are considered part of the CCA classification. CCA is characterized by a desmoplastic nature, scarce cellularity, a pleiotropic marker expression, and frequent neuroendocrine differentiation [4–6]. A progressive increase in CCA worldwide incidence and mortality has been described [7, 8]. However, epidemiologic data are biased by a number of pitfalls including the absence of specific markers or specific radiologic features, the biologic and histologic heterogeneity, and, mainly, the lack of uniform classification [7, 8]. CCA still represents a challenge for clinicians at both the diagnostic and therapeutic levels [9].

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So far, basic science studies on CCA have been limited with scarce translation into the clinical setting, and this is particularly true for diagnostic and prognostic biomarkers [4, 10–13]. Recently, using a molecular approach, CCA has been demonstrated to represent the predominant cause of distant metastases when the primary malignancy is unknown, thus confirming a general belief among clinicians and oncologists [14]. This is a further demonstration of how basic science studies may impact general practice, and of the importance of promoting such studies.

Molecular profiling is the classification of pathological tissues for diagnostic or prognostic purposes based on multiple gene expression and is currently utilized to clarify tumor etiopathogenesis or to support diagnosis and targeted treatment [15, 16]. However, the use of these tests for clinical decisions presents many challenges since assay development and data analysis are strongly affected by a number of variables. Frequently, the performance of a certain assay is emphasized in basic studies, while the absolute sensitivity and specificity remain modest when tested in validation studies. With the exception of breast cancer, the real usefulness of molecular profiling is so far limited, especially in terms of cost-effectiveness [16]. Nevertheless, the potential of molecular technology deserves attention in the near future, and this is particularly relevant in the setting of cancer, where the etiopathogenesis is extremely complex. In CCA, molecular studies are only at the beginning, and this is further complicated by the epidemiologic, biologic, and pathological heterogeneity of this cancer. In addition, the availability of good quality CCA samples is mandatory for clinicopathological or basic science studies, but, unfortunately, the desmoplastic nature and the anatomical location make sampling very difficult in most cases.

2 Molecular Profiling and the Origin of Cholangiocarcinoma

Identification of key genetic and epigenetic signatures could aid the identification of biomarkers for diagnosis, screening, surveillance of CCA in categories at risk, and, finally, the development of potential therapeutic strategies [10–12]. In addition, these studies could provide insights into the mechanisms underlying neoplastic transformation of cholangiocytes. However, enormous geographic and racial differences exist with CCA [8]. As far as risk factors are concerned, for example, liver flukes represent the main risk factor in east countries, while hepatitis viruses and primary sclerosing cholangitis (PSC) represent main risk factors in western countries [8], but, in the majority of CCA cases, no risk factor is found [17]. This implies that molecular studies performed in a certain population are not always globally applicable.

Chronic inflammation is considered the background, which favors the emergence of the majority of primitive liver cancers, and this is even truer for CCA [4, 11, 17]. Indeed, all the putative risk factors so far identified for CCA share, as a common variable, the chronic inflammation of bile ducts [11]. However, only 40–50 % of CCA emerges in the setting of chronic liver disease or parasitic infestation; the remaining CCA cases emerge in the absence of an evident chronic liver disease [8, 11, 17]. To explain this variability, two models have been proposed for liver carcinogenesis [17]. According to the so-called clonal evolution model, sequential genetic and epigenetic changes in a cell in the setting of chronic inflammatory stimuli determine a multistep process of tumor development from precancerous lesions to metastatic carcinoma [17]. The alternative model contemplates the involvement of individual genetic and environmental factors [17].

Since all known CCA risk factors are associated with chronic bile duct inflammation, it is conceivable that molecular studies have focused on genetic/epigenetic abnormalities involving inflammation-related genes other than genes involved in the control of DNA repair, cell cycle, apoptosis, and proliferation [10–12, 17].

P53 is a pivotal cell cycle regulator at the G1/S regulation checkpoint, but it is also involved in controlling DNA repair and apoptosis [10, 11]. Nault and Zucman-Rossi observed that substitutions, insertions, or deletions associated with loss of heterozygosity (LOH) may occur in biliary tract cancers [10]. However, differences in *P53* mutations have not been reported when IH-, EH-CCAs (Table 1) and gallbladder cancer are compared [18]. Studies concerning *P53* in CCA highly reflect the complexity and heterogeneity of this cancer at molecular level and further sustain the relevance of the two models of carcinogenesis. Indeed, over 90 different types of *P53* mutations have been described in CCA [18]. As reported in Table 1, a total number of 330 CCA patients have been investigated by sequencing studies [18–31]. Studies from Europe, America, and Asia showed a 34 % (112/330 patients) overall percentage of *P53* mutations [18–31]. Overall, the most commonly reported type of mutation in CCA interests CpG sites. Mutation pattern showed G:C>A:T at CpG sites in 29.3 % of CCAs [18]. Interestingly, alkylating agents, such as N-nitroso compounds, tend to induce G:C–A:T transitions in *P53* via the formation of O-6-methylguanine. In northeast Thailand, the traditional habit of eating nitrosamine- and liver fluke-contaminated foods exposes the population to a synergistic effect of chemical carcinogens and liver fluke infection (*Opisthorchis viverrini*). Nitrosamines are assumed to act as genotoxicants, while liver flukes are assumed to play epigenetic role in CCA development in this exposed population. Consistently, Kamikawa et al. [19] found that mutational spectra are highly correlated with each carcinogen. A lower overall percentage

Table 1 *P53* mutations in human cholangiocarcinoma: sequencing studies

References	Country	No. of patients	CCA site	Overall number of patients with <i>P53</i> mutations (%)	Notes
Jonas et al. [23]	Germany	12	Perihilar	2 (16.6)	<i>P53</i> exons 5–8 evaluated
Sturm et al. [22]	USA	27	Perihilar	7 (26.31)	<i>P53</i> exons 5–8 evaluated
Petmitr et al. [21]	Thailand	20	IH-CCA	1 (5)	<i>P53</i> exons 5–8 evaluated
Kang et al. [24]	Korea	40	IH-CCA	14 (35.7)	<i>P53</i> exons 5–8 evaluated
Furubo et al. [25]	Japan	15	IH-CCA (peripheral) and perihilar	3 ^a (20)	<i>P53</i> exons 5–8 evaluated
Kamikawa et al. [19]	Japan	22	IH-CCA	9 (41.6)	<i>P53</i> exons 5–8 evaluated; thorotrast exposed patients
Della Torre et al. [26]	Italy)	13	Not specified	2 (15.3)	<i>P53</i> exons 5–8 evaluated
Tullo et al. [27]	Europe	29	Perihilar	7 (24)	<i>P53</i> exons 5–8 evaluated 3/7 cases carried germline heterozygous polymorphism in tumoral and non-tumoral DNA
Momoi et al. [28]	Japan	28	IH-CCA	2 (7.1)	<i>P53</i> exons 5–8 evaluated
Khan et al. [18]	UK	31	IH-CCA	24 (76)	Complete <i>P53</i> mutational signatures Three new frameshift mutations and two new intron mutations discovered
Liu et al. [29]	China	36	Not specified	22 (62)	<i>P53</i> exons 5–8 evaluated
Kiba et al. [20] ^c	Thailand	26	IH-CCA	9 (35.7)	<i>P53</i> exons 5–8 evaluated 2 patients with <i>KRAS</i> mutations, none carrying both <i>P53</i> and <i>KRAS</i> mutations
Kiba et al. [20] ^c	Japan	12	IH-CCA	4 (33.3)	<i>P53</i> exons 5–8 evaluated 7 patients with <i>KRAS</i> mutations, none carrying both <i>P53</i> and <i>KRAS</i> mutations
Imai et al. [30] ^c	Japan	7	IH-CCA ^b	2 (28.5)	<i>P53</i> exons 5–8 evaluated
Itoi et al. [31] ^c	Japan	12	Not specified	4 (33.3)	6 patients with <i>KRAS</i> mutations, none carrying both <i>P53</i> and <i>KRAS</i> mutations <i>KRAS</i> and <i>P53</i> abnormalities not detected in non-neoplastic biliary tract tissues The same mutation patterns detected in bile and neoplastic tissue
		Total 330		Total 112 (34.0)	

Abbreviations: *IH-CCA* intrahepatic cholangiocarcinoma^a 1 case perihilar-type and 2 cases not defined^b Combined hepatocarcinoma-CCA; 1 patient with *KRAS* mutations, none carrying both *P53* and *KRAS* mutations^c Studies where also *KRAS* mutations were evaluated

of *P53* mutations were seen in CCA cases from European studies (14 %) with respect to Asian studies (23 %) [18]. Also, the pattern of mutations shows large geographic differences. For example, Kiba et al. [20] found that over 50 % of *P53* mutations in their Thai patients were G:C→A:T transitions at CpG sites, whereas a study on Korean patients found the same pattern in only 17 % of cases [24].

In the absence of definite environmental risk factors, *P53* mutations are more frequent in areas with high CCA incidence (United States of America high-incidence cluster area = 67 %) than in areas with low incidence (United States low-incidence cluster = 20 %) [22]. This could reflect the exposure to unidentified mutagen triggering *P53*, in high-incidence areas. Unfortunately, very little is still

known on environmental mutagens, and our current capability to disclose *P53* impairment is limited. In the western world, similar rates (on average, 51 %) of *P53* mutation have been found in CCA associated or not with PSC, indicating the lack of a PSC–CCA-specific molecular signature in *P53* gene. It has been previously suggested that *P53* alterations in CCA may be mediated by abnormal intracellular signaling cascades caused by cytotoxic biliary constituents [18]. In PSC, changes in bile composition are associated with bile duct inflammation and enhanced cholangiocyte proliferation, and this could favor, according to the clonal model of carcinogenesis, accumulation of mutations up to the threshold of neoplastic transformation. The alternative model of cholangiocarcinogenesis contemplates the involvement of individual genetic and environmental factors [17]. Several *P53* polymorphisms have been so far described. Their relevance is unclear, and only two of these variants are associated with abnormal amino acid sequence of the *P53* protein [18]. The lack of a specific *P53* molecular signature in sporadic CCA could be explained if a definite gene polymorphism predisposes to *P53* alterations in the presence of the pathological milieu (i.e., inflammation) determined by CCA risk factors. In comparison with the sporadic form, CCA associated with thorotrast exposure showed a different pattern of *P53* mutations [18, 19]. It is, however, important to note that the full-length *P53* cDNA has been insufficiently investigated. Indeed of the fourteen *P53* sequencing studies, thirteen have evaluated only *P53* exons 5–8, whereas the only study that evaluated the complete *P53* mutational signatures disclosed three new frameshift mutations and two new intron mutations and demonstrated the highest mutation rate in *P53* gene never reported (76 %).

In conclusion, the frequency and type of *P53* mutations occurring in CCA patients depends from environmental factors, including the nature and dose of exposure to environmental carcinogens, which vary in different populations [18].

Growth factors and growth factor receptors (e.g., the ErbB family, insulin-like growth factors (IGF), and hepatocyte growth factor (HGF/MET)) are pivotal growth signal regulators in cancers of different origin [10]. Among the pathways involved in the pathogenesis of IH-CCA, the family of ErbB receptors is perhaps the most relevant [10, 11]. ErbB-2 is an epidermal growth factor receptor (EGFR) homologue and is able to homodimerize or heterodimerize with other members of the EGFR superfamily, resulting in activation of the Raf/MAPK pathway [10, 11]. The most notable are the aberrant regulation of ErbB2 and the EGFR signaling [10, 11]. Constitutive overexpression of ErbB2 and/or ErbB1 in malignant cholangiocytes has been documented in more than 50 % of IH-CCA [32, 33]. In addition, experimental models of IH-CCA in rodents are associated with constitutive ErbB2 overexpression [11]. ErbB2 and

ErbB1 interact with different relevant molecular signaling pathways associated with IH-CCA development and progression, including bile acids, IL (interleukin)-6/gp130, transmembrane mucins, HGF/MET, and vascular endothelial growth factor (VEGF) signaling [11, 32, 33]. Hydrophobic bile salts, such as deoxycholate, may play a carcinogenetic role through transactivation of EGFR and impairment of Mcl-1 functions, and this has been considered a mechanism favouring the intraductal pattern of growth characterizing a subset of CCAs [11]. The relevance of ErbB2- or ErbB1-related pathways in CCA has raised interest in exploring, for the treatment of CCA, agents selectively targeting these receptors. However, current experience with ErbB-targeted therapies produced only modest responses in patients with biliary tract cancers [10, 11]. Activation of EGFR triggers downstream Ras/Raf/Mek/Erk and PI3K/PTEN/Akt, two major cell survival pathways. Ras proteins (K-Ras, N-Ras, H-Ras, B-Raf), responsible for signal transduction downstream to growth factor receptors, have been largely investigated in CCA, and in this regard, *KRAS*-activating mutations represent one of the most frequent genetic alterations found in CCA (10–75 % of CCA cases) [34]. After binding and activation by GTP, Ras proteins recruit Raf that, in turn, activates, by phosphorylation, MAP kinases (MEK1/2 and ERK1/2) [10, 11]. Activation of MAP kinase pathways leads to enhanced proliferation and inhibition of apoptosis.

As reported in Table 2, a total number of 218 CCA patients have been investigated by sequencing studies aimed to identify *KRAS* mutations [20, 30, 31, 35–39, 40, 41]. Studies from 1992 to 2011 have evaluated CCA patient cohorts from Europe, America, and Asia, as shown in Table 2 [20, 30, 31, 35–39, 40, 41]. The total number of CCA patients with *KRAS* mutations resulted 88, the 40.4 % of all the CCAs. When classified by tumor site, 17 % of peripheral type CCAs were positive for *KRAS* mutations with the most frequent alteration in codon 12. Importantly, the incidence of mutations was higher in the hilar-type tumors (53 %) [34]. It is noteworthy that the frequency of *KRAS* mutations increases with tumor stage (stage I, 8 %; stage II, 15 %; stage III, 31 %; stage IV, 46 %) [39].

Another recently proposed mechanism linking chronic inflammation with CCA development is related to activation-induced cytidine deaminase (AID), a member of the DNA/RNA editing enzyme family, implicated in human cancerogenesis via its mutagenic activity [42]. AID was found to be increased in biopsies from patients with PSC or CCA, whereas only trace amounts of AID were detected in the normal liver [11, 42]. In in vitro studies, in human CCA cell lines, AID was induced by tumor necrosis factor- α that, in turn, was stimulated via I κ B kinase-dependent nuclear factor- κ B (NF- κ B) pathway [11]. The aberrant expression of AID in biliary cells resulted in the

Table 2 *KRAS* mutations in human cholangiocarcinoma: sequencing studies

References	Country	No. of patients	CCA site	Overall number of patients with <i>KRAS</i> mutations (%)	Notes
Tada et al. [35]	Japan	18	IH-CCA (peripheral) and perihilar	9 (50)	The incidence of mutations higher in the perihilar CCA
Tannapfel et al. [37]	Germany	41	IH-CCA	22 (54)	All 22 cancers with <i>KRAS</i> mutations also exhibited methylated <i>P16</i> ; in 2 cases, mutations were detected in non-neoplastic liver tissue surrounding the tumor (germline mutations)
Ahrendt et al. [38]	USA	12	Not specified	12 (33)	Patients with PSC-associated CCA Overall survival shorter in patients with <i>KRAS</i> mutation
Xu et al. [39]	China	13	Not specified	5 (38.2)	2 patients (5.9 %) harbored both <i>KRAS</i> and <i>PIK3CA</i> mutations
Isa et al. [41]	Japan	23	IH-CCA (peripheral) and perihilar	9 (39.1)	Patients with <i>KRAS</i> mutations worst survival rates; <i>KRAS</i> mutation rates higher in perihilar (6/8, 75.0 %) than in peripheral (3/5, 20.0 %) CCA
Rashid et al. [40]	China	33	Not specified	5 (15.2)	Mean survival of patients with <i>KRAS</i> mutations shorter (3.0 months) compared with patients without mutation (15.5 months)
Kiba et al. [20] ^b	Thailand	26	IH-CCA	2 (7.6)	<i>P53</i> exons 5–8 also evaluated; 9 patients (35.7 %) with <i>P53</i> mutations
Kiba et al. [20] ^b	Japan	12	IH-CCA	7 (58.4)	<i>P53</i> exons 5–8 also evaluated and the overall number of patients with <i>P53</i> mutations was 4 (33.3 %)
Ohashi et al. [36] ^b	Japan	21	IH-CCA	10 (48)	<i>P53</i> exons 5–8 also evaluated; 2 patients (7.1 %) with <i>P53</i> mutations; <i>KRAS</i> mutations were prominent in the periductal growing CCA (4/6; 67 %) with respect to the mass-forming CCA (0/5)
Imai et al. [30] ^b	Japan	7	IH-CCA ^a	1 (14.2)	<i>P53</i> exons 5–8 also evaluated; 2 patients (28.5 %) with <i>P53</i> mutations
Itoi et al. [31] ^b	Japan	12	Not specified	6 (50)	<i>P53</i> exons 5–8 also evaluated; 4 patients (33.3 %) with <i>P53</i> mutations <i>KRAS</i> abnormalities were not detected in non-neoplastic tissues The same mutation patterns detected in bile and neoplastic tissues
		Total 218		88 (40.4)	

Abbreviations: *IH-CCA* intrahepatic cholangiocarcinoma, *PSC* primary sclerosing cholangitis^a Combined HCC-CCA^b Studies where also *P53* mutations were evaluated

generation of somatic mutations in tumor-related genes, including *P53*, *c-Myc*, and the promoter region of the *INK4A/P16* sequences [10, 11]. In contrast with hepatocellular carcinoma (HCC), mutations activating β -catenin are rarely found in CCA (0–8 % of CCA cases) [10]. Other genes such as *IDH1*, *SMAD4*, and *KEAP1* have been described to be frequently mutated in CCA tissue, but with large differences among studies. [10, 11, 43]. Aberrant epigenetic regulation, such as promoter hypermethylation, was demonstrated in numerous important cancer-associated genes in CCA [44, 45]. Promoter methylation of *P14*,

a regulator of *P53*, has been found in CCA [10]. *P16* (*CDKN2*) is frequently silenced in CCA by genetic or epigenetic mechanisms [37].

The interleukin-6 (IL-6) is one of the most investigated genes in the pathogenesis of CCA, where it could be involved by different mechanisms [10, 11]. IL-6 is produced at high levels in CCA cells and elevated IL-6 serum concentrations have been reported in CCA patients [10, 11]. Constitutive activation of the IL-6/STAT3 pathway has been described in CCA cells, and this was associated with silencing of *SOC3*. The methylation of *SOC3* promoters

occurs in 61 % of IH-CCA together with down-regulation of gp130, a membrane protein that, when associated with SOCS3 protein product, inhibits the IL-6 pathway [44]. By autocrine and paracrine mechanisms, IL-6 activates via STAT3 the prosurvival *P38* mitogen-activated protein kinase [10, 11]. STAT3 is an activator of p44/42 and *P38* mitogen-activated protein kinase that has been frequently found, by immunohistochemistry, to be activated in IH-CCA [10, 11]. In addition, IL-6 up-regulated the expression of myeloid cell leukemia-1 (Mcl-1) through STAT3- and AKT-related signaling pathways [46, 47]. Mcl-1 increases cell resistance to TRAIL apoptotic signals [48]. Moreover, IL-6-related pathways can modulate epigenetic fate of the cells through DNA (cytosine-5)-methyltransferase 1 (DNMT1), and this has been demonstrated for IL-6-mediated up-regulation of EGFR and for down-regulation of *P53* expression, which occur by promoter hypo- or hypermethylation, respectively [10, 12]. Finally, IL-6 may act in CCA by autocrine and paracrine pathways since it is secreted by malignant cholangiocytes [11]. In light of these findings, IL-6 has been explored in the diagnostic setting and, in fact, serum levels of IL-6 have been correlated with tumor burden in CCA patients [13]. However, although these findings are encouraging, it should be considered that serum IL-6 is also elevated in many patients with HCC, benign biliary disease, and metastatic lesions, and therefore, the specificity of high IL-6 serum levels for CCA is still debated [13]. Recently, the induction of progranulin (PGRN) has been advanced as another mechanism by which IL-6 could enter CCA pathogenesis [49]. PGRN is involved in multiple steps of the tumor progression cascade, including cellular proliferation, anchorage independence, invasiveness, resistance to apoptosis, and promotion of resistance to certain cytotoxic drugs. In addition, PGRN may also act by promoting neoangiogenesis with a direct effect on endothelial cells as well as an indirect effect on VEGF synthesis. The expression and secretion of PGRN are up-regulated in human CCA, and this in part occurs via IL-6-mediated activation of the Erk1/2/Rsk1/C/EBP β pathway [49]. Serum PGRN levels were higher in patients with CCA than in non-neoplastic controls, but it is unknown if this can discriminate CCA with respect to benign biliary pathologies, including PSC and benign strictures of the biliary tree [13]. IL-6 and other mediators of inflammation, including TNF- α , may enter CCA pathogenesis by inducing or synergizing a number of different growth factors [10, 11].

Cyclooxygenase 2 (COX-2), the rate-limiting enzyme in prostaglandin biosynthesis from arachidonic acid, activated by inflammatory cytokines and nitric oxide (NO), accelerates cell cycle via prostaglandin E₂ (PGE₂) and inhibits different apoptotic cascades. Indeed, increased COX-2 immunohistochemical expression has been documented in more than 70 % of CCA samples [50], and the COX-2 gene

is frequently affected by epigenetic (methylation) perturbations in CCA. COX-2 is activated by oxysterols, oxygenated cholesterol derivatives formed in the bile of patients with inflammatory diseases of the biliary tree, and by hydrophobic bile acids [11]. Another COX-2-inducing molecule is the tyrosine kinase ErbB-2, which is overexpressed in CCA and involved in CCA origin and progression [11]. Current evidence supports a primary role played by NO, induced by proinflammatory cytokines (TNF- α , IL-6, etc.) [51]. These cytokines are able to activate inducible nitric oxide synthase (iNOS), which, at the immunohistochemical level, is overexpressed in more than 70 % CCA [11]. Increased iNOS activity results in generations of NO and reactive oxygen species, which are known to interact with cellular DNA and to inhibit DNA reparative mechanisms, thus triggering oncogenetic mutations. NO together with different cytokines can also inhibit cholangiocyte apoptosis by nitrosylation of caspase-9 and may also induce proliferation, thus favouring accumulation of somatic mutations [11]. Very recently, a relevant role in modulating CCA growth and proliferation has been attributed to estrogens, IGF1, leptin, opioid receptor modulators, endothelin, and serotonin [11]. As far as estrogens are concerned, recent studies suggest their synergistic action with growth factors (IGF1, VEGF) in sustaining the cholangiocyte proliferative machinery and in depressing apoptosis [52, 53]. Indeed, a cross talk between IGF1 and estrogens has been demonstrated to modulate CCA proliferation, whereas estrogens act at several points of the IGF1 signal transduction pathway [52]. In addition, it has been shown that the estrogen proliferative effect on CCA cells is also due to the stimulation of VEGF synthesis and secretion [52, 53]. In agreement with these data, IGF1 have been explored as CCA markers in a diagnostic setting. The IGF1 biliary concentration was shown to be capable of completely discriminating CCA from benign biliary pathologies and pancreatic cancer [54].

Recent technical improvement in molecular profiling platforms is adding new insights into the current knowledge of cholangiocarcinogenesis favoring the integration of the different proposed models. Unfortunately, few comparative genomic hybridization (CGH) studies on CCA have been performed during the past decade, and these studies are biased by the heterogeneous population investigated that included IH-CCA, EH-CCA, or even gallbladder cancers, making difficult any accurate interpretation. Evaluation of DNA copy number (CN) demonstrated CN gains in the region of several molecular targets: *ERBB2*, *MEK2*, *PDGFB*, *MTOR*, *VEGFR-3*, *PDGFA*, *RAF1*, *VEGFA*, and *EGFR* [55]. Technological advances also allow the differential characterization of genomic and genetic features of CCA epithelial and stromal compartments [56]. The tumor epithelium was defined by deregulation of the HER2 network and frequent

overexpression of EGFR, the HGF/MET receptor, pRPS6, and Ki67, whereas stroma was enriched in inflammatory cytokines [56]. Recently, the comparative evaluation of gene expression profile (transcriptome), clinicopathological traits, and patient outcomes in IH-CCA cases has allowed the identification of 2 main biologic classes of IH-CCA: (1) the inflammation class (38 % of IH-CCA), characterized by activation of inflammatory signaling pathways, overexpression of cytokines, and STAT3 activation and (2) the proliferation class (62 % of IH-CCA), characterized by activation of oncogenic signaling pathways (i.e., RAS, MAP kinase, and HGF/MET), DNA amplifications at 11q13.2, deletions at 14q22.1, mutations in *KRAS* and *BRAF*, and gene expression signatures previously associated with poor outcomes for patients with HCC [57]. As previously discussed, an optimal approach to CCA molecular profiling should be the comparative investigation of subtypes such as CCA emerging in a definite category at risk, including PSC or liver fluke infestation. Unfortunately, very few studies followed this type of approach. PSC is a major risk factor for IH- and EH-CCAs, and these patients experienced a cumulative risk of 11.2 %, 10 years after diagnosis [7]. Unfortunately, predictive factors or standardized screening or surveillance strategies are lacking. Different molecular signatures of the high oncogenic risk have been described in PSC patients. *KRAS* mutations have been found in 30 % of bile fluid of PSC patients without evidence of CCA [58]. Since *KRAS* mutations are frequently observed in CCA, this could be an early event of bile duct carcinogenesis in PSC patients. Notably, mutational profiling can be performed in cell-free DNA of bile supernatant [59]. The inflammatory microenvironment has also been associated with an aberrant DNA methylation profile in PSC-derived CCA, which provides survival signals for the tumor [60]. Genetic susceptibility of PSC patients for CCA development has been demonstrated by studies concerning the natural killer cell receptor G2D receptor [61], where specific genetic variants have been described in PSC patients.

The association between liver flukes and CCA has been evaluated by the International Agency for Research on Cancer (IARC) since 1994. *Opisthorchis viverrini* (OV) infestation, endemic in Southeast Asia, is now considered a definitive carcinogen. The molecular mechanism of OV-associated CCA has been also studied in experimental models. Up-regulation of 23 transcripts and down-regulation of 1 transcript related to CCA induced in OV-infected hamsters has been identified. The up-regulated genes include signal transduction protein kinase A regulatory subunit Ia (PRKAR1a), myristoylated alanine-rich protein kinase C substrate, transcriptional factor LIM-4-only domain, oxysterol-binding protein involved in lipid metabolism, splicing regulatory protein 9, ubiquitin-conjugating enzyme involved in protein degradation, β -tubulin, β -actin, and collagen type VI. Interestingly, PRKAR1a expression tended to increase

during the progression from hyperplasia to precancerous lesions and to CCA [62]. In humans, molecular studies of IH-CCA associated with liver flukes demonstrated overexpression of genes involved in xenobiotic metabolism (*UGT2B11*, *UGT1A10*, *CHST4*, *SULT1C1*), whereas, in contrast, non-OV-associated IH-CCA showed enhanced expression of genes related to growth factor signaling (*TGFBI*, *PGF*, *IGFBP1*, *IGFBP3*). Thus, the evaluation of the putative signature of OV-associated IH-CCA in OV-infected patients could help in screening and surveillance, with the perspective of an early diagnosis [63]. The draft genome of *Clonorchis sinensis* and transcriptomes of *Clonorchis sinensis* and OV have been recently elucidated [64, 65]. Recently, a study in a large IH-CCA cohort (N = 102) associated with liver fluke infection demonstrated promoter hypermethylation in a handful of target genes, when CCA specimens were compared with adjacent non-tumoral tissues [66]. These results could help in identifying molecules linked with the development of liver fluke-induced CCA. CCA genetic susceptibility has been investigated in geographic areas endemic for liver flukes. In these studies, specific haplotypes of COX-2-coding gene (PTGS2) or IL8RB have been recently associated with a significant risk of CCA development [67].

3 Molecular Profiling and the Diagnosis of Cholangiocarcinoma

Immunohistochemical markers specific to CCA are lacking, and the definite diagnosis in bioptic or surgical samples is still based on a panel of markers aimed at excluding HCC or metastatic cancer. Therefore, for many years, studies have been focused on the search for CCA-specific markers. Different proposals appear in recent literature, but none of these reached clinical routine application. Recently, high-throughput techniques based on DNA microarray technology [68] have been tested in human CCA samples. The first study using DNA microarray technology (Affymetrix U133A) in a series of surgically resected biliary cancers, biliary cancer cell lines, and biliary epithelial scrapings was carried out in 2003 by Hansel et al. [69]. They reported 282 genes overexpressed threefold or greater in biliary malignancies or cancer cell lines, including proliferation and cell cycle-related genes (e.g., cyclins D2 and E2, *cdc2/p34*, and *geminin* genes), transcription factors (e.g., *homeobox B7* and *islet-1*), growth factors and growth factor receptors (e.g., *hepatocyte growth factor*, *amphiregulin*, and *insulin-like growth factor 1 receptor*), two important downstream mediators of the mitogenic Akt/mTOR signaling pathway (*ribosomal protein S6 kinase* and *eukaryotic translation initiation factor 4E*), enzymes modulating sensitivity to chemotherapeutic agents (e.g., *cystathionine beta synthase*,

dCMP deaminase, and CTP synthase), and cytosolic phospholipase A2 [69]. After this first report, other studies aimed to investigate the utility of transcriptomic in CCA diagnosis have been performed. A genome-wide cDNA microarray containing 27,648 cDNAs carried out in IH-CCA specimens and non-cancerous biliary tissues, showed 52 genes up-regulated and 421 genes down-regulated. The overexpressed genes are related to a variety of functions, such as signal transduction (*GNAS*, *MDK*), transcription (*FOXM1*, *HOXB7*, *DRIL1*), DNA synthesis (*TOP2A*, *TOP2B*, *NAV2*, *BUB1B*, *CKS2*), antiapoptosis (*BIRC5*, *S100P*), angiogenesis (*ECGF1*), cytoskeleton (*FSCN1*, *PRCI*, *ANLN*, *KIF2C*), and cytokinesis or adhesion (*CDH3*, *CIT*, *ECT2*). On the contrary, the down-regulated genes are mainly involved in growth suppression (*EGR1* and *EGR2*, *AXIN1*, *AXUD1*, *DLCL1*, *DOC1*). From the 52 up-regulated genes, P-cadherin and survivin were selected for further investigation, and the enhanced expression of their protein products in CCA tissues was demonstrated by immunohistochemical staining [70]. Recently, oligonucleotide arrays (Affymetrix U133A) were used to establish a specific gene expression profile of IH-CCA in comparison with adjacent non-malignant liver tissue. Most of the strongly overexpressed genes are related to cell cycle regulation and DNA replication (15 genes, including *ribonucleosidediphosphate reductase M2*, *calgizzarin*, *calcyclin*, *BUB1B*) intracellular signaling (15 genes, including *CD24* and *MARCKS*), genes encoding transcription factors (6 genes, such as *SOX9*), or genes involved in nuclear organization and nucleic metabolism (13 genes, such as *thymidylate synthetase*). Other up-regulated genes include those coding for extracellular matrix and cell adhesion molecules (37 genes, for example *OPN*, *ADAM9*, *thymosin beta-10*, *integrin alpha-6*), cytoskeleton structure proteins (16 genes, such as *tropomyosin 2*, *cytokeratin 7* and *19*), or enzymes involved in protein biosynthesis (4 genes). The gene encoding for OPN was identified as the highest and most consistently overexpressed gene (33.5-fold change) in all analyzed CCA samples. Most of the genes encoding proteins involved in cellular apoptosis (7 genes including growth arrest-specific protein 2, *CIDE-B*) were found to be down-regulated in IH-CCA [71]. The genes overexpressed in IH-CCA, have been confirmed at protein level by immunohistochemical analysis, and included osteopontin, *P38 δ*/MAPK-13, cadherin, and survivin. In conclusion, oligonucleotide microarray analysis shows a specific gene expression profile of IH-CCA, which could discriminate this cancer with respect to other malignancies or non-malignant lesions. These data, however, need further validation in independent cohorts of samples.

The differential diagnosis between IH-CCA and some subtypes of HCC is frequently challenging because of the existence of many overlapping features. Indeed, detailed

studies on immunohistochemical profile have revealed that a whole range of phenotypical traits of hepatocytes, cholangiocytes, and progenitor cells can be shared by IH-CCA, combined HCC-CCA, fibrolamellar HCC, and HCC with stem cell features. This is consistent with a common origin of these cancers from the hepatic stem cell compartment within canals of Hering [72]. A substantial number of HCCs, ranging from 28 to 50 % of human HCCs, express markers of progenitor cells or cholangiocytes including CK7, CK19, and OV6, which suggest an origin from bipotential stem/progenitor cells located within canals of Hering [73]. Some of these markers in HCC, especially CK19, have been associated with a worse prognosis and higher rates of recurrence after surgical treatment [73]. The emergence of HCC and IH-CCA in the same pathological context of chronic liver diseases does not help in differential diagnosis, and radiologic features may overlap. Differential diagnosis between HCC and IH-CCA deserves important clinical implications since, for example, IH-CCA is excluded from liver transplantation programs. Recently, mutations of *BRAF* and *KRAS* were evaluated in 25 HCC and in 69 CCA by direct DNA sequencing analyses after microdissection. Using this molecular profiling approach, *RAS* or *BRAF* mutations have been detected in approximately 62 % of CCA, but not in HCC [74]. The diagnostic utility of evaluation of active intermediates of the MAPK pathway was assessed by microarray gene expression. The study identified a *P38* MAP kinase, *P38 δ* (also known as MAPK13 or SAPK4) as a protein that is up-regulated in CCA relative to HCC and to normal biliary tract tissues. Consistently, *P38 δ* immunohistochemical staining distinguished CCA from HCC with a sensitivity of 92.6 % and a specificity of 90.7 %. *P38 δ* is important for motility and invasion of CCA cells, suggesting an important role in CCA metastasis. Therefore, *P38 δ* could represent a novel diagnostic marker for CCA and may also serve as a new target for molecular-based targeted therapy [75]. Evaluation of markers of apoptosis and cell proliferation, such as bcl-2, c-myc, Fas, Lewis(y), and *P53* in human CCA and HCC, showed that Lewis(y) antigen was expressed in some CCA, whereas it was not found in HCC [76]. The diagnostic workup of EH-CCA usually starts with the evidence of biliary tract obstruction [2, 9]. The definitive diagnosis is obtained during endoscopic retrograde cholangiopancreatography (ERCP) with cytology on bile samples, brushing, or endoscopic biopsies. Unfortunately, endoscopic biopsies can be obtained almost exclusively in the case of CCA with an intraductal pattern of growth and located at the distal part of the bile duct [2, 9]. Furthermore, these samples are often of poor quality given the scarce cellularity of this tumor. For the same reasons, cytology on bile samples or brushing has a low diagnostic yield, which is markedly increased by fluorescent in situ hybridization (FISH) analysis of

chromosomal aberrations (mainly polysomy) [2, 9]. Even recent guidelines indicate FISH analysis of chromosomal aberrations in cells collected by bile sampling or brushing as the procedure to be performed during the diagnostic workup of EH-CCA [2, 9]. Another unresolved issue is the differential diagnosis of biliary strictures, especially in the setting of PSC. Recently, microarray analysis has been applied to endoscopic biliary brushing from patients with benign and malignant biliary disease. Despite the variable quantity and poor quality of analyzed RNA, a differential gene expression profile by microarray analysis was demonstrated in patients with CCA with respect to benign pathologies. Specifically, comparing malignant versus benign biliary strictures by quantitative polymerase chain reaction (qPCR) and microarray analysis of endoscopic biliary brushings, 45 up-regulated genes have been identified in malignant strictures including various *HOX* genes, collagens, *PVT1*, *MUC4*, *MUC5AC*, and *LEF1*. Immunohistochemistry of surgically resected tissues showed elevated CD9, Serpina, and PNMA2 protein expression in CCA [77]. Notably, mutational profiling of cell-free DNA in residual supernatant fluid improves sensitivity of microscopic examination of biliary cytobrush specimens and demonstrated *KRAS* mutations as distinctive feature of CCA with respect to benign biliary strictures. Molecular analyses of biliary brushings using microarray and qPCR have the potential to provide valuable information on the biology of biliary diseases [78]. As a clinical translation of studies exploring CCA pathogenesis, the IGF1 biliary concentration was shown to be capable of completely discriminating CCA from benign biliary pathologies and pancreatic cancer [54].

4 Molecular Profiling and the Prognosis of Cholangiocarcinoma

CCA prognostic factors represent the basis for recently proposed staging systems, but not without certain criticisms and controversies. In general, the histologic grade, the size and number of the primary tumor, the tumor growth type, the depth of tumor invasion, local and distant metastatic disease, tumor-associated vascularization, vascular encasement, and lobar atrophy have been considered factors affecting survival. Biomarkers and molecular markers of local invasiveness and early metastatic behavior would help to assess prognosis as well as the eligibility of CCA patients to potential curative treatments, but, to this regard, still little is known. Indeed, no molecular marker entered the staging systems so far proposed for IH- or EH-CCA. [2, 6, 9, 11]. Several molecular markers have been investigated in relationship to CCA prognosis, and some of these have been found of potential clinical utility, including P-cadherin, p27,

Skp2, *P16*, matrix metalloproteinases, and vitamin D receptor [79]. The frequency of *KRAS* mutations progressively increases with increasing tumor stage (stage I, 8 %; stage II, 15 %; stage III, 31 %; stage IV, 46 %) [39]. Molecular profiling could open new perspectives for identifying valid and reproducible predictors of survival based on protein or gene profiles. Gene expression profiling demonstrated the periostin gene as markedly overexpressed in CCA, and, by multivariate analysis, high levels of periostin were found to represent an independent negative prognostic factor, also predictive of chemoresistance [80]. Moreover, recent studies of gene expression profiling in node-positive with respect to node-negative CCA cases have shown a significantly higher expression of the genes coding for: BRCA1-associated protein 1, cyclin I, collagen type IV alpha-1 chain, collagen type IV alpha-2 chain, DR3, TL1A, heparin-binding EGF-like growth factor, urocortin receptor, bradykinin receptor B1, calpain 1, nitric oxide synthase 2, RAB10, and scavenger receptor class B member 1. In contrast, the following gene products were found down-regulated: caspase-7, BCL2/adenovirus E1B 19kD-interacting protein 1, cadherin-8, phosphodiesterase 4D, c-Abl, MEK Kinase-4 [81]. The same authors were able to select several expressed genes capable of predicting, in 100 % of the cases, the perineural invasion: *MMP-14*, *HSD3B*, *Wip1*, *COL2A1*, *CNP*, *Integrin 4*, *ING1*, *Wnt-10b*, *IL15RA*, *Fbn-1*, *Spectrin*, *ARF1* [81]. Recently, gene expression cluster analysis performed in large series of IH-CCA demonstrated how CCA could be separated into two distinct subclasses with large different survival (5-year survival rate after resection: 72 % in cluster 1 vs. 30 % in cluster 2). Major networks controlled by key molecules, such as tumor necrosis factor, transforming growth factor, and mitogen-activated protein kinase-1/2, were found to be deregulated in the poor prognosis cluster. Thirty-six genes were strongly associated with poor survival, and these genes were found to be enriched in key networks controlled by *VEGF/ERRB*, *CTNNB1/MYC*, and *TNF*. At a protein level, three of the survival genes (*ITGA2*, *TMPRSS4*, *CEACAM6*) as well as pRPS6, a marker of mTOR, and Ki67 staining showed significant over expression in CCA with poor prognosis. Moreover, all patients with mutated *KRAS/BRAF* have been retrieved in poor prognosis cluster [57]. These new insights received confirmation by another independent study, which showed two main biologic classes of IH-CCA. The so-called proliferation class (62 % of IH-CCA), characterized by activation of oncogenic signaling pathways (including RAS, mitogen-activated protein kinase, and MET), DNA amplifications at 11q13.2, deletions at 14q22.1, and mutations in *KRAS* and *BRAF*, showed reduced survival with respect to the so-called inflammation class (38 % of IH-CCA), which is characterized by activation of inflammatory signaling pathways, overexpression

of cytokines, and STAT3 activation [82]. In this study, an association of various genes with the histopathological grading has been demonstrated. Indeed, a trend toward higher expression of specific cell surface proteins (EMP1, EVA1, proteoglycan2) and intermediate filaments (cytokeratin 6, 7, 13, 15, 17) in well-differentiated tumors (G1–G2) was observed, whereas samples of high-grade (G3) IH-CCA showed an elevated expression of genes involved in G-protein signaling and nuclear transcription [71].

Stem cell markers have been extensively investigated as prognostic markers in CCA. The expression of SALL4, for example, correlates with tumor growth and resistance to 5-fluorouracil, while its suppression results in differentiation and delayed tumor growth [83]. The expression of neural cell adhesion molecule 1 (NCAM1), a known hepatic stem/progenitor cell marker, has been found to be predictive of poor overall survival in patients with IH-CCA [84]. In immunohistochemical investigated specimens, strong expression of CD133, a cancer stem cell marker, was strictly associated with lymph node involvement and positive surgical margins in resected CCA [72]. Recently, S100A4, a member of the S100 family of small calcium-binding proteins, expressed by macrophages and epithelial cells in mesenchymal transition, was proposed as a biomarker of increased metastasization and reduced survival after resection in CCA [5].

MicroRNA (miRNA) profile analyses have identified various microRNAs associated with either the progression or prognosis of CCA. MicroRNAs can thus serve as potential prognostic biomarkers. Recently, a transcriptomic profile has revealed hepatic stem-like gene signatures and interplay of miR-200c and epithelial–mesenchymal transition in IH-CCA. Integrative analyses of the IH-CCA-specific mRNA and microRNA expression profiles revealed that a common signaling pathway linking miR-200c signaling with epithelial–mesenchymal transition (EMT) was preferentially activated in IH-CCA with stem cell trait and poor prognosis [84].

5 Molecular Profiling and Classification of Cholangiocarcinoma

The distinction between IH- and EH-CCA, which has been reported for many years in different classifications, has become increasingly important since these two CCA forms showed enormous differences in epidemiologic features (i.e., incidence and risk factors), biologic and pathological characteristics, and clinical course [7, 8]. Recent studies comparing clinicopathological features with molecular profiling are bringing new insights into CCAs classification, further supporting the concept that IH- and EH-CCAs are two different tumors. Indeed, in vitro studies on cell cultures

prepared from IH-CCA or EH-CCA have shown that they express different cellular proteins, cellular shape, doubling time, chromosome karyotype, and chemosensitivity [85]. Consistently, researchers from France have demonstrated that perihilar EH-CCA expresses with respect to IH-CCA higher levels of MUC5AC (60 vs. 22 %), Akt2 (64 vs. 36 %), K8 (98 vs. 82 %), annexin (56 vs. 44 %), and less VEGF (22 vs. 78 %) [86]. At a molecular level, distinct patterns of genetic mutations, methylation, and expression profiling may differentiate IH-CCA from EH-CCA. IH-CCAs, for example, were significantly more frequently *bcl-2+* and *P16+*, whereas EH-CCAs were more often *P53+* [87]. Miller et al. [88] investigated gene expression and copy number in biliary cancers and correlated their changes with the anatomical site of origin, histopathology, and outcomes. They revealed 545 genes with altered expression in EH-CCA and 2,354 in IH-CCA. Mutations in *IDH1* and *IDH2* were found only in IH-CCA ($n = 9$), but in none of the examined EH-CCA ($n = 22$) and gallbladder cancer ($n = 75$) [43]. *KRAS*-activating mutations appear to be less frequent in EH-CCA (9–33 %) than in IH-CCA (21–54 %). As far as epigenetic abnormalities are concerned, methylation of *RASSF1A* was more common in EH- than in IH-CCAs, while the opposite was demonstrated for methylation of *GSTP* gene [89].

More recently, new updated classifications of CCAs are emerging in which the IH-CCA is comprised of a pure mucin-secreting form similar to EH-CCA and a peripheral non-mucin-secreting form [4, 72, 90, 91]. These new classifications are based on cells of origin. Their rationale derives from recent scientific advances in the heterogeneity of cholangiocytes lining bile ducts of different diameters and in the nature and distribution of stem cell niches along the biliary tree [4, 72]. As far as cholangiocyte heterogeneity is concerned, small bile ducts are lined by cuboidal non-mucin-secreting cells, while large intrahepatic and extrahepatic bile ducts are lined by cylindric mucin-secreting cells. Molecular profiling of small and large mouse bile ducts have been analyzed by Alpini's group [92]. Isolated total RNAs were hybridized with microarrays, which detect 4850 cDNA expressions. Of these, 230 cDNAs were differentially expressed between small and large cholangiocytes, with aquaporin 8, IL-2 receptor beta chain, and caspase-9 being strongly expressed by large cholangiocytes [92]. In general, this study demonstrated how genes controlling proliferative activities were strongly expressed in cholangiocytes lining small ducts, while genes controlling transport processes were strongly expressed in large cholangiocytes lining large ducts. These findings are consistent with the role of small cholangiocytes as precursor cells linked with liver regeneration. As far as stem cell niches are concerned, two types have been so far identified in the biliary tree. The first type is located in the canals of

Hering and bile ductules and is composed of bipotential progenitor cells, named human hepatic stem/progenitor cells (hHpSCs) [93, 94]. The second type is located in the peribiliary glands (PBGs) and is composed of multipotent stem cells of endodermal origin, named human biliary tree stem/progenitor cells (hBTSCs) [95, 96]. Based on these concepts, the clinicopathological heterogeneity of CCAs could reflect the different lineage of origin. Nakanuma et al. [90] stressed the concept of CCA heterogeneity and proposed a small duct type (peripheral type) and a large bile duct type (or perihilar type) IH-CCA [90], with the first type originating from canals of Hering/hHpSCs and the second from peribiliary glands/hBTSCs in large ducts. The small duct type IH-CCA is mainly described as a tubular adenocarcinoma, while the large bile duct type involves the IH large bile ducts and is composed of mucin-producing elements [90]. Aishima et al. [97] investigated 87 cases of IH-CCA smaller than 5 cm in diameter and described a perihilar type showing IH large bile duct involvement within the tumor and a peripheral type containing a preserved architecture of the portal triad. They demonstrated that the frequency of perineural invasion, lymph node metastasis, vascular invasion, intrahepatic metastasis, and recurrence of IH-CCA from large ducts were significantly higher than that of IH-CCA from small ducts. In addition, the survival of patients with IH-CCA from large ducts was worse than that of patients with IH-CCA from small ducts [97]. Recently, Roskams et al. [91] carried out a study investigating the CCA histologic diversity in relation to the heterogeneity of cholangiocytes lining the biliary tree: perihilar mucin-producing cells versus peripheral cuboidal ductular cells or hHpSCs. They investigated the clinicopathological and molecular features of 79 resected CCAs and their relationship with hHpSCs and compared the spectrum of CCAs with respect to K19-positive or K19-negative HCCs. They described a subtype IH-CCA with mixed features (mixed CCAs) showing a peripheral location, a larger tumor size, less microvascular invasion, and less lymph node involvement when compared to pure mucin-producing CCAs which, in contrast, showed a hilar location, a smaller tumor size, more microvascular invasion, and more lymph node involvement. S100P expression was seen only in mucin CCAs, while neural cell adhesion molecule (NCAM) expression was only present in mixed CCAs [91]. Phenotype profiling showed high homology between mixed CCAs and K19-positive HCCs, suggesting that these two primitive liver cancers could arise from the same cell type, i.e., hHpSCs. In keeping, indeed, in 2006 Lee et al. [98], analyzing the transcriptional characteristics of HCCs by integrating gene expression or rat fetal hepatoblasts, adult hepatocytes, and HCCs from human and mouse models, showed that a gene expression profile that distinguishes HCC subtypes with poor prognosis includes well-known

markers of progenitor cells (i.e., KRT7, KRT19, and VIM). This probably reflects the derivation of these HCCs from hepatic progenitor cells. Notably, at multivariate analyses where all relevant pathological and molecular variables were included, only the hepatoblast subtype was independently associated with both recurrence and worse overall survival [98].

These recent results are opening a completely new scenario and break many paradigms in the field of primitive liver cancers. Indeed, the large bile duct mucin-producing IH-CCA has similarities with EH-CCA. In contrast, the small bile duct type (peripheral) or mixed type IH-CCA has features in common with ductular type cholangiolocellular carcinoma and with CK19+ HCC [99], further reflecting the different cells of origin [4, 72]. The clinical implications of these recent advances in terms of diagnostic tools, targeted therapy and indications for surgery or transplantation need accurate evaluations in the near future. In substance, the existence of two different stem cell compartments and the associated cell lineages may result in multiple cells of origin of CCA and could represent the basis of the clinicopathological, epidemiologic, and molecular heterogeneity of CCA.

6 Molecular Profiling of Gallbladder Cancer

Mutations and epigenetic alterations of K-ras, *P53*, and *P16* have been frequently considered to be involved in the development of gallbladder cancer (GBC) and precancerous lesions [31, 100–109]. As reported in Table 3, a total number of 327 patients affected by GBC have been investigated by sequencing studies to evaluate *KRAS* mutations [104–109], with a 25 % (80 patients) overall rates of mutations. A high heterogeneity of the mutation rates among studies is clearly evident. The observed differences may recognize several causes including methods, the quality of DNA, the diversity of the ethnic background, and the different etiologies of the GBC under investigation [102]. Adenoma and dysplasia are considered to represent precancerous lesions, the latter being frequently associated with carcinoma. The mutation rates of *KRAS* in GBC, dysplasia, and adenoma have been reported, in different studies, to be 0–73 %, 0–59 %, and 0 %, respectively [102]. Controversy exists on whether *KRAS* mutations may participate in early step of cancerogenesis or, alternatively, drives adenoma formation. To this regard, two recent studies achieved opposite results. Indeed, Kim et al. [102] demonstrated that *KRAS* gene was mutated in 20 % of the GBC, but never in dysplasia or adenoma [102]. In sharp contrast, Pai et al., in 29 GBC, 16 adenomas, and 5 cases of high-grade dysplasia, analyzed for activating missense

Table 3 Sequencing studies detailing *P53* and/or *KRAS* mutations in human gallbladder cancer

References	Country	No. of patients	Overall number of patients with <i>P53</i> mutations (%)	Overall number of patients with <i>KRAS</i> mutations (%)	Notes
Yokoyama et al. [100]	Japan	22	13 (58)	ND	<i>P53</i> exons 5–8 evaluated
Yokoyama et al. [100]	Chile	20	12 (60)	ND	<i>P53</i> exons 5–8 evaluated
Nigam et al. [101]	North India	22	2 (9.1)	ND	<i>P53</i> exons 5–8 evaluated
Itoi et al. [31]	Japan	7	3 (42.9)	4 (57)	<i>P53</i> exons 5–8 evaluated; none patient with both <i>P53</i> and <i>KRAS</i> mutations <i>KRAS</i> and <i>P53</i> abnormalities not detected in non-neoplastic tissues
Kim et al. [102]	South Korea	15	3 (20.0)	5 (35.7)	<i>P53</i> exons 5–8 evaluated; none patient with both <i>P53</i> and <i>KRAS</i> mutations <i>P53</i> and <i>KRAS</i> mutations were not found in five dysplasias around cancers and three adenomas; 30.7 % of GBC patients carried also <i>P16</i> mutations
Nagahashi et al. [103]	Japan	22	11 (50)	0 (0)	<i>P53</i> exons 5–8 evaluated; None patient with both <i>P53</i> and <i>KRAS</i> mutations Dysplastic epithelia obtained from gallstone patients demonstrated less frequent <i>P53</i> mutations (11 %)
Nagahashi et al. [103]	Hungary	18	6 (33.3)	1 (5.5)	<i>P53</i> exons 5–8 evaluated; none patient caring both <i>P53</i> and <i>KRAS</i> mutations Dysplastic epithelia obtained from gallstone patients demonstrated less frequently <i>P53</i> mutations (11 %)
Imai et al. [104]	Japan	23	ND	9 (39)	No mutations detected in normal, hyperplastic, dysplastic epithelium, adenomyomatous hyperplasia, cholesterol polyps, and cystitis glandularis proliferans
Ajiki et al. [105]	Japan	51	ND	30 (59)	Mutations in <i>KRAS</i> detected also in 8/11 gallbladder dysplasias in gallstone patients but not in normal gallbladder epithelium
Hanada et al. [106]	Japan	39	ND	15 (38)	In GBC associated with anomalous junction of the pancreaticobiliary duct (AJPBD) the prevalence of <i>KRAS</i> mutations were 100 % (stage II–IV carcinomas), whereas in the GBC without AJPBD were 38 %
Rashid et al. [40]	China	75	ND	2 (2.7)	Mean survival of GBC with <i>KRAS</i> mutation shorter (3.0 months) in comparison with GBC without mutation (15.5 months)
Parwani et al. [108]	USA	27	ND	8 (30)	
Saetta et al. [107]	Greece	21	ND	4 (19)	<i>BRAF</i> mutations observed in 7/21 (33 %) GBC; <i>KRAS</i> and <i>BRAF</i> mutations never in the same specimen
Pai et al. [109]	USA	29	ND	2 (7)	
			Total 50/126 (39.6)	Total 80/327 (25)	

Abbreviations: *ND* not determined, *GBC* gallbladder cancer

mutations in *KRAS* codons 12 and 13 and *BRAF* V600E mutations, demonstrated that *KRAS* mutations were infrequently found in GBC (2/29, 7 %) or high-grade dysplastic lesions (0/5, 0 %) but, in more than 30 % (5/16, 31 %) adenomas where, *KRAS* codon 12 mutations have been detected [107]. Based on these controversial findings, the

role played by *KRAS* mutations in the stepwise malignant transformation of dysplasia to carcinoma or as mutational event in adenoma formation is still indefinite. However, it is possible that controversial findings depend on the background favoring GBC emergence. To this regard, *KRAS* mutations have been reported more frequently in GBC

arising in patients with anomalous union of the pancreaticobiliary duct (AUPBD) (50 %) than without AUPBD (6 %) [106]. In the study by Kim et al. [102], the high frequency of *KRAS* mutation in GBC was found in patients without gallstones, but this is not the case in patients investigated by Pai et al. [109]. The polymorphisms of *KRAS* gene were investigated in different studies. For example, Pramanik et al. analyzed 60 GBC (13 men and 47 women) with histologically proven diagnosis and 90 controls (14 men and 76 women) in eastern India. They found a novel polymorphism in codon 25 of the *KRAS* gene associated with GBC. This novel polymorphism was found at codon 25 (CAG>CAT; Gln25His) in exon 1 of the *KRAS* gene in both germline and tissue DNA and appeared significantly associated with GBC also in multivariable logistic regression analysis after adjustment for age and sex. Silico analysis validated the *KRAS* p.Q25H polymorphism as a disease-causing variant [109].

As far as *P53* is concerned (Table 3), using sequencing methodology, several rates of *P53* mutations (0, 30, 37.5, and 50 %) have been described in GBC but not in gallbladder adenoma [102]. As reported in Table 3, a total number of 126 patients affected by GBC have been investigated by sequencing studies to evaluate *P53* mutations [31, 100–103] with a 39.6 % overall mutation rate (50 patients). It is, however, important to note that the full-length *P53* cDNA has been insufficiently investigated. Indeed, all the studies have evaluated only *P53* exons 5–8. *P53* mutations have been found mostly in the advanced stages of GBC, and therefore, *P53* has been considered to be involved only in the late events of GBC carcinogenesis favoring an aggressive behavior. Reports concerning *P16* point mutation in GBC showed alteration rate of 40 and 80 %. Similar to *P53*, the *P16* mutations or down-regulation occurred only at the advanced stage of GBC [102]. Point mutations of serine or threonine phosphorylation sites in exon 3 of β -catenin have been detected at higher rates in GBC than in bile duct carcinomas [110]. Finally, substitution and deletion of the *CTTNB1* gene causing Wnt/ β -catenin activation and associated with chromosomal stability has been described in the majority of GBC (from 58 to 62 %), while substitution and insertion in the *KEAP1* gene have been described only in 30 % of GBC cases. Mutations of *PIK3CA* have been also described in GBC [111]. A mass spectrometry-based platform evaluating common cancer-associated mutations across a panel of 77 formalin-fixed paraffin-embedded biliary tree cancer specimens (32 GBC, 45 CCA) demonstrated how activating mutations in *PIK3CA* occur only in GBC (4/32, 12.5 %) [111]. This was confirmed in a recent study by sequencing analysis where even higher rates of *PIK3CA* mutations (32.4 %) were found in GBC [39]. Finally, LOHs of multiple

chromosomes have been described not only in GB cancers, but also in the dysplastic lesions of gallbladder mucosa.

Given the silent clinical presentation, early diagnosis of GBC is very difficult. In light of the discussed findings, the screening and surveillance of patients affected by serious risk factors such as AUPBD could be performed by searching for *KRAS* p.Q25H polymorphism, but this needs further evaluations in different geographic areas. Biomarkers helping diagnosis have been recently investigated by evaluating gene and protein expression profile (proteomic) of GBC, compared with benign pathologies or normal tissues. A largely different profile of proteins expression marks GBC, since 46 differentially expressed proteins have been individuated by two-dimensional gel electrophoresis and by mass spectrometry. The increased level of PEBP1 protein in GBC with respect to normal mucosa has been confirmed by immunohistochemical analysis [112]. The connective tissue growth factor (CTGF) transcripts were significantly overexpressed in microdissected GBC when compared to non-neoplastic gallbladder epithelium by real-time qPCR [113]. Using a similar proteomic analysis, it has been shown that annexin A3 expression is significantly higher in GBC cancer than in chronic cholecystitis (74.0 vs. 21.1 %) [112].

Molecular profiling of GBC has been also investigated in relation to prognostic factors. Different studies suggest that gene expression or proteomic profiles can be predictive of progression and invasiveness of GBC. For example, gene expression profile evaluated by cDNA array technology showed a significantly higher expression in node-positive with respect to node-negative GBC cases of the following genes: arginine vasopressin receptor 2, sulfotransferase family, cytosolic 2B member 1, CD152 antigen. In contrast, phosphodiesterase 4C and CD1A antigen were markedly down-regulated [81]. By a proteomic evaluation, overexpression of annexin A3 gene resulted correlated significantly with lymphonode positivity or distant metastasis (40.9 vs. 100 %) or a shorter survival time after operation (50.0 vs. 93.8 %) [112]. Connective tissue growth factor (CTGF) gene overexpression has been observed in microdissected primary GBC, but not in metastatic GBC, compared with non-neoplastic gallbladder epithelium. High CTGF antigen labeling by immunohistochemistry has been significantly associated with better survival on univariate analysis [113]. The expression of MK-1, a tumor-associated antigen encoded by the *GA733-2* gene, was demonstrated in 79 % of GBCs but with large changes in relation to histologic grade. MK-1 expression, in fact, occurred in approximately 90 % of well-differentiated tubular adenocarcinomas but only in approximately 10 % of poorly differentiated adenocarcinomas. In addition, multivariate analysis showed that MK-1 expression is an independent prognostic marker, significantly correlated with increased

overall survival [114]. Therefore, MK-1 could be a useful prognostic marker for GBC. Recently, CD44 and CD133 emerged as cell surface markers for CSCs in GBC [115].

7 Conclusions

The biliary tract and gallbladder cancers are still a challenge for scientists and clinicians. These tumors usually progress insidiously, are difficult to diagnose, and have a bad prognosis. Unfortunately, treatment options are discouraging. In fact, radical surgery, the only effective treatment, is applicable in a minority of patients due to the late clinical presentation and diagnosis. Thus, to improve survival, the early detection of biliary tract and gallbladder cancers seems to be essential. Molecular biomarkers or gene polymorphisms allowing screening and surveillance of population at risk represent a necessity for the near future. Furthermore, molecular profiling analyses providing a detailed tissue evaluation for diagnosis, prognosis, and staging other than guiding therapeutic decisions are absolutely demanding. As discussed in this article, several studies have evaluated gene mutations in CCA and GBC and their impact as diagnostic or prognostic tool. Unfortunately, conclusive data are limited by the small number of samples analyzed, the CCA heterogeneity, and, mainly, the requirement of validation studies in independent cohorts of samples.

References

1. Nakanuma Y, Sripa B, Vatanasapt V et al (2000) Intrahepatic cholangiocarcinoma. In: Hamilton SR, Aaltonen LA (eds) World Health Organization classification of tumours: pathology and genetics of tumours of the digestive system. IARC Press, Lyon, pp 173–180
2. Blechacz B, Komuta M, Roskams T et al (2011) Clinical diagnosis and staging of cholangiocarcinoma. *Nat Rev Gastroenterol Hepatol* 8:512–522
3. Razumilava N, Gores GJ (2013) Classification, diagnosis, and management of cholangiocarcinoma. *Clin Gastroenterol Hepatol* 11(13–21):e1
4. Cardinale V, Carpino G, Reid L et al (2012) Multiple cells of origin in cholangiocarcinoma underlie biological, epidemiological and clinical heterogeneity. *World J Gastrointest Oncol* 4:94–102
5. Fabris L, Alvaro D (2012) The prognosis of perihilar cholangiocarcinoma after radical treatments. *Hepatology* 56:800–802
6. Fabris L, Cadamuro M, Moserle L et al (2011) Nuclear expression of S100A4 calcium-binding protein increases cholangiocarcinoma invasiveness and metastasization. *Hepatology* 54:890–899
7. Cardinale V, Semeraro R, Torrice A et al (2010) Intra-hepatic and extra-hepatic cholangiocarcinoma: new insight into epidemiology and risk factors. *World J Gastrointest Oncol* 2:407–416
8. Bragazzi MC, Cardinale V, Carpino G et al (2012) Cholangiocarcinoma: epidemiology and risk factors. *Transl Gastrointest Cancer* 1:21–32. doi:10.3978/j.issn.2224-4778.2011.11.04
9. Khan SA, Davidson BR, Goldin RD et al (2012) Guidelines for the diagnosis and treatment of cholangiocarcinoma: an update. *Gut* 61:1657–1669
10. Nault JC, Zucman-Rossi J (2011) Genetics of hepatobiliary carcinogenesis. *Semin Liver Dis* 31:173–187
11. Gatto M, Alvaro D (2010) New insights on cholangiocarcinoma. *World J Gastrointest Oncol* 2:136–145
12. Chen CP, Haas-Kogan D (2010) Neoplasms of the hepatobiliary system: clinical presentation, molecular pathways and diagnostics. *Expert Rev Mol Diagn* 10:883–895
13. Alvaro D (2011) Progranulin and cholangiocarcinoma: another bad boy on the block! *Gut* 61:170–171
14. Hainsworth JD, Rubin MS, Spigel DR et al (2013) Molecular gene expression profiling to predict the tissue of origin and direct site-specific therapy in patients with carcinoma of unknown primary site: a prospective trial of the Sarah Cannon research institute. *J Clin Oncol* 31:217–223
15. Ismael G, de Azambuja E, Awada A (2006) Molecular profiling of a tumor of unknown origin. *N Engl J Med* 355:1071–1072
16. Ioannidis JP (2007) Is molecular profiling ready for use in clinical decision making? *Oncologist* 12:301–311
17. Kumar M, Zhao X, Wang XW et al (2012) Molecular carcinogenesis of hepatocellular carcinoma and intrahepatic cholangiocarcinoma: one step closer to personalized medicine? *Hepatology* 56:800–802
18. Khan SA, Thomas HC, Toledano MB et al (2005) p53 Mutations in human cholangiocarcinoma: a review. *Liver Int* 25:704–716
19. Kamikawa T, Amenomori M, Itoh T et al (1999) Analysis of genetic changes in intrahepatic cholangiocarcinoma induced by thorotrast. *Radiat Res* 152:S118–S124
20. Kiba T, Tsuda H, Pairojkul C et al (1993) Mutations of the p53 tumor suppressor gene and the ras gene family in intrahepatic cholangiocellular carcinomas in Japan and Thailand. *Mol Carcinog* 8:312–318
21. Petmitr S, Pinlaor S, Thousungnoen A et al (1998) K-ras oncogene and p53 gene mutations in human cholangiocarcinoma from Thai patients. *Southeast Asian J Trop Med Public Health* 29:71–75
22. Sturm PD, Baas IO, Clement MJ et al (1998) Alterations of the p53 tumor-suppressor gene and K-ras oncogene in perihilar cholangiocarcinomas from a high-incidence area. *Int J Cancer* 78:695–698
23. Jonas S, Springmeier G, Tauber R et al (1998) p53 mutagenesis in Klatskin tumors. *Hum Pathol* 29:955–960
24. Kang YK, Kim WH, Lee HW et al (1999) Mutation of p53 and K-ras, and loss of heterozygosity of APC in intrahepatic cholangiocarcinoma. *Lab Invest* 79:477–483
25. Furubo S, Harada K, Shimonishi T et al (1999) Protein expression and genetic alterations of p53 and ras in intrahepatic cholangiocarcinoma. *Histopathology* 35:230–240
26. Della Torre G, Pasquini G, Pilotti S et al (2000) TP53 mutations and mdm2 protein overexpression in cholangiocarcinomas. *Diagn Mol Pathol* 9:41–46
27. Tullo A, D'Erchia AM, Honda K et al (2000) New p53 mutations in hilar cholangiocarcinoma. *Eur J Clin Invest* 30:798–803
28. Momoi H, Itoh T, Nozaki Y et al (2001) Microsatellite instability and alternative genetic pathway in intrahepatic cholangiocarcinoma. *J Hepatol* 35:235–244
29. Liu XF, Zhang H, Zhu SG et al (2006) Correlation of p53 gene mutation and expression of P53 protein in cholangiocarcinoma. *World J Gastroenterol* 12:4706–4709
30. Imai Y, Oda H, Arai M et al (1996) Mutational analysis of the p53 and K-ras genes and allelotype study of the Rb-1 gene for

- investigating the pathogenesis of combined hepatocellular-cholangiocellular carcinomas. *Jpn J Cancer Res* 87:1056–1062
31. Itoi T, Takei K, Shinohara Y et al (1999) K-ras codon 12 and p53 mutations in biopsy specimens and bile from biliary tract cancers. *Pathol Int* 49:30–37
 32. Lai GH, Zhang Z, Shen XN et al (2005) erbB-2/neu transformed rat cholangiocytes recapitulate key cellular and molecular features of human bile duct cancer. *Gastroenterology* 129:2047–2057
 33. Aishima SI, Taguchi KI, Sugimachi K et al (2002) c-erbB-2 and c-Met expression relates to cholangiocarcinogenesis and progression of intrahepatic cholangiocarcinoma. *Histopathology* 40:269–278
 34. Andersen JB, Thorgeirsson SS (2012) Genetic profiling of intrahepatic cholangiocarcinoma. *Curr Opin Gastroenterol* 28:266–272
 35. Tada M, Omata M, Ohto M (1992) High incidence of ras gene mutation in intrahepatic cholangiocarcinoma. *Cancer* 69:1115–1118
 36. Ohashi K, Nakajima Y, Kanehiro H et al (1995) Ki-ras mutations and p53 protein expressions in intrahepatic cholangiocarcinomas: relation to gross tumor morphology. *Gastroenterology* 109:1612–1617
 37. Tannapfel A, Benicke M, Katalinic A et al (2000) Frequency of p16(INK4A) alterations and K-ras mutations in intrahepatic cholangiocarcinoma of the liver. *Gut* 47:721–727
 38. Ahrendt SA, Rashid A, Chow JT et al (2000) p53 overexpression and K-ras gene mutations in primary sclerosing cholangitis-associated biliary tract cancer. *J Hepatobiliary Pancreat Surg* 7:426–431
 39. Xu RF, Sun JP, Zhang SR et al (2011) KRAS and PIK3CA but not BRAF genes are frequently mutated in Chinese cholangiocarcinoma patients. *Biomed Pharmacother* 65:22–26
 40. Rashid A, Ueki T, Gao YT et al (2002) K-ras mutation, p53 overexpression, and microsatellite instability in biliary tract cancers: a population-based study in China. *Clin Cancer Res* 8:3156–3163
 41. Isa T, Tomita S, Nakachi A et al (2002) Analysis of microsatellite instability, K-ras gene mutation and p53 protein overexpression in intrahepatic cholangiocarcinoma. *Hepato-gastroenterology* 49(45):604–608
 42. Komori J, Marusawa H, Machimoto T et al (2008) Activation-induced cytidine deaminase links bile duct inflammation to human cholangiocarcinoma. *Hepatology* 47:888–896
 43. Borger DR, Tanabe KK, Fan KC et al (2012) Frequent mutation of isocitrate dehydrogenase (IDH)1 and IDH2 in cholangiocarcinoma identified through broad-based tumor genotyping. *Oncologist* 17:72–79
 44. Isomoto H, Mott JL, Kobayashi S et al (2007) Sustained IL-6/STAT-3 signaling in cholangiocarcinoma cells due to SOCS-3 epigenetic silencing. *Gastroenterology* 132:384–396
 45. Sandhu DS, Shire AM, Roberts LR (2008) Epigenetic DNA hypermethylation in cholangiocarcinoma: potential roles in pathogenesis, diagnosis and identification of treatment targets. *Liver Int* 28:12–27
 46. Isomoto H, Kobayashi S, Werneburg NW et al (2005) Interleukin 6 upregulates myeloid cell leukemia-1 expression through a STAT3 pathway in cholangiocarcinoma cells. *Hepatology* 42:1329–1338
 47. Kobayashi S, Werneburg NW, Bronk SF et al (2005) Interleukin-6 contributes to Mcl-1 up-regulation and TRAIL resistance via an Akt-signaling pathway in cholangiocarcinoma cells. *Gastroenterology* 128:2054–2065
 48. Kim SH, Ricci MS, El-Deiry WS (2008) Mcl-1: a gateway to TRAIL sensitization. *Cancer Res* 68:2062–2064
 49. Frampton G, Invernizzi P, Bernuzzi F et al (2012) Interleukin-6-driven progranulin expression increases cholangiocarcinoma growth by an Akt dependent mechanism. *Gut* 61:268–277
 50. Endo K, Yoon BI, Pairojkul C et al (2002) ERBB-2 overexpression and cyclooxygenase-2 up-regulation in human cholangiocarcinoma and risk conditions. *Hepatology* 36:439–450
 51. Jaiswal M, LaRusso NF, Burgart LJ et al (2000) Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by a nitric oxide-dependent mechanism. *Cancer Res* 60:184–190
 52. Alvaro D, Barbaro B, Franchitto A et al (2006) Estrogens and insulin-like growth factor 1 modulate neoplastic cell growth in human cholangiocarcinoma. *Am J Pathol* 169:877–888
 53. Mancino A, Mancino MG, Glaser SS et al (2009) Estrogens stimulate the proliferation of human cholangiocarcinoma by inducing the expression and secretion of vascular endothelial growth factor. *Dig Liver Dis* 41:156–163
 54. Alvaro D, Macarri G, Mancino MG et al (2007) Serum and biliary insulin-like growth factor I and vascular endothelial growth factor in determining the cause of obstructive cholestasis. *Ann Intern Med* 147:451–459
 55. McKay SC, Unger K, Pericleous S et al (2011) Array comparative genomic hybridization identifies novel potential therapeutic targets in cholangiocarcinoma. *HPB (Oxford)* 13:309–319
 56. Seol MA, Chu IS, Lee MJ et al (2011) Genome-wide expression patterns associated with oncogenesis and sarcomatous transdifferentiation of cholangiocarcinoma. *BMC Cancer* 11:78. doi: [10.1086/1471-2407-11-78](https://doi.org/10.1086/1471-2407-11-78)
 57. Andersen JB, Spee B, Blechacz BR et al (2012) Genomic and genetic characterization of cholangiocarcinoma identifies therapeutic targets for tyrosine kinase inhibitors. *Gastroenterology* 142:1021–1031
 58. Kipp BR, Fritcher EG, Clayton AC et al (2010) Comparison of KRAS mutation analysis and FISH for detecting pancreaticobiliary tract cancer in cytology specimens collected during endoscopic retrograde cholangiopancreatography. *J Mol Diagn* 12:780–786
 59. Huang L, Frampton G, Liang LJ et al (2010) Aberrant DNA methylation profile in cholangiocarcinoma. *World J Gastrointest Pathophysiol* 1:23–29
 60. Huang L, Frampton G, Rao A et al (2012) Monoamine oxidase a expression is suppressed in human cholangiocarcinoma via coordinated epigenetic and IL-6-driven events. *Lab Invest* 92:1451–1460
 61. Sakoda LC, Gao YT, Chen BE et al (2006) Prostaglandin-endoperoxide synthase 2 (PTGS2) gene polymorphisms and risk of biliary tract cancer and gallstones: a population-based study in Shanghai, China. *Carcinogenesis* 27:1251–1256
 62. Sripa B, Kaewkes S, Sithithaworn P et al (2007) Liver fluke induces cholangiocarcinoma. *PLoS Med* 4:e201
 63. Jinawath N, Chamgramol Y, Furukawa Y et al (2006) Comparison of gene expression profiles between *Opisthorchis viverrini* and non-*Opisthorchis viverrini* associated human intrahepatic cholangiocarcinoma. *Hepatology* 44:1025–1038
 64. Wang X, Chen W, Huang Y et al (2011) The draft genome of the carcinogenic human liver fluke *Clonorchis sinensis*. *Genome Biol* 12:R107
 65. Young ND, Campbell BE, Hall RS et al (2010) Unlocking the transcriptomes of two carcinogenic parasites, *Clonorchis sinensis* and *Opisthorchis viverrini*. *PLoS Negl Trop Dis* 4:e719. doi: [10.1371/journal.pntd.0000719](https://doi.org/10.1371/journal.pntd.0000719)
 66. Sriraksa R, Zeller C, El-Bahrawy MA et al (2011) CpG-island methylation study of liver fluke-related cholangiocarcinoma. *Br J Cancer* 104:1313–1318

67. Melum E, Karlsen TH, Schrumpf E et al (2008) Cholangiocarcinoma in primary sclerosing cholangitis is associated with NKG2D polymorphisms. *Hepatology* 47:90–96
68. Wang Z, Gerstein M, Snyder M (2009) RNA-Seq: a revolutionary tool for transcriptomics. *Nature Rev Genetics* 10(1):57–63
69. Hansel DE, Rahman A, Hidalgo M et al (2003) Identification of novel cellular targets in biliary tract cancers using global gene expression technology. *Am J Pathol* 163(1):217–229
70. Obama K, Ura K, Li M et al (2005) Genome-wide analysis of gene expression in human intrahepatic cholangiocarcinoma. *Hepatology* 41:1339–1348
71. Hass HG, Nehls O, Jobst J et al (2008) Identification of osteopontin as the most consistently over-expressed gene in intrahepatic cholangiocarcinoma: detection by oligonucleotide microarray and real-time PCR analysis. *World J Gastroenterol* 14:2501–2510
72. Carpino G, Cardinale V, Reid L et al (2012) Cells of origin and cancer stem cells in cholangiocarcinoma. *Transl Gastrointest Cancer* 1:33–43
73. Roskams T (2006) Liver stem cells and their implication in hepatocellular and cholangiocarcinoma. *Oncogene* 25:3818–3822
74. Tannapfel A, Sommerer F, Benicke M et al (2003) Mutations of the BRAF gene in cholangiocarcinoma but not in hepatocellular carcinoma. *Gut* 52:706–712
75. Tan FL, Ooi A, Huang D et al (2010) p38delta/MAPK13 as a diagnostic marker for cholangiocarcinoma and its involvement in cell motility and invasion. *Int J Cancer* 126:2353–2361
76. Terada T, Nakanuma Y (1996) Expression of apoptosis, proliferating cell nuclear antigen, and apoptosis-related antigens (bcl-2, c-myc, Fas, Lewis(y) and p53) in human cholangiocarcinomas and hepatocellular carcinomas. *Pathol Int* 46:764–770
77. Chapman MH, Tidswell R, Dooley JS et al (2011) Whole genome RNA expression profiling of endoscopic biliary brushings provides data suitable for biomarker discovery in cholangiocarcinoma. *Genome Biol* 12:R107
78. Finkelstein SD, Bibbo M, Loren DE et al (2012) Molecular analysis of centrifugation supernatant fluid from pancreaticobiliary duct samples can improve cancer detection. *Acta Cytol* 56:439–447
79. Malouf G, Dreyer C, Guedj N et al (2009) Prognosis factors of cholangiocarcinoma: contribution of recent molecular biology tools. *Bull Cancer* 96:405–415
80. Utispan K, Thuwajit P, Abiko Y et al (2010) Gene expression profiling of cholangiocarcinoma-derived fibroblast reveals alterations related to tumor progression and indicates periostin as a poor prognostic marker. *Mol Cancer* 9:13
81. Murakawa K, Tada M, Takada M et al (2004) Prediction of lymph node metastasis and perineural invasion of biliary tract cancer by selected features from cDNA array data. *J Surg Res* 122:184–194
82. Sia D, Hoshida Y, Villanueva A et al (2013) Integrative molecular analysis of intrahepatic cholangiocarcinoma reveals 2 classes that have different outcomes. *Gastroenterology*. doi:10.1053/j.gastro.2013.01.001
83. Oikawa T, Kamiya A, Zeniya M et al (2012) SALL4, a stem cell biomarker in liver cancers. *Hepatology*. doi:10.1002/hep.26147
84. Oishi N, Kumar MR, Roessler S et al (2012) Transcriptomic profiling reveals hepatic stem-like gene signatures and interplay of miR-200c and epithelial-mesenchymal transition in intrahepatic cholangiocarcinoma. *Hepatology* 56:1792–1803
85. He XR, Wu XP (2008) et al Difference in biological characteristics and sensitivity to chemotherapy and radiotherapy between intrahepatic and extrahepatic cholangiocarcinoma cells in vitro. *Chin Med Sci J* 23:54–59
86. Guedj N, Zhan Q, Perigny M (2009) et al. Comparative protein expression profiles of hilar and peripheral hepatic cholangiocarcinomas. *J Hepatol* 51:93–101
87. Karamitopoulou E, Tornillo L, Zlobec I et al (2008) Clinical significance of cell cycle- and apoptosis-related markers in biliary tract cancer: a tissue microarray-based approach revealing a distinctive immunophenotype for intrahepatic and extrahepatic cholangiocarcinomas. *Am J Clin Pathol* 130:780–786
88. Miller G, Socci ND, Dhall D et al (2009) Genome wide analysis and clinical correlation of chromosomal and transcriptional mutations in cancers of the biliary tract. *J Exp Clin Cancer Res* 28:62
89. Yang B, House MG, Guo M et al (2005) Promoter methylation profiles of tumor suppressor genes in intrahepatic and extrahepatic cholangiocarcinoma. *Mod Pathol* 18:412–420
90. Nakanuma Y, Sato Y, Harada K et al (2010) Pathological classification of intrahepatic cholangiocarcinoma based on a new concept. *World J Hepatol* 2:419–427
91. Komuta M, Govaere O, Vandecaveye V et al (2012) Histological diversity in cholangiocellular carcinoma reflects the different cholangiocyte phenotypes. *Hepatology* 55:1876–1888
92. Ueno Y, Alpini G, Yahagi K et al (2003) Evaluation of differential gene expression by microarray analysis in small and large cholangiocytes isolated from normal mice. *Liver Int* 23:449–459
93. Schmelzer E, Zhang L, Bruce A et al (2007) Human hepatic stem cells from fetal and postnatal donors. *J Exp Med* 204:1973–1987
94. Turner R, Lozoya O, Wang Y et al (2011) Human hepatic stem cell and maturational liver lineage biology. *Hepatology* 53:1035–1045
95. Cardinale V, Wang Y, Carpino G et al (2011) Multipotent stem/progenitor cells in human biliary tree give rise to hepatocytes, cholangiocytes, and pancreatic islets. *Hepatology* 54:2159–2172
96. Cardinale V, Wang Y, Carpino G et al (2012) The biliary tree—a reservoir of multipotent stem cells. *Nat Rev Gastroenterol Hepatol* 9(4):231–240
97. Aishima S, Kuroda Y, Nishihara Y et al (2007) Proposal of progression model for intrahepatic cholangiocarcinoma: clinicopathologic differences between hilar type and peripheral type. *Am J Surg Pathol* 31:1059–1067
98. Lee JS, Heo J, Libbrecht L et al (2006) A novel prognostic subtype of human hepatocellular carcinoma derived from hepatic progenitor cells. *Nat Med* 12:410–416
99. Komuta M, Spee B, Vander Borgh S et al (2008) Clinicopathological study on cholangiolocellular carcinoma suggesting hepatic progenitor cell origin. *Hepatology* 47:1544–1556
100. Yokoyama N, Hitomi J, Watanabe H et al (1998) Mutations of p53 in gallbladder carcinomas in high-incidence areas of Japan and Chile. *Cancer Epidemiol Biomarkers Prev* 7:297–301. doi:10.1186/1471-2407-11-78
101. Nigam P, Misra U, Negi TS et al (2010) Alterations of p53 gene in gallbladder cancer patients of North India. *Trop Gastroenterol* 31:96–100
102. Kim YT, Kim J, Jang YH et al (2001) Genetic alterations in gallbladder adenoma, dysplasia and carcinoma. *Cancer Lett* 169:59–68
103. Nagahashi M, Ajioka Y, Lang I et al (2008) Genetic changes of p53, K-ras, and microsatellite instability in gallbladder carcinoma in high-incidence areas of Japan and Hungary. *World J Gastroenterol* 14:70–75
104. Imai M, Hoshi T, Ogawa K (1994) K-ras codon 12 mutations in biliary tract tumors detected by polymerase chain reaction denaturing gradient gel electrophoresis. *Cancer* 73:2727–2733

105. Ajiki T, Fujimori T, Onoyama H et al (1996) K-ras gene mutation in gall bladder carcinomas and dysplasia. *Gut* 38:426–429
106. Hanada K, Tsuchida A, Iwao T et al (1999) Gene mutations of K-ras in gallbladder mucosae and gallbladder carcinoma with an anomalous junction of the pancreaticobiliary duct. *Am J Gastroenterol* 94:1638–1642
107. Saetta AA, Papanastasiou P, Michalopoulos NV et al (2004) Mutational analysis of BRAF in gallbladder carcinomas in association with K-ras and p53 mutations and microsatellite instability. *Virchows Arch* 445:179–182
108. Parwani AV, Geradts J, Caspers E et al (2003) Immunohistochemical and genetic analysis of non-small cell and small cell gallbladder carcinoma and their precursor lesions. *Mod Pathol* 16:299–308
109. Pai RK, Mojtahed K, Pai RK et al (2011) Mutations in the RAS/RAF/MAP kinase pathway commonly occur in gallbladder adenomas but are uncommon in gallbladder adenocarcinomas. *Appl Immunohistochem Mol Morphol* 19:133–140
110. Pramanik V, Sarkar BN, Kar M et al (2011) A novel polymorphism in codon 25 of the KRAS gene associated with gallbladder carcinoma patients of the eastern part of India. *Genet Test Mol Biomarkers* 15:431–434
111. Kimura Y, Furuhashi T, Mukaiya M et al (2011) Mutational profiling reveals PIK3CA mutations in gallbladder carcinoma. Frequent beta-catenin alteration in gallbladder carcinomas. *BMC Cancer* 11:60
112. Tan Y, Meng HP, Wu Q et al (2010) Proteomic study of gallbladder cancer, with special reference on the expression and significance of annexin A3. *Zhonghua Bing Li Xue Za Zhi* 39:382–386
113. Alvarez H, Corvalan A, Roa JC et al (2008) Serial analysis of gene expression identifies connective tissue growth factor expression as a prognostic biomarker in gallbladder cancer. *Clin Cancer Res* 14:2631–2638
114. Ikeda T, Nakayama Y, Hamada Y et al (2009) FU-MK-1 expression in human gallbladder carcinoma: an antigenic prediction marker for a better postsurgical prognosis. *Am J Clin Pathol* 132:111–117
115. Shi C, Tian R, Wang M et al (2010) CD44+ CD133+ population exhibits cancer stem cell-like characteristics in human gallbladder carcinoma. *Cancer Biol Ther* 10:1182–1190

Overview of Current Strategies for Diagnostic Imaging of Biliary Tract and Gallbladder Tumors

Heljä Oikarinen

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Abstract

Early diagnosis of biliary cancers would be important to improve their prognosis, and accurate staging would help to choose the best possible treatment. However, biliary cancers present specific diagnostic challenges. Imaging modalities, imaging-guided fine-needle aspiration, and endoscopic brush samples play a crucial role in the diagnostic work-up. However, there is no single modality capable of reliably detecting and accurately staging biliary cancers; hence, complementary modalities are usually needed. Transabdominal ultrasound (US) is often the first imaging modality applied to patients with jaundice or nonspecific gastrointestinal complaints. US visualizes bile duct obstruction accurately and is a suitable method for assessing even mild symptoms, and it is noninvasive, nonradiative, and commonly available. If a biliary malignancy is suspected, further investigations are usually performed after US. Magnetic resonance imaging (MRI) and multidetector computed tomography (MDCT) may yield additional information of the tumor and/or its extent. Fast-imaging techniques have made MRI potentially more valuable, and magnetic resonance cholangiography (MRC) is the least invasive mode of cholangiography, which is useful with MRI in the case of biliary obstruction. MDCT can produce multiplanar reconstructions of good quality but it has exposed patients to relatively high dose of radiation. In ambiguous cases, both MRI and MDCT may be needed. Direct cholangiography may provide the most accurate anatomic information of the bile ducts. It is also needed for therapeutic purposes in the case of bile duct obstruction. Further, positron emission tomography (PET), PET/CT, and endoscopic or intraductal US may help in the diagnostic work-up, when available.

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1 Introduction

Carcinoma of the gallbladder is the most common biliary malignancy. Cancers of the bile ducts are less common, but their incidence has been increasing. Bile duct tumors can be classified as intrahepatic (or peripheral) cholangiocarcinoma (ICC), hilar (or Klatskin) tumors, and extrahepatic tumors. Klatskin tumors are the most common. Most biliary tumors (tumors of the gallbladder and the bile ducts) are malignant adenocarcinomas, the prognosis for which has been dismal. Early diagnosis of biliary tumors would be important to improve their prognosis, and accurate staging would help to choose the best possible treatment. However, biliary tumors present specific diagnostic challenges. Their symptoms may be mild or unspecific, such as abdominal pain, malaise, mild fever, or weight loss. In the case of bile duct obstruction, jaundice may be the presenting sign. The differences in the clinical behavior of bile duct cancers are due to variation in the location and size of the tumor at the time of diagnosis. A tumor of the papilla of Vater or the distal common bile duct may cause jaundice at an early stage, while ICC—or gallbladder carcinomas—is often advanced before causing symptoms of obstruction. Gallbladder carcinoma is often found incidentally in a resected cholecystectomy specimen. Gallstones are present in most of the affected patients [1–3].

Imaging modalities, image-guided fine-needle aspiration (FNA), and endoscopic brush samples play a crucial role in the diagnostic work-up, although laboratory findings or tumor markers may also be suggestive of a tumor. However, there is no single modality capable of reliably detecting and accurately staging biliary cancers; hence, complementary modalities are usually needed.

This chapter will concentrate on the potential of different imaging modalities to respond to the challenge of how to diagnose and stage biliary cancers. The current state-of-the-art strategies are also discussed. Similar imaging modalities and diagnostic strategies are mainly used for both carcinoma of the gallbladder and carcinoma of the bile ducts. Therefore, the possibilities of each imaging method in both cancer types are presented under the subheadings of the modalities. Although jaundice with bile duct obstruction is typical for cancer of the bile ducts, it is also common in advanced gallbladder cancer.

The accuracy for diagnosing a bile duct carcinoma has been up to 84 % for ultrasound (US), 94 % for computed tomography (CT), and 95 % for magnetic resonance imaging (MRI) with magnetic resonance cholangiopancreatography (MRCP) [4]. However, comparative studies of the accuracies of modern MRI with MRCP and multidetector computed tomography (MDCT) in biliary cancers are sparse. It is also challenging to compare existing studies due to the differences in study design, algorithms, or equipment and the differences

in the origin of the tumors. There is also rapid emergence of novel technology. The classification and nomenclature of bile duct tumors and classification for operability are variable, as well. Nevertheless, the conclusions of the pertinent literature are highlighted and discussed.

2 Spread, Staging, and Treatment of Biliary Cancers

With the exception of ampullary carcinoma, the prognosis of biliary carcinomas has been poor. In biliary cancers, the histologic type, the staging, and, in the case of carcinoma of the bile ducts, the location of the tumors are the most important prognostic factors. Papillary-type carcinomas have the most favorable prognosis. In general, resection provides the only chance of cure, and since advanced surgical techniques are increasingly used, there is a need for accurate preoperative staging and determination of the best therapeutic option.

Gallbladder carcinoma spreads early in its course. It invades the wall of the gallbladder and into the liver and spreads into the lymph nodes. Common bile duct, hepatic artery, portal vein, stomach, duodenum, transverse colon, pancreas, and omentum are at risk of tumor extension. It usually metastasizes to the peritoneum and liver and, occasionally, to the lungs and pleura.

ICC may spread to other intrahepatic locations, vessels, common bile duct, regional and more distant lymph nodes, adjacent organs, peritoneum, abdominal wall, diaphragm, lungs, and pleura. Klatskin tumors have a tendency to spread to adjacent hepatic parenchyma, vessels, bile ducts, and regional lymph nodes, especially hilar and pericholedochal nodes. It is characterized by intrahepatic ductal extension and spread along perineural and periductal lymphatic channels. Liver metastases are common and klatskin tumors may also metastasize to the peritoneal cavity, lungs, brains, and bones.

Distal bile duct cancer can spread to the vessels, lymph nodes, pancreas, duodenum, stomach, colon, or omentum. Distant metastases may occur in the liver, peritoneum, and lungs. Ampullary carcinoma may spread to the regional lymph nodes and adjacent structures, such as duodenum, the head of the pancreas, and extrahepatic bile ducts. Metastases may occur in the liver, peritoneum, lungs, and pleura.

In the case of a bile duct tumor, in addition to the spread, it is important to evaluate the location, length, and local invasion of the tumor. The TNM classifications of biliary tumors are used for staging. There are different staging systems. Separate staging schemes for gallbladder carcinoma, ICC, Klatskin tumors, distal tumors, and ampullary carcinoma may be used [1–3, 5].

Table 1 Primary Tumor (T) [3] (Used with the permission of the American Joint Committee on Cancer (AJCC), Chicago, Illinois)

TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor invades lamina propria or muscular layer
T1a	Tumor invades lamina propria
T1b	Tumor invades muscular layer
T2	Tumor invades perimuscular connective tissue; no extension beyond serosa or into liver
T3	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
T4	Tumor invades main portal vein or hepatic artery or invades two or more extrahepatic organs or structures

The original source for this material is the AJCC Cancer Staging Handbook, Seventh Edition (2010) published by Springer Science and Business Media LLC, <http://www.springerlink.com>.)

There are various practices for the treatment of biliary cancers. In gallbladder carcinomas, surgery is the only curative therapy in properly selected patients. T1–2 tumors are potentially resectable, while T4 tumors are usually regarded as unresectable (Table 1). Patients with advanced cancer or significant comorbidities are candidates for biliary enteric bypass or biliary drainage, and adjuvant or palliative chemotherapy and radiotherapy are also possible [6, 7]. In the case of ICC, surgical exploration is carried out if imaging reveals that a complete resection is possible. A poor prognosis is associated with regional lymph node involvement and incomplete resection in patients treated with resection [3].

There is no universally accepted surgical approach for Klatskin tumors, and variable practices are employed. Bismuth staging is often used to describe the extent of tumor involvement within the ductal system (Table 2). Criteria for unresectability have also been published and are presented in Table 3. The operative goal is complete resection with negative histologic margins, which is the most important predictor of long-term survival. However, the proximity to the hepatic artery, portal vein, and hepatic parenchyma makes excision challenging. Partial hepatic resection or total hepatectomy with transplantation are also possible. Most patients have logoregional extension or distant metastasis that precludes resection. Factors impairing survival include vascular invasion, lobar atrophy, and lymph node metastasis [3, 6, 8]. The operative procedure for distal bile duct cancers consists of pancreaticoduodenectomy or local bile duct excision. Ampullary carcinoma is usually treated by pancreaticoduodenectomy. Endoscopic treatment or transduodenal excision may also be possible [6].

Table 2 Bismuth classification of hilar cholangiocarcinoma [6]

Type I	Confluence of the right and left hepatic ducts not involved
Type II	Tumor involves the confluence of the hepatic ducts
Type III	Tumor involves the confluence of the hepatic ducts and extends into the right (IIIA) or left duct (IIIB)
Type IV	Tumor extends into both hepatic ducts and the confluence

Table 3 Criteria for unresectability in patients with hilar cholangiocarcinoma [8]

Medical comorbidities limiting the patient's ability to undergo major surgery
Significant underlying liver disease prohibiting liver resection necessary for curative surgery based on preoperative imaging
Bilateral tumor extension to secondary biliary radicals
Encasement or occlusion of the main portal vein
Lobar atrophy with contralateral portal vein involvement
Contralateral tumor extension to secondary biliary radicals
Evidence of metastases to N2 level lymph nodes ^a
Presence of distant metastases

^a N2 lymph nodes, metastasis in the peripancreatic (head only), paraduodenal, periportal, celiac, superior mesenteric, and/or posterior pancreaticoduodenal lymph nodes.

Patients with unresectable bile duct carcinoma may need palliative treatment for jaundice, which can be accomplished by biliary enteric bypass, percutaneous biliary drainage, or by inserting a plastic or metallic stent percutaneously or endoscopically. Catheters suffer from the risk of infection or dislodgement, and the major problems with plastic stents are displacement and occlusion with sludge. Self-expandable metallic stents inserted by radiologists have advantages over plastic stents, as they can be introduced on a small delivery catheter, have a large inner diameter, and remain in a fixed position after release. However, they may also cause infections or become occluded by tumor ingrowth or overgrowth. Radiotherapy and/or chemotherapy is used as adjuvant therapy or palliation, and photodynamic therapy and thermoablative procedures are also options [4, 9–11].

A few biostatistical terms will be defined here, as they are used widely in the literature and in subsequent chapters. Sensitivity is the proportion of true positives (TP) that are correctly identified by the test, and specificity is the proportion of true negatives (TN) that are correctly identified by the test. Positive predictive value is the proportion of patients with positive test results who are correctly diagnosed, and negative predictive value is the proportion of patients with negative test results who are correctly diagnosed. Accuracy is

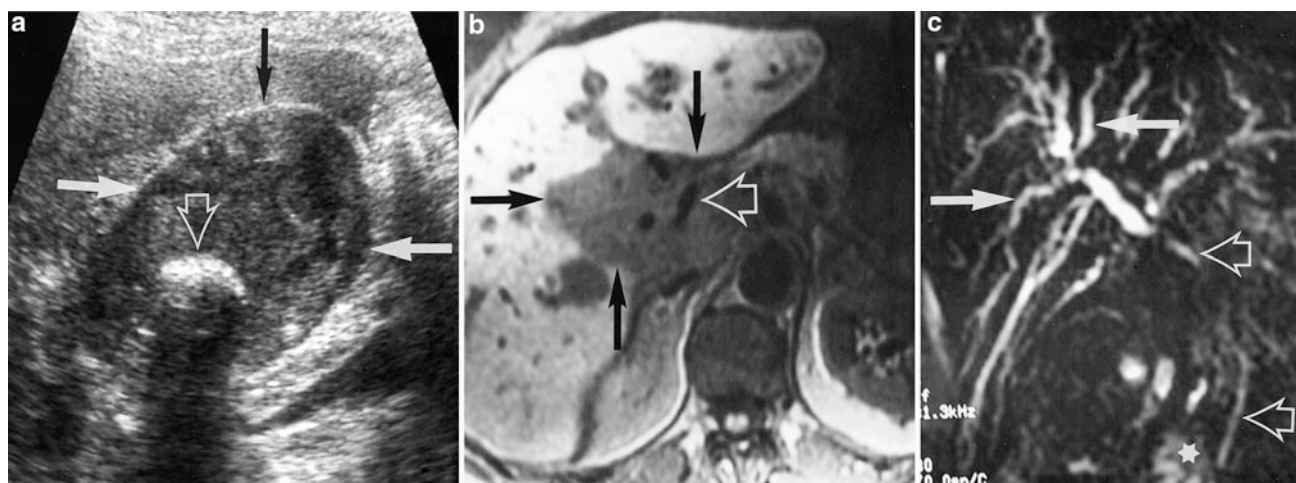


Fig. 1 Gallbladder carcinoma. **a** Sonography reveals tumorous tissue replacing the gallbladder (arrows). A gallstone is also seen (open arrow). **b** MRI (T1 fat-saturated gradient echo) shows tumorous tissue even in the hilar area (arrows). There are vessels inside the tumorous

area (open arrow). **c** MRC reveals intrahepatic bile duct dilatation (arrows). Extrahepatic bile ducts are seen only partly (open arrows) because of the strictures caused by tumorous tissue. Duodenum (*) [2]

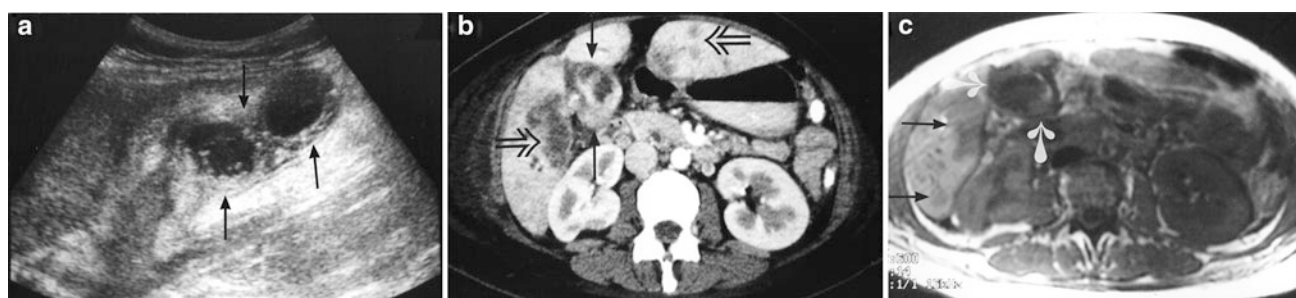


Fig. 2 Gallbladder carcinoma. **a** The gallbladder is thick-walled and deformed (arrows) due to carcinoma (sonography). **b** CT (arterial phase) also reveals a tumorous gallbladder (arrows) and metastases of

the liver (open arrows). **c** The thickened gallbladder wall (white arrows) seen in MRI (T1 spin echo). Liver metastases are also visible (black arrows) [2]

the proportion of true results in the population. It is defined as a ratio of $TP + TN$ and $TP + FP + FN + TN$ (FP = false positive and FN = false negative).

3 Ultrasound

Transabdominal US is often the first imaging modality applied to patients with nonspecific gastrointestinal complaints or jaundice. It is a suitable method for even mild symptoms, and it is commonly available. US does not include any radiation, the examination can be performed at bedside, and it is relatively inexpensive. However, the value of US depends on the experience of the operator and the quality of the equipment. It may also be problematic in the case of obese patients and in the presence of bowel gas. The sensitivity of US to reveal a primary tumor of the

gallbladder or the bile ducts has increased to over 90 % with technical development of the equipment, although problems do occur, especially with small bile duct tumors [12, 13].

3.1 Carcinoma of the Gallbladder

A tumor of the gallbladder may appear on US as a mass of variable echogenicity filling the entire lumen of the gallbladder (exophytic type) (Fig. 1). There may be tumor necrosis, and echogenic foci may be related to gallstones, porcelain gallbladder, air, or calcification of the tumor itself. Other manifestations are focal or diffuse thickening of the gallbladder wall, which can be hypo- or hyperechoic and often irregular (infiltrating type) (Fig. 2), or an intraluminal fungate mass with a nodular or smooth contour and variable

echogenicity (polypoid type). The mass type is the most common, and the infiltrating type has been the most difficult to detect by US. Gallstones may sometimes disturb the visualization of tumors [14–16].

US- or CT-guided FNA is necessary to reveal the malignant nature of the tumor. This technique has a diagnostic accuracy of 95 %. For differential diagnosis, tumorous sludge, other causes of wall thickening (e.g., cholecystitis), benign polyps, and other malignancies should be noticed.

Early-stage cancers have been difficult to detect sonographically. However, it has been reported that most early cancerous lesions appear polypoid at US, and high-resolution US can detect even small lesions. Single polyps, broad-based sessile polyps, or lesions larger than 1 cm are more likely to be malignant. There have been efforts to differentiate benign from malignant lesions with Doppler, and the results are suggestive at best [7, 17, 18].

For detailed analysis, endoscopic US (EUS) or intraductal US (IDUS) has also been promising. High-frequency EUS can provide high-resolution images, and it can reveal the layered structure of the gallbladder and gallbladder masses. It has been useful in differentiating polyps or wall thickening. In the presence of polyps, the internal echogenicity and contour of polypoid lesions are analyzed. EUS is also used to guide FNA procedures. However, EUS or IDUS are more invasive, less widely available, and more examiner-dependent. Contrast-enhanced US has also been a valuable adjunct for the differential diagnosis of polypoid lesions, and laparoscopic US may help to detect unsuspected cancer during laparoscopic cholecystectomy [19–22].

3.2 Carcinoma of the Bile Ducts

The most frequently seen abnormality due to carcinoma of the bile ducts at US is dilatation of the intrahepatic bile ducts, which may also accompany advanced gallbladder carcinoma (Fig. 3). In fact, such dilatation can be an indirect sign of a biliary tumor. The accuracy of US to define the level and cause of obstruction with surgical obstructive jaundice has been 95 and 88 %, respectively. Malignancies are found especially in obstructions at the distal or hilar level. The zone of transition from a dilated to a nondilated or nonvisualized duct should be evaluated regardless of the imaging modality. Bile duct carcinoma can also be visible as a mass (exophytic, nodular), an infiltrating tumor (sclerosing, periductally infiltrating), or a polypoid growth (papillary, intraductal growth). The infiltrating type has been especially difficult to detect. The polypoid type is rare and of low-grade malignancy [23–26].

The mass-forming type of ICC, Klatskin tumor, or extrahepatic carcinoma may present as a tumor mass with

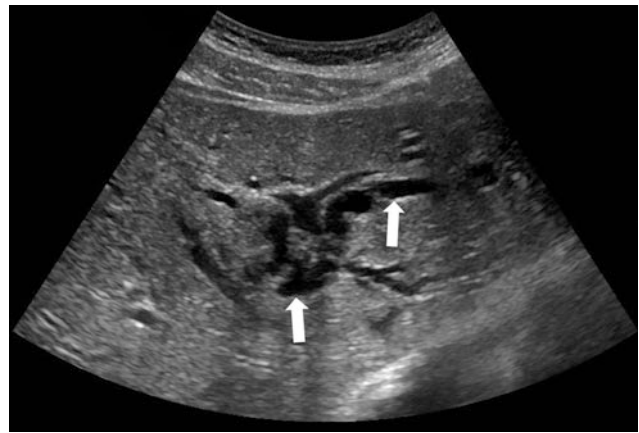


Fig. 3 Intrahepatic bile duct dilatation (arrows) seen at US

variable echogenicity (Figs. 4, 5). Carcinomas of the distal common bile duct are often small. The architecture is also dependent on the amount of fibrous tissue, mucin, calcification, and necrosis. An infiltrating tumor may show a diffusely abnormal liver echo pattern or focal irregularity of the ducts. However, in these two types, US may only reveal bile duct dilatation—a small mass or bile duct wall thickening may not be depicted. Intraductal carcinomas have a variety of imaging features. They may be single or multiple with variable echoes, and a mucin-secreting tumor (intraductal papillary mucinous tumor) may present as a cystic mass and sometimes severe bile duct dilatation. With bile duct cancers, peripheral bile duct dilatation, necrosis, satellite nodules, calcification, lobar atrophy, pressure effects, and, in the case of Klatskin tumors, segmental dilatation and nonunion of the right and left ducts may also be seen. Lobar atrophy may be caused by vascular or biliary obstruction [24, 27–29].

Contrast-enhanced US has been introduced to characterize focal liver lesions and has shown hyperperfusion in the arterial phase and punched-out defects in the late portal venous phase with ICC. It has also improved the detection and staging of malignant hilar obstruction (mostly caused by biliary malignancies) compared with unenhanced sonography [30, 31]. US- or CT-guided FNA may reveal the malignant nature of the tumor. However, FNA can be hazardous in the case of hilar tumors due to the adjacent big vessels. Differential diagnosis of bile duct cancer includes other malignant diseases (e.g., liver and lymph node metastases, hepatocellular carcinoma, pancreatic cancer, or gallbladder carcinoma), bile duct stones, and benign tumors or strictures (e.g., primary sclerosing cholangitis). Extrinsic tumors may displace, encircle, obstruct, or invade the bile ducts visualized by different modalities.

To get detailed information, laparoscopic US or EUS may show the presence and origin of a small hilar or common bile duct tumor. IDUS has also been valuable in

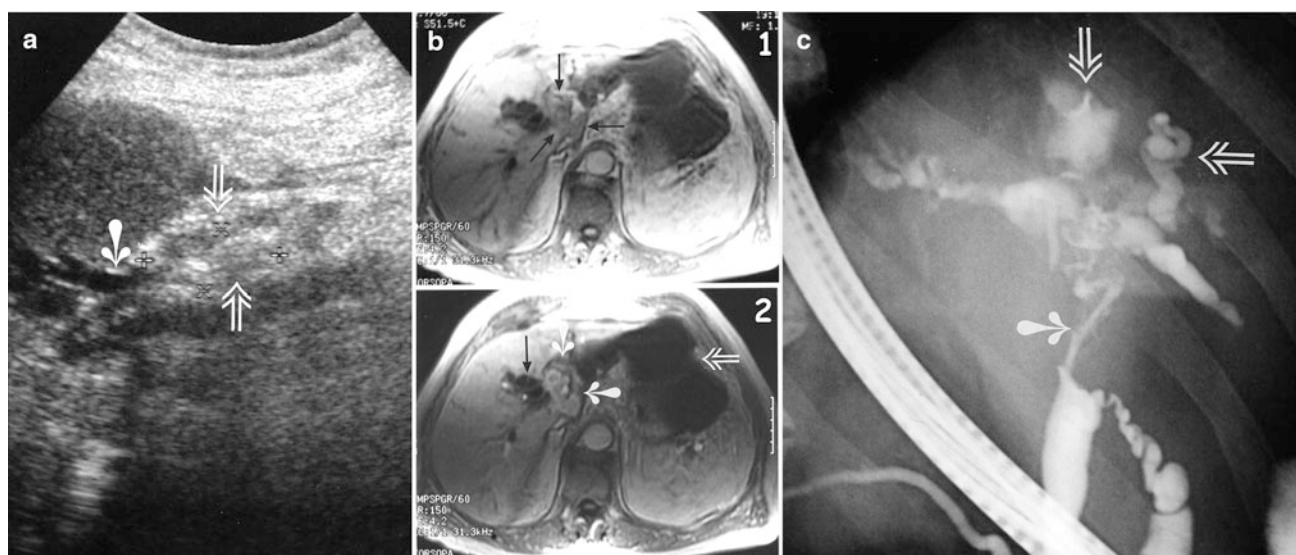


Fig. 4 Klatskin tumor. **a** Intrahepatic biliary dilatation (white arrow) ends in the hilar area, where sonography shows an unclear heterogeneous mass (open arrows). **b** MRI (T1 gradient echo) reveals slightly different tissue in the hilar area (arrows). 2 Gadolinium-enhanced MRI (T1 gradient echo) shows nonhomogeneously enhanced tissue in the

hilar area (white arrows). Intrahepatic bile duct dilatation (black arrow) and a biloma (open arrow) are also shown. **c** ERC shows a long stricture of the common hepatic duct (arrow) and intrahepatic bile duct dilatation (open arrows) [2]

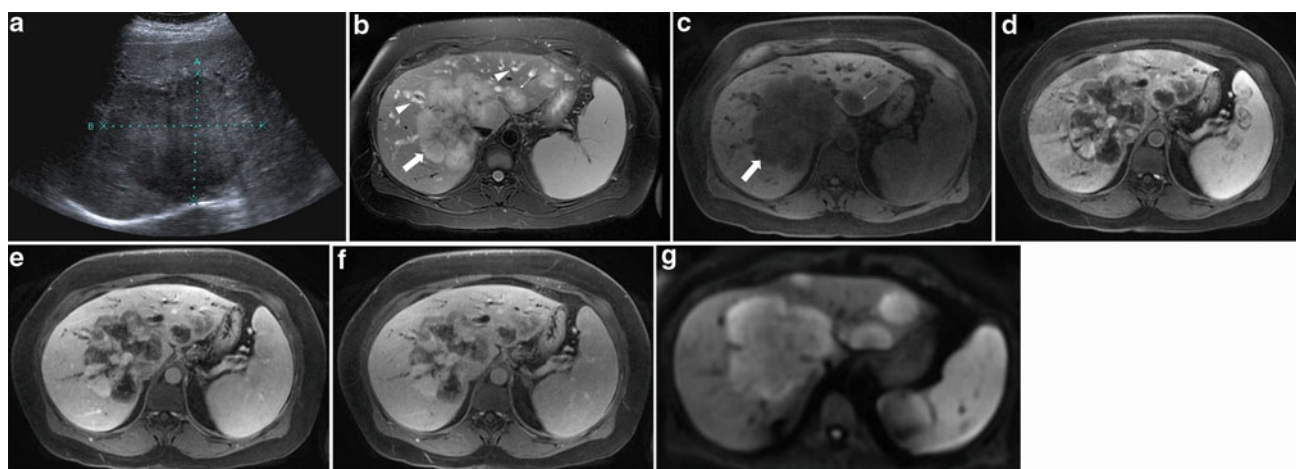


Fig. 5 Cholangiocarcinoma. **a** A large hypoechoic, heterogenic expansion is seen in the central area of the liver at US. **b** MRI (fat-saturated T2 axial FSE) reveals a large hyperintense, lobulated tumor (thick arrow) with intrahepatic metastases (thin arrow) and bile duct dilatation (arrowheads). **c–f** Gadolinium-enhanced MRI (T1 fat-

saturated gradient echo) reveals heterogenic dynamic enhancement of the tumor (thick arrow) and the metastasis (thin arrow) (unenhanced, arterial, portovenous, and delayed phases). **g** Diffusion-weighted image shows the tumor and metastases

biliary strictures, and it can show tumor extension. EUS-guided FNA is useful in bile duct tumors, too. EUS with FNA has had a greater sensitivity for detecting malignant strictures than endoscopic retrograde cholangiopancreatography (ERCP) with brushings. However, it also has the potential for tumor seeding [9, 20, 22, 32–34].

In the case of an ampullary tumor, transabdominal US may only reveal the double-duct sign (dilatation of the bile duct and the pancreatic duct). Endoscopy with a biopsy,

EUS, or IDUS may show the tumor itself, and EUS and IDUS are able to define the size, invasion, and extension of the tumor [34, 35].

3.3 Staging of Biliary Cancers by US

US may help to reveal the spread of a suspected malignancy. Doppler can be used to analyze hepatic vessels. In gallbladder carcinoma or Klatskin tumors, US with

Doppler can detect spread into the liver, the portal vein, and the bile ducts rather well, but it is not equally good in the detection of lymph node and especially peritoneal metastases. Advanced gallbladder carcinoma has been understaged by US. There are also controversial results about US in liver and lymph node invasion in gallbladder carcinoma. At any rate, other imaging modalities are also involved in the difficult analysis of pathologic, but normal-sized lymph nodes [12, 14, 27].

More invasive EUS, IDUS, or laparoscopic or intraoperative US has improved staging. EUS and IDUS are useful especially in evaluating the bile duct, the regional lymph nodes, or the vessels, but they are not suitable for the detection of distant metastases. EUS with FNA may be useful in lymphadenopathy. In addition, transabdominal or endoscopic US elastography might help to reveal malignancy of the tumors or the lymph nodes. Malignant expansions are stiffer than benign tissue [9, 13, 22, 33, 34].

4 Computed Tomography

Further investigations are usually desirable after US. Recent technological developments have led to improvements in CT and MRI. We lack large-scale comparative reports of MDCT and modern MRI with MRCP on the sensitivity and accuracy of finding and staging biliary cancers, which makes it difficult to rank these two methods. The choice of modality also depends on local expertise, capacity, and facilities. Sometimes both modalities are needed.

With MDCT, the liver can be imaged in a single breathhold, which eliminates artifacts from respiratory motion and slice misregistration. Thin, high-resolution images and high-quality multiplanar reformations of even curved structures are produced. The arterial and portovenous phases can be separated, and vascular structures can be displayed. CT angiography (CTA) with high-resolution three-dimensional (3D) angiograms, virtual CT cholangioscopy, or CT cholangiography with cholangiographic contrast medium are also possible. CT protocol should include CT acquisition (with intravenous contrast medium) with the early and late arterial phases and the portovenous phase. The early arterial phase is able to reveal the anatomy of the vessels. An additional delayed phase might reveal specific signs in the case of a bile duct tumor [36]. In spite of the marked improvement in image quality, modern MDCT has suffered from the high levels of radiation and possible allergy to the iodinated contrast medium.

4.1 Carcinoma of the Gallbladder

The sensitivity of CT in the detection of gallbladder carcinoma has been about 90 %. MDCT has been accurate in the diagnosis of the local extent of the cancer. The findings of gallbladder carcinoma may include a heterogeneous mass replacing the gallbladder, wall thickening (Fig. 2), or a fungate (polypoid) tumor. The mass may have various retaining enhancement, an ill-defined contour, and low-attenuation areas of necrosis or calcification. Wall thickening may be irregular and enhance markedly. A polypoid tumor may also enhance, and the adjacent gallbladder wall may be thickened. There have been differences in the enhancement of the wall thickening between carcinoma and chronic cholecystitis. Protrusion of the quadrate lobe with lymphadenopathy has been reported to be unique to gallbladder carcinoma [16, 37–40].

4.2 Carcinoma of the Bile Ducts

Bile duct carcinoma often shows abrupt termination of bile duct dilatation at CT, which can be a finding in advanced gallbladder carcinoma as well. The accuracy of CT to determine the level and cause of obstruction has been 97 and 94 %, respectively. The sensitivity of CT to find bile duct carcinoma has been about 90 % [41]. However, CT may not readily detect a small mass or bile duct wall thickening.

A mass type tumor (Fig. 6) manifests as a low-attenuation mass, which may show peripheral enhancement during the arterial and portal venous phases. Delayed images with concentric retention of contrast are typical of highly fibrous content, and some tumors may only visualize on delayed images. This feature may help to differentiate them from hepatocellular carcinoma. Focal, eccentric wall thickening may have various enhancement patterns (Figs. 7, 8). A polypoid type tumor can be a single or multiple intraductal lesions with increased enhancement. In the case of excessive amounts of mucin, accumulated mucin can cause significant ductal dilatation, direct continuity of a cystic tumor to the ducts, and increased attenuation of the ducts caused by tumor casts or by diffuse spreading of the tumor. CT may have an important role in the diagnosis of papillary tumors [23, 24, 29, 42–46].

In the case of an ampullary tumor, CT may reveal both the double-duct sign and the tumor itself (Fig. 9) [35]. Bile duct carcinoma may also show calcification, biliary dilatation, nonunion of the right and left hepatic ducts, satellite

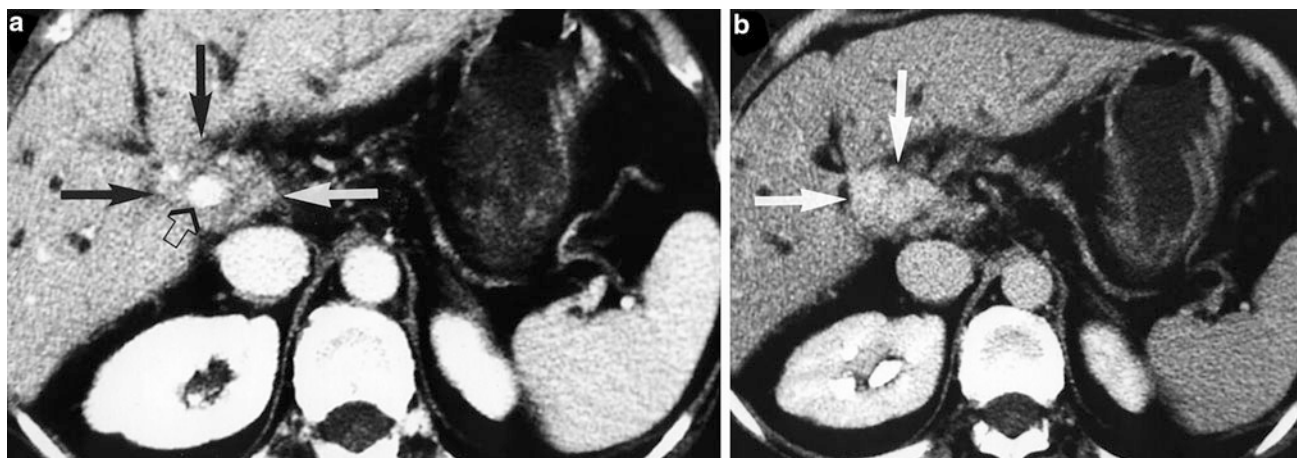


Fig. 6 Bile duct carcinoma. **a** CT in the venous phase shows a heterogeneous mass (*arrows*) in the hilar area around the portal vein (*open arrow*). The common bile duct is not seen because of

obliteration caused by the tumor. **b** In the delayed phase, the mass shows enhancement (*arrows*) [2]

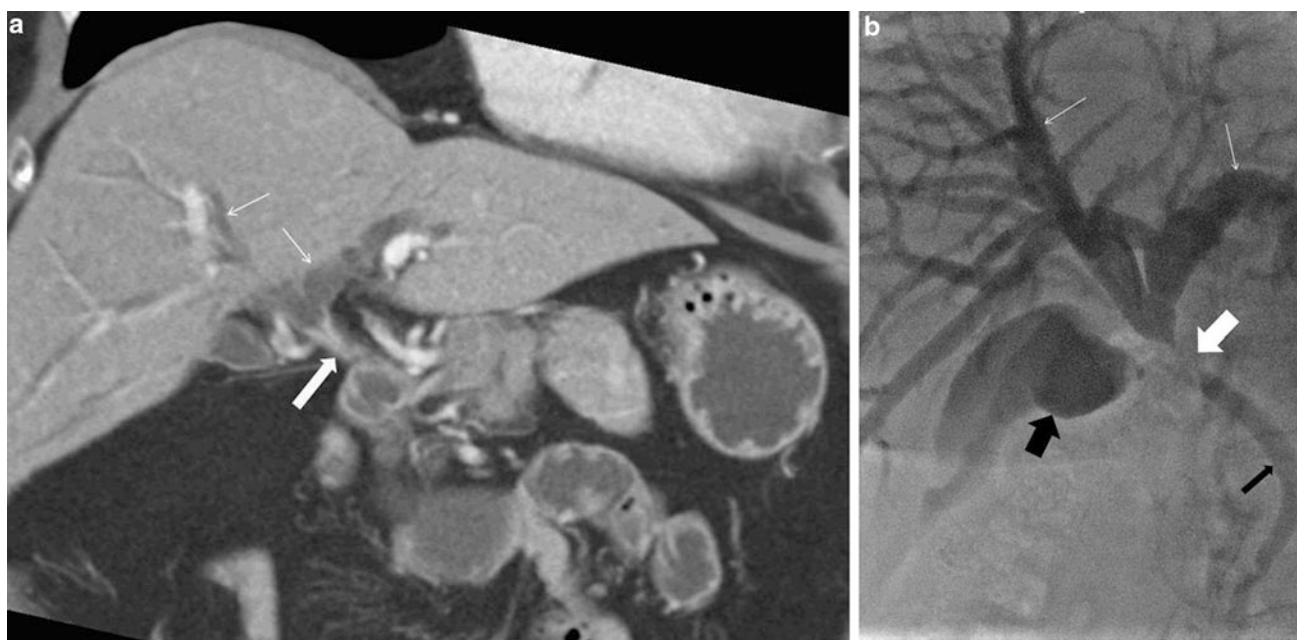


Fig. 7 Cholangiocarcinoma. **a** Coronal view reconstruction of contrast-enhanced CT shows a stricture with enhanced wall thickening of the common hepatic duct (*thick arrow*) and intrahepatic bile duct dilatation (*thin arrows*). **b** A stricture of the common hepatic duct

(*white thick arrow*) and dilatation of the intrahepatic bile ducts (*white thin arrows*) are revealed by PTC. The gallbladder (*black thick arrow*) and normal-sized common bile duct (*black thin arrow*) are also seen

lesions, lobar atrophy, and capsular retraction. Stents inserted to relieve jaundice may limit the usefulness of CT in diagnosis and staging.

4.3 Staging of Biliary Cancers by CT

CT has been quite sensitive in assessing liver, vascular, and bile duct invasion of gallbladder carcinoma (Fig. 2) or bile duct tumor (Fig. 6), but not as good or variable in

carcinomatous spread into lymph nodes, omentum, and peritoneum. In practice, however, CT seems to be the best modality for assessing peritoneal spread. As mentioned earlier, especially MDCT has provided good accuracy in the diagnosis of the local extent of carcinomas of the gallbladder (T staging). Invading gallbladder carcinoma may show irregular enhancement with regions of necrosis. The accuracy for local staging has been better for intraluminal mass types than for thickened wall-type tumors. Dual-phase helical CT has been reported to be a useful tool in assessing

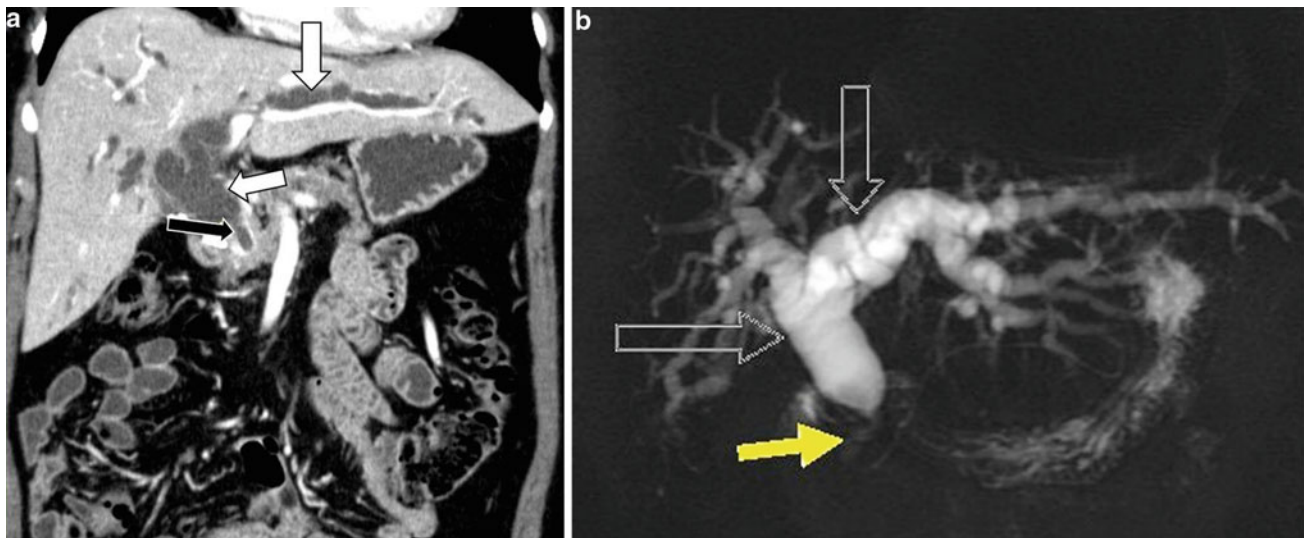


Fig. 8 Carcinoma of the common bile duct. **a** Coronal view reconstruction of contrast-enhanced CT reveals enhancement of the thickened wall of a 2-cm stricture in the distal common bile duct (black arrow) and marked intra- and extrahepatic bile duct dilatation

(white arrows). **b** MRCP (4-cm-thick slab) visualizes a stricture in the distal common bile duct (arrow) and marked intra- and extrahepatic bile duct dilatation (open arrows)

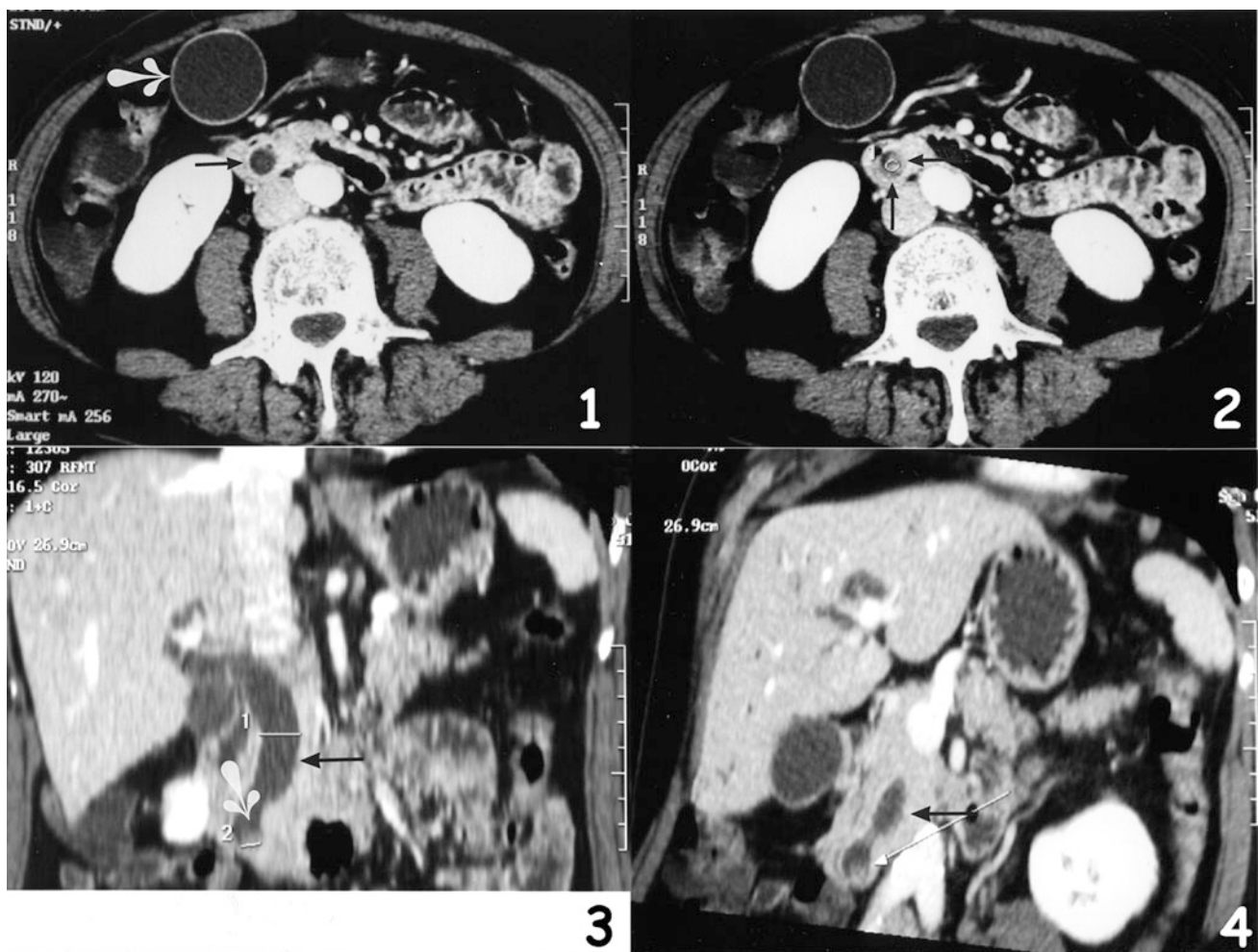


Fig. 9 Carcinoma of the papilla of Vater. Enhanced CT reveals a dilated gallbladder (white arrow) and a common bile duct (black arrow) (1) with an enhancing small mass in the ampullary area

(arrows) (2). The coronary reconstructions show similar findings: the mass (white arrow) and the dilated common bile duct (black arrow) (3, 4) [2]

the resectability of gallbladder cancers [28, 37, 38, 40, 41, 47–49].

The accuracy of MDCT has been 77 % in T staging of extrahepatic bile duct carcinoma, 63 % in N staging, and 97 % in M staging. In one report, 3D MDCT angiography and cholangiography with biliary contrast agent through a transhepatic drainage catheter showed the degree of vascular and biliary involvement of a Klatskin tumor. The diagnostic accuracy of portal vein and hepatic artery invasion was 94 and 89 %, respectively. Combined CT with direct cholangiography in Klatskin tumors has revealed 75 % accuracy for prediction of resectability. The accuracy for portal vein, arterial, and lymph node invasion was 86, 93, and 84 %, respectively. In general, metastatic lymph nodes are suspected if the short-axis diameter of a lymph node is longer than 10 mm, if central necrosis is present, or if attenuation is greater than for the hepatic parenchyma in the portal venous phase [49–52].

5 Magnetic Resonance Imaging and Magnetic Resonance Cholangiography

Fast-imaging techniques have made MRI more useful in biliary imaging. T1- and T2-weighted images of the liver can be obtained within a single breathhold. Using gadolinium chelate, it is possible to obtain images in the arterial, portal venous, and delayed phases.

Magnetic resonance cholangiography (MRC) is the least invasive mode of cholangiography, and it can show a detailed map of the biliary tree. Many studies consider MRC to be equally diagnostic as direct cholangiography in biliary diseases [53]. It is often a noninvasive alternative to ERCP or percutaneous transhepatic cholangiography (PTC), or when direct cholangiography fails. MR imaging also has high soft-tissue contrast and multiplanar capability, and it does not cause any ionizing radiation. However, there are certain contraindications to MRI as well, and interventions are usually not available.

Intrahepatic segmentary ducts are visible up to the first-order branches at MRC, and more peripheral ducts are seen in the case of dilatation. The accuracy of MRC to diagnose the presence and level of obstruction approaches 100 %, and it can show the bile ducts both above and below the obstruction as well as the severity of dilatation (Figs. 1, 8). Information on adjacent organs or extrinsic masses is also provided by MRC. However, the evaluation of obstruction in the case of bile duct carcinoma or advanced gallbladder carcinoma requires not only MRC, but also T1 and T2 images with gadolinium. Combined MRI/MRC has been superior to MRC or endoscopic retrograde cholangiography (ERC) alone in identifying

malignant strictures in Klatskin tumors. Magnetic angiography (MRA) is able to provide images that resemble standard angiography [28, 36, 54, 55].

5.1 Carcinoma of the Gallbladder

There are only a few reports of MRI in the diagnosis of gallbladder carcinoma, but it has been considered a promising method. The tumor has been hypointense on T1 images (Fig. 2) and hyperintense or heterogenous on T2 images compared with the liver. With gadolinium, there may be early irregular enhancement, which persists throughout the dynamic study. Irregular wall thickening may also enhance markedly. Dynamic MRI has been used to differentiate different malignant gallbladder lesions from benign changes based on the enhancement pattern. The method has been promising [39, 56–58].

5.2 Carcinoma of the Bile Ducts

At MRC, bile duct carcinoma may typically show an irregular, asymmetric biliary stricture or obstruction with a dilatation above it (Figs. 8, 10). The morphology and length of the stricture can be evaluated by MRC. The accuracy of MRCP to differentiate extrahepatic bile duct cancer from benign stricture has been comparable with that of ERCP. However, differential diagnosis of a stricture may be difficult with MRC alone, and the discovery of a tumor at MRI may help to suspect a malignancy. MRC/MRI may show a mass type tumor, a polyp-type tumor, or a wall thickening. In view of recent technical improvements, a combination of single-shot thick-slab MRCP and thin-slice MRCP with MIP is the best choice for MRCP today. Biliary drainage can make bile duct assessment difficult, and MRC should hence be performed before biliary drainage [44, 59–62].

ICC and hilar tumors have been hypo- or isointense on T1 images (Figs. 4, 5), while the former have been hyperintense and the latter variable on T2 images. ICC may also have a hypointense central scar. There may be peripheral enhancement by gadolinium and concentric enhancement in the delayed phase. A high mucin content can cause high signal intensity on T2 images. Periductal infiltrating cancer with a thickened wall may show persistent enhancement (Fig. 10), and a papillary tumor may also enhance. Dilated ducts (Fig. 4), capsular retraction, satellite lesions, and lobar atrophy may also be seen, and segmental cholestasis may cause segmental hyperintensity on T1 images [29, 42, 43, 63].

An extrahepatic mass is often hypointense in both T1 and T2 images, and the malignancy may show strong enhancement in the delayed phase. A papillary tumor or wall thickening may also enhance. MRCP and 3D fat-saturated

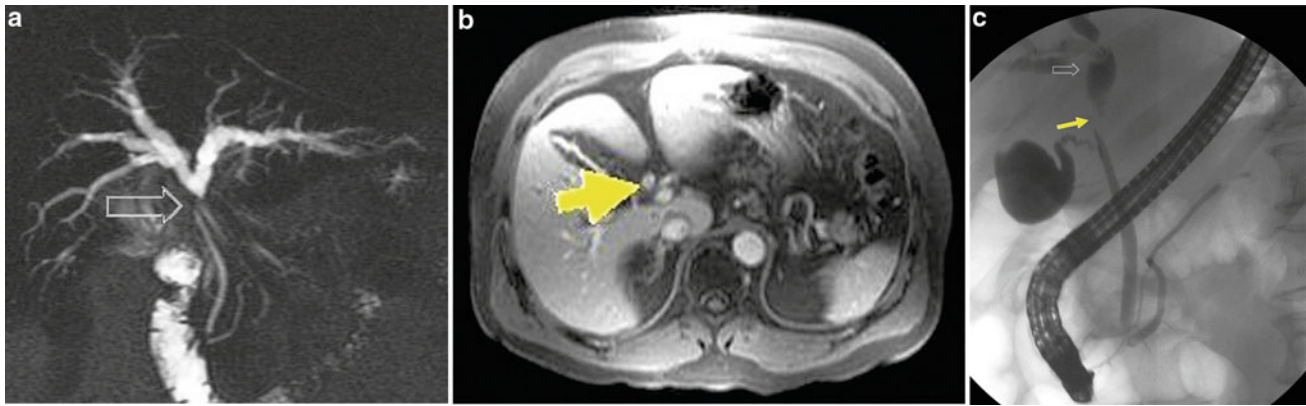


Fig. 10 Klatskin tumor. **a** MRCP (2-cm-thick slab) shows intrahepatic bile duct dilatation and a short, tight stricture in the common hepatic duct next to the bifurcation (*arrow*). **b** Gadolinium-enhanced MRI (T1 fat-saturated gradient echo) reveals enhancement of the wall

thickening of the stricture (*arrow*). **c** ERC shows a short stricture in the common hepatic duct (*arrow*) and dilated intrahepatic bile ducts (*open arrow*)

thin-slice T1-weighted imaging with intravenous contrast at 3T MRI with enhanced spatial and temporal resolution has been superior in defining tumor margins and involvement of vascular and adjacent structures. Ampullary carcinomas have had low signal intensity on T1 and T2 images and have enhanced less than the pancreas. MRCP may reveal the double-duct sign. MRI with MRC is also useful in the differential diagnosis of periampullary carcinomas [44, 61, 63, 64].

5.3 Staging of Biliary Cancers by MRI

There is only scant information about the accuracy of MRI/MRC in the staging of biliary cancers. MRI with MRC and MRA has revealed liver invasion and spread into the bile ducts, vessels, lymph nodes, peritoneum, or pancreas and liver metastases in bile duct cancers. In gallbladder carcinoma, it has been sensitive in at least the first three groups of spread (Figs. 1, 2), but its status in for instance lymph node spread is still unclear. Dynamic MRI has been used to assess the depth of carcinoma invasion in gallbladder carcinoma. The signal intensity of the tumor in the liver is similar to that of the primary tumor. The T1 signal intensity contrast between the tumor and the surrounding tissues also facilitates the detection of tumor extension into surrounding structures.

The diagnostic accuracy of MRI with MRCP has been similar to that of MDCT with direct cholangiography for biliary and vascular involvement, lymph node metastases, and resectability in bile duct carcinomas. Both MRI and MDCT have had limitations in the assessment of lymph node and peritoneal metastases. When MRA and digital subtraction angiography have been compared for their ability to reveal arterial and venous invasion in bile duct

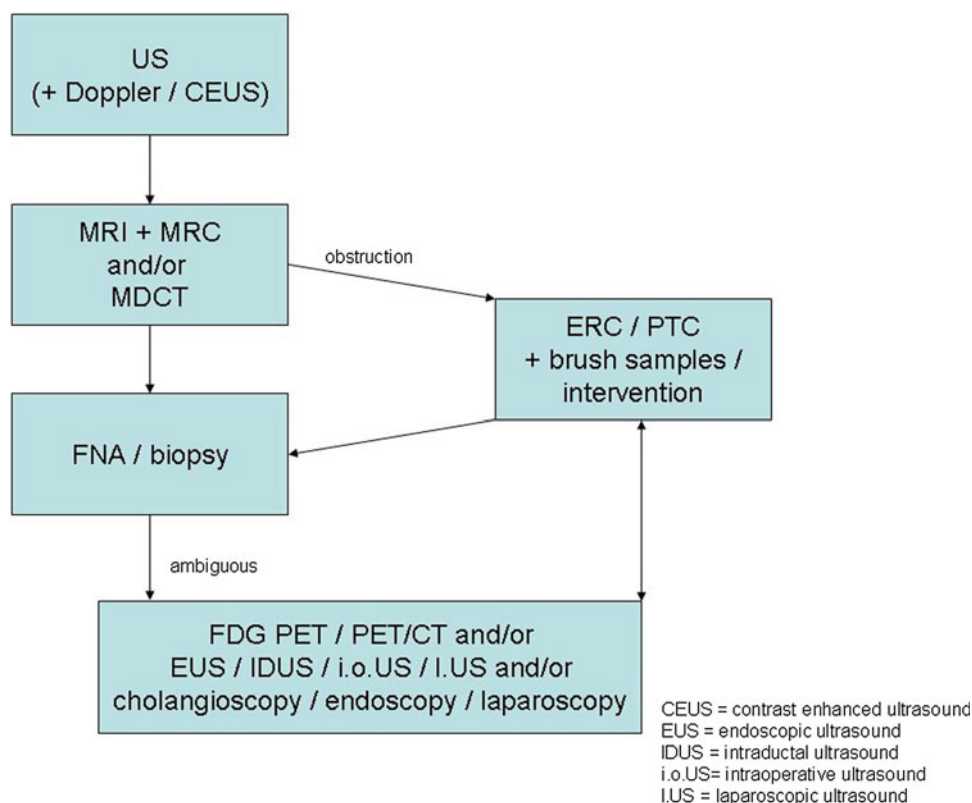
carcinoma, similar diagnostic accuracies have been obtained [59, 61, 65–69].

6 Cholangiography

Traditionally, tumors causing biliary obstruction have been evaluated with direct cholangiography, i. e. ERC (Figs. 4, 10) or PTC (Fig. 7). This technique provides a detailed view of the anatomy of the biliary tree and detects the level of obstruction in 100 % of cases. It may provide the most accurate anatomic information because of its better spatial resolution compared to MRC. Brushings, biopsies, or bile cytology may also be simultaneously obtained, or more advanced cytologic techniques may be used to facilitate the final diagnosis. Cholangiography is performed for therapeutic purposes as well: A plastic or metallic stent may be inserted either endoscopically or percutaneously, or percutaneous biliary drainage can be accomplished. Direct cholangiography is still one of the main examinations, especially in the case of bile duct obstruction [9, 70].

At cholangiography, bile duct carcinoma may appear as an irregular stricture of variable length, a diffuse sclerosing change, or polypoid filling defects, or it may obstruct the duct (Figs. 4, 7). Luminal narrowing is usually abrupt, irregular, or uneven. Cholangiography can be essential to evaluate the disease extent. Advanced gallbladder malignancy may show bile duct changes or cause external bile duct compression. In ampullary carcinoma, PTC may show stenosis, obstruction, or an irregular polypoid filling defect, and ERCP may reveal the double-duct sign and the tumor itself. In a very small ampullary tumor, ERCP with its dynamic capability may be more diagnostic than MRCP [35, 70, 71].

Fig. 11 Imaging strategies utilized in a typical case of a suspected biliary malignancy



However, direct cholangiography has its drawbacks. In cases of total obstruction, ERC does not show the cranial extent of the stricture, and PTC does not show the caudal extent. They are invasive procedures, not always possible, and carry the risk of complications. ERCP is associated with significant morbidity—pancreatitis, cholangitis, hemorrhage, perforation, and sepsis—and a mortality of 0.2–1 %. Cholangiography requires contrast medium and ionizing radiation, the technique is operator dependent, and it only provides information on the bile ducts.

7 Other Modalities

Angiography has had a major role in revealing encasement of the portal vein and hepatic artery by the malignancy. The recently improved versions of helical CT and MRI are increasingly replacing traditional angiography, unless there is a lack of capacity and facilities.

The 2-fluoro-2-deoxy-D-glucose-positron emission tomography (FDG PET) technique is based on the uptake of a radioactive-labeled glucose analog by rapidly metabolizing tumors. PET/CT combines functional and structural imaging. The sensitivity of FDG PET or PET/CT in revealing gallbladder or bile duct carcinoma has been quite high. FDG PET has also been helpful in assessing wall thickening of the gallbladder. However, its sensitivity in

bile duct carcinoma has been dependent on the tumor subtype, being higher for the mass type than the infiltrating type and for the peripheral tumors than the hilar or distal tumors. FDG PET and PET/CT have improved diagnostics of regional lymph node metastases and distant metastases when compared with CT in biliary cancers. They may reveal occult metastases that have not been found by standard imaging. PET/CT has also had a slightly better accuracy than MDCT in assessing resectability in incidental gallbladder cancer with no distant metastases. Unfortunately, FDG PET has only limited spatial resolution and is not widely available [36, 66, 72–76].

Cholangioscopy with biopsies may reveal a small tumor or the longitudinal extent of a bile duct tumor. In the case of an ampullary tumor, endoscopy and biopsy may significantly contribute to the diagnosis. Sometimes, even laparoscopy and biopsies are necessary to reveal the extent of the biliary malignancy, e.g., to detect occult lymph node and peritoneal metastases.

8 Strategies of Imaging

A flow diagram of imaging strategies in a typical case of a suspected biliary malignancy is shown in Fig. 11. The prognosis of biliary cancers has been mainly dismal. However, recent advances in surgical techniques have led to

a need for improved detection and staging of these cancers. There has also been rapid development of radiological techniques, which has improved the diagnostic possibilities. Early diagnosis would be important in improving the prognosis, and careful staging would help in choosing the best possible treatment. All of this still remains a challenge. There is no single modality capable of reliably detecting and especially staging biliary cancers. In spite of these major advances, each modality seems to have its restrictions, and there are variable capacities and practices. Detailed recommendations cannot be given, and continuing advances will still modify the practice.

Transabdominal US is often the first imaging modality applied to patients with jaundice or nonspecific gastrointestinal complaints. It is noninvasive, nonradiative, and commonly available, and it is a suitable method for assessing even mild symptoms. US visualizes bile duct obstruction accurately, and it is able to reveal a gallbladder or bile duct tumor in about 90 % of cases, but less well able to reveal, especially small bile duct lesions. If a biliary malignancy is suspected, US-guided FNA is often able to confirm the final diagnosis. US is helpful, but of limited value, in staging.

Further investigations are usually performed after US. Technological developments have led to improvements, especially in MRI and CT. Both methods may yield additional information of the tumor and/or its extent. Fast-imaging techniques have made MRI potentially more valuable, and MRC is the least invasive mode of cholangiography, which is useful with MRI in the case of biliary obstruction. It is practical especially in patients who are unlikely to require any therapeutic intervention. The technique should include T1 and T2 sequences and gadolinium, often with MRC. There is ongoing discussion about the ranking of MRI and modern CT. The advantage of MRI is the absence of ionizing radiation.

Modern MDCT can produce multiplanar reconstructions of good quality, but the relatively high dose of radiation has made it problematic. The protocol should include triphasic CT acquisition when vascular structures can also be displayed. Simultaneously, CT of the thorax may reveal metastases of the lungs. Since comparative reports of the accuracies of modern MRI with MRCP and MDCT in biliary cancers are sparse, it is difficult to rank these methods. The choice also depends on the contraindications, local expertise, facilities, relative cost, and capacity. In ambiguous cases, both methods may be needed.

Direct cholangiography (PTC or ERCP) is still often necessary and may provide the most accurate anatomic information of the bile ducts. It is also needed for therapeutic purposes in the case of bile duct obstruction. Brushings, biopsies, or bile cytology may be obtained simultaneously, unless imaging-guided FNA is available.

However, cholangiography is invasive, includes ionizing radiation, and carries the risk of complications.

Further, EUS and IDUS might help in the diagnosis and staging of the tumor as well, but they are invasive and not widely available and do not reveal distant metastases. PET and PET/CT have been promising ways to reveal the tumor and regional lymph node and distant metastases, and cholangioscopy may determine the longitudinal extent of a bile duct change. Sometimes, even laparoscopy with biopsies or laparoscopic or intraoperative US, when available, may be needed.

Detection of preneoplastic lesions of the gallbladder and microscopic tumor extension is a big challenge for the future. Again, large-scale comparison studies of the accuracies of MRI/MRC and MDCT in biliary cancers and their staging would be helpful. And the development of even better spatial resolution of MRC, tumor-targeted molecular imaging, and intervention-compatible MRI scanners and instruments would also be welcome.

Acknowledgments The author expresses sincere gratitude to Dr. Eija Pääkkö, MD, PhD, for useful comments of the manuscript.

References

1. Choi BI, Lee JM (2008) Neoplasms of the gallbladder and biliary tract. In: Gore RM, Levine MS (eds) Textbook of gastrointestinal radiology. Saunders Elsevier, Philadelphia, pp 1467–1487
2. Oikarinen H (2006) Diagnostic imaging of carcinomas of the gallbladder and the bile ducts. *Acta Radiol* 47:345–358. doi:10.1080/02841850600580317
3. In: Edge SB, Byrd DR, Compton CC (eds) (2010) AJCC cancer staging handbook, 7th edn. Springer, New York
4. Vogl TJ, Zangos S, Eichler K, Gruber-Rouh T, Hammerstingl RM, Trojan J et al (2012) Radiological diagnosis and intervention of cholangiocarcinomas (CC). *Fortschr Röntgenstr* 184:883–892
5. Bosman FT, Carneiro F, Hruban RH, Theise ND (eds) (2010) Tumours of the gallbladder and extrahepatic bile ducts. In: WHO classification of tumours of the digestive system. IARC, Lyon, pp 263–278
6. Choi H, Loyer EM, Charnsangavej C (2004) Neoplasms of the liver and the bile ducts. *Semin Roentgenol* 39:412–427
7. Miller G, Jarnagin WR (2008) Gallbladder carcinoma. *Eur J Surg Oncol* 34:306–312
8. Chamberlain RS, Blumgart LH (2000) Hilar cholangiocarcinoma: a review and commentary. *Ann Surg Oncol* 7:55–66
9. Parodi A, Fisher D, Giovannini M, Baron T, Conio M (2012) Endoscopic management of hilar cholangiocarcinoma. *Nat Rev Gastroenterol Hepatol* 9:105–112
10. Lee K-H, Lee DY, Kim KW (2004) Biliary intervention for cholangiocarcinoma. *Abdom Imaging* 29:581–589
11. Oikarinen H, Leinonen S, Karttunen A, Tikkakoski T, Hetemaa T, Mäkelä J et al (1999) Patency and complications of percutaneously inserted metallic stents in malignant biliary obstruction. *J Vasc Interv Radiol* 10:1387–1393
12. Bach AM, Loring LA, Hann LE, Illescas FF, Fong Y, Blumgart LH (1998) Gallbladder cancer: can ultrasonography evaluate extent of disease? *J Ultrasound Med* 17:303–309

13. Yarmenitis SD (2002) Ultrasound of the gallbladder and the biliary tree. *Eur Radiol* 12:270–282
14. Oikarinen H, Päivänsalo M, Lähde S, Tikkakoski T, Suramo I (1993) Radiological findings in cases of gallbladder carcinoma. *Eur J Radiol* 17:179–183
15. Rooholamini SA, Tehrani NS, Razavi MK, Au AH, Hansen GC, Ostrzega N et al (1994) Imaging of gallbladder carcinoma. *Radiographics* 14:291–306
16. Levy AD, Murkata LA, Rohrmann CA (2001) Gallbladder carcinoma: radiologic-pathologic correlation. *Radiographics* 21:295–314
17. Chijiwa K, Sumiyoshi K, Nakayama F (1991) Impact of recent advances in hepatobiliary imaging techniques on the preoperative diagnosis of carcinoma of the gallbladder. *World J Surg* 15:322–327
18. Gore RM, Yaghmai V, Newmark GM, Berlin JW, Miller FH (2002) Imaging benign and malignant disease of the gallbladder. *Radiol Clin North Am* 40:1307–1323
19. Matsumoto J (1998) Endoscopic ultrasonography diagnosis of gallbladder lesions. *Endoscopy* 30(Suppl 1):A124–A127
20. Meara RS, Jhala D, Eloubeidi MA, Eltoun I, Chhieng DC, Crowe DR et al (2006) Endoscopic ultrasound-guided FNA biopsy of bile duct and gallbladder: analysis of 53 cases. *Cytopathology* 17:42–49
21. Inui K, Yoshino J, Miyoshi H (2011) Diagnosis of gallbladder tumors. *Intern Med* 50:1133–1136
22. Mishra G, Conway JD (2009) Endoscopic ultrasound in the evaluation of radiologic abnormalities of the liver and biliary tree. *Curr Gastroenterol Rep* 11:150–154
23. Baron LR, Tublin ME, Peterson MS (2002) Imaging the spectrum of biliary tract disease. *Radiol Clin North Am* 40:1325–1354
24. Lim JH (2003) Cholangiocarcinoma: morphologic classification according to growth pattern and imaging findings. *Am J Roentgenol* 181:819–827
25. Mittelstaedt CA (1997) Ultrasound of the bile ducts. *Semin Roentgenol* 32:161–171
26. Päivänsalo M, Oikarinen H, Tikkakoski T, Puumala K, Suramo I (1993) Radiological findings in bile duct carcinoma. *Fortschr Röntgenstr* 159:4–9
27. Hann LE, Greatrex KV, Bach AM, Fong Y, Blumgart LH (1997) Cholangiocarcinoma at the hepatic hilus: sonographic findings. *Am J Roentgenol* 168:985–989
28. Choi J-Y, Kim M-J, Lee JM, Kim KW, Lee JY, Han JK et al (2008) Hilar cholangiocarcinoma: role of preoperative imaging with sonography, MDCT, MRI, and direct cholangiography. *Am J Roentgenol* 191:1448–1457
29. Takanami K, Yamada T, Tsuda M, Takase K, Ishida K, Nakamura Y et al (2011) Intraductal papillary mucinous neoplasm of the bile ducts: multimodality assessment with pathologic correlation. *Abdom Imaging* 36:447–456
30. Dietrich CF (2004) Characterisation of focal liver lesions with contrast enhanced ultrasonography. *Eur J Radiol* 51:S9–S17
31. Khalili K, Metser U, Wilson SR (2003) Hilar biliary obstruction: preliminary results with Levovist-enhanced sonography. *Am J Roentgenol* 180:687–693
32. Inui K, Miyoshi H (2005) Cholangiocarcinoma and intraductal sonography. *Gastrointest Endosc Clin North Am* 15:143–155
33. Levy MJ, Heimbach JK, Gores GJ (2012) Endoscopic ultrasound staging of cholangiocarcinoma. *Curr Opin Gastroenterol* 28:244–252
34. Fujita N, Noda Y, Kobayashi G, Ito K, Horaguchi J, Koshita S et al (2009) Intraductal ultrasonography (IDUS) for the diagnosis of biliopancreatic diseases. *Best Pract Res Clin Gastroenterol* 23:729–742
35. Buck JL, Elsayed AM (1993) From the archives of the AFIP. Ampullary tumors: radiologic-pathologic correlation. *Radiographics* 13:193–212
36. Zech CJ, Schoenberg SO, Reiser M, Helmberger T (2004) Cross-sectional imaging of biliary tumors: current clinical status and future developments. *Eur Radiol* 14:1174–1187
37. Ohtani T, Shirai Y, Tsukada K, Muto T, Hatakeyama K (1996) Spread of gallbladder carcinoma: CT evaluation with pathologic correlation. *Abdom Imaging* 21:195–201
38. Yoshimitsu K, Honda H, Shinozaki K, Aibe H, Kuroiwa T, Irie H et al (2002) Helical CT of the local spread of carcinoma of the gallbladder: evaluation according to the TNM system in patients who underwent surgical resection. *Am J Roentgenol* 179:423–428
39. Furlan A, Ferris JV, Hosseinzadeh K, Borhani AA (2008) Gallbladder carcinoma update: multimodality imaging evaluation, staging, and treatment options. *Am J Roentgenol* 191:1440–1447
40. Kim SJ, Lee JM, Lee JY, Choi JY, Kim SH, Han JK et al (2008) Accuracy of preoperative T-staging of gallbladder carcinoma using MDCT. *Am J Roentgenol* 190:74–80
41. Feydy A, Vilgrain V, Denys A, Sibert A, Belghiti J, Vullierme M-P et al (1999) Helical CT assessment in hilar cholangiocarcinoma: correlation with surgical and pathologic findings. *Am J Roentgenol* 172:73–77
42. Choi BI, Lee JM, Han JK (2004) Imaging of intrahepatic and hilar cholangiocarcinoma. *Abdom Imaging* 29:548–557
43. Han JK, Lee JM (2004) Intrahepatic intraductal cholangiocarcinoma. *Abdom Imaging* 29:558–564
44. Lim JH, Lee WJ, Takehara Y, Lim HK (2004) Imaging of extrahepatic cholangiocarcinoma. *Abdom Imaging* 29:565–571
45. Tillich M, Mischinger H-J, Preisegger K-H, Rabl H, Szolar DH (1998) Multiphasic helical CT in diagnosis and staging of hilar cholangiocarcinoma. *Am J Roentgenol* 171:651–658
46. Chung YE, Kim M-J, Park YN, Choi J-Y, Pyo JY, Kim YC et al (2009) Varying appearances of cholangiocarcinoma: radiologic-pathologic correlation. *Radiographics* 29:683–700
47. Kalra N, Suri S, Gupta R, Natarajan SK, Khandelwal N, Wig JD et al (2006) MDCT in the staging of gallbladder carcinoma. *Am J Roentgenol* 186:758–762
48. Kumaran V, Gulati S, Paul B, Pande K, Sahni P, Chattopadhyay K (2002) The role of dual-phase helical CT in assessing resectability of carcinoma of the gallbladder. *Eur Radiol* 12:1993–1999
49. Kim HJ, Lee DH, Lim JW, Ko YT (2009) Multidetector computed tomography in the preoperative workup of hilar cholangiocarcinoma. *Acta Radiol* 50:845–853
50. Park M-S, Lee DK, Kim M-J, Lee WJ, Yoon D-S, Lee SJ et al (2006) Preoperative staging accuracy of multidetector row computed tomography for extrahepatic bile duct carcinoma. *J Comp Ass Tomogr* 30:362–367
51. Chen HW, Pan AZ, Zhen ZJ, Su SY, Wang JH, Yu SCH et al (2006) Preoperative evaluation of resectability of Klatskin tumor with 16-MDCT angiography and cholangiography. *Am J Roentgenol* 186:1580–1586
52. Lee HY, Kim SH, Lee JM, Kim S-W, Jang J-Y, Han JK et al (2006) Preoperative assessment of resectability of hepatic hilar cholangiocarcinoma: combined CT and cholangiography with revised criteria. *Radiology* 239:113–121
53. Varghese JC, Farrell MA, Courtney G, Osborne H, Murray FE, Lee MJ (1999) A prospective comparison of magnetic resonance cholangiopancreatography with endoscopic retrograde cholangiopancreatography in the evaluation of patients with suspected biliary tract disease. *Clin Radiol* 54:513–520
54. Holzknecht N, Gauger J, Sackmann M, Thoeni RF, Schurig J, Holl J et al (1998) Breath-hold MR cholangiography with snapshot techniques: prospective comparison with endoscopic retrograde cholangiography. *Radiology* 206:657–664
55. Vogl TJ, Schwarz WO, Heller M, Herzog C, Zangos S, Hintze RE et al (2006) Staging of Klatskin tumours (hilar cholangiocarcinomas): comparison of MR cholangiography, MR

- imaging, and endoscopic retrograde cholangiography. *Eur Radiol* 16:2317–2325
56. Sagoh T, Itoh K, Togashi K, Shibata T, Minami S, Noma S et al (1990) Gallbladder carcinoma: evaluation with MR imaging. *Radiology* 174:131–136
57. Tseng JH, Wan YL, Hung CF, Ng KK, Pan KT, Chou AS et al (2002) Diagnosis and staging of gallbladder carcinoma. Evaluation with dynamic MR imaging. *Clin Imaging* 26:177–182
58. Yoshimitsu K, Honda H, Kaneko K, Kuroiwa T, Irie H, Ueki T et al (1997) Dynamic MRI of the gallbladder lesions: differentiation of benign from malignant. *J Magn Reson Im* 7:696–701
59. Hänninen EL, Pech M, Jonas S, Ricke J, Thelen A, Langrehr J et al (2005) Magnetic resonance imaging including magnetic resonance cholangiopancreatography for tumor localization and therapy planning in malignant hilar obstructions. *Acta Radiol* 46:462–470
60. Park M-S, Kim TK, Kim KW, Park SW, Lee JK, Kim J-S et al (2004) Differentiation of extrahepatic bile duct cholangiocarcinoma from benign stricture: findings at MRCP versus ERCP. *Radiology* 233:234–240
61. Takehara Y (2004) Preoperative assessment of extrahepatic cholangiocarcinoma with imaging. *Abdom Imaging* 29:572–580
62. Kim JY, Lee JM, Han JK, Kim SH, Lee JY, Choi JY et al (2007) Contrast-enhanced MRI combined with MR cholangiopancreatography for the evaluation of patients with biliary strictures: differentiation of malignant from benign bile duct strictures. *J Magn Reson Imaging* 26:304–312
63. Li N, Liu C, Bi W, Lin X, Jiao H, Zhao P (2012) MRCP and 3D LAVA imaging of extrahepatic cholangiocarcinoma at 3 T MRI. *Clin Radiol* 67:579–586
64. Semelka RC, Kelekis NL, John G, Ascher SM, Burdeny D, Siegelman ES (1997) Ampullary carcinoma: demonstration by current MR techniques. *J Magn Reson Imaging* 7:153–156
65. Kaza RK, Gulati M, Wig JD, Chawla YK (2006) Evaluation of gallbladder carcinoma with dynamic magnetic resonance imaging and magnetic resonance cholangiopancreatography. *Australas Radiol* 50:212–217
66. Rodriguez-Fernandez A, Gomez-Rio M, Medina-Benitez A, Villar-Del Moral J, Ramos-Font C, Ramia-Angel JM et al (2006) Application of modern imaging methods in diagnosis of gallbladder cancer. *J Surg Oncol* 93:650–664
67. Schwartz LH, Black J, Fong Y, Jarnagin W, Blumgart L, Gruen D et al (2002) Gallbladder carcinoma: findings at MR imaging with MR cholangiopancreatography. *J Comp Ass Tomogr* 26:405–410
68. Park HS, Lee JM, Choi J-Y, Lee MW, Kim HJ, Han JK et al (2008) Preoperative evaluation of bile duct cancer: MRI combined with MR cholangiopancreatography versus MDCT with direct cholangiography. *Am J Roentgenol* 190:396–405
69. Misra S, Chaturvedi A, Misra NC, Sharma ID (2003) Carcinoma of the gallbladder. *Lancet Oncol* 4:167–176
70. Brugge WR (2005) Endoscopic techniques to diagnose and manage biliary tumors. *J Clin Oncol* 23:4561–4565
71. Geier A, Nguyen HN, Gartung C, Matern S (2000) MRCP and ERCP to detect small ampullary carcinoma. *Lancet* 356:1607–1608
72. Petrowsky H, Wildbrett P, Husarik DB, Hany TF, Tam S, Jochum W et al (2006) Impact of integrated positron emission tomography and computed tomography on staging and management of gallbladder cancer and cholangiocarcinoma. *J Hepatol* 45:43–50
73. Rosenbaum SJ, Stergar H, Antoch G, Veit P, Bockisch A, Kuhl H (2006) Staging and follow-up of gastrointestinal tumors with PET/CT. *Abdom Imaging* 31:25–35
74. Chung YE, Kim M-J, Park YN, Lee Y-H, Choi J-Y (2008) Staging of extrahepatic cholangiocarcinoma. *Eur Radiol* 18:2182–2195
75. Sacks A, Peller PJ, Surasi DS, Chatburn L, Mercier G, Subramaniam RM (2011) Value of PET/CT in the management of primary hepatobiliary tumors, part 2. *Am J Roentgenol* 197:W260–W265
76. Lee SW, Kim HJ, Park JH, Park DI, Cho YK, Sohn CI et al (2010) Clinical usefulness of 18F-FDG PET-CT for patients with gallbladder cancer and cholangiocarcinoma. *J Gastroenterol* 45:560–566

Imaging of Hilar Cholangiocarcinoma for Liver Transplantation

Victor M. Zaydfudim, David M. Nagorney, and Charles B. Rosen

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Abstract

Liver transplantation and resection for hilar cholangiocarcinoma are mutually exclusive therapeutic pathways, without possibility of cross over. Interpretation of relevant imaging is essential for accurate preoperative diagnosis, operative planning, management of complications during neoadjuvant therapy, and post-transplant surveillance of patients with hilar cholangiocarcinoma. Preoperatively, careful and dynamic interpretation of both cross-sectional imaging and cholangiography is needed to appreciate tumor presence, location, and vascular involvement. While many post-transplant vascular complications can be managed with percutaneous endovascular techniques, a number of specific vascular complications are preferentially treated operatively. An experienced multidisciplinary team is required for successful treatment of hilar cholangiocarcinoma with liver transplantation.

Hilar cholangiocarcinoma requires accurate imaging for both diagnosis and choice of treatment. The goals of imaging are to (1) confirm clinical suspicion of cancer and its location; (2) assess local extent of disease including vascular and biliary involvement; and (3) detect regional and distant metastatic disease that may affect decisions regarding treatment. The Bismuth–Corlette classification (Fig. 1), is the most widely adopted system used to describe tumor location and biliary involvement [1]. Tumor location and biliary involvement are assessed by cholangiography with endoscopic (ERC), percutaneous (PTC), and/or cross-sectional imaging studies such as MRCP and CT cholangiography.

Appreciation of the tumor location within the biliary system is critical for operative management of patients with hilar cholangiocarcinoma. Understanding of biliary anatomy as well as appreciation of nomenclature is also paramount: Biliary anatomy can be viewed proximal to distal based on either embryologic development or bile drainage. For the purpose of this chapter, we will refer to biliary bifurcation based on embryologic development and proximal to distal

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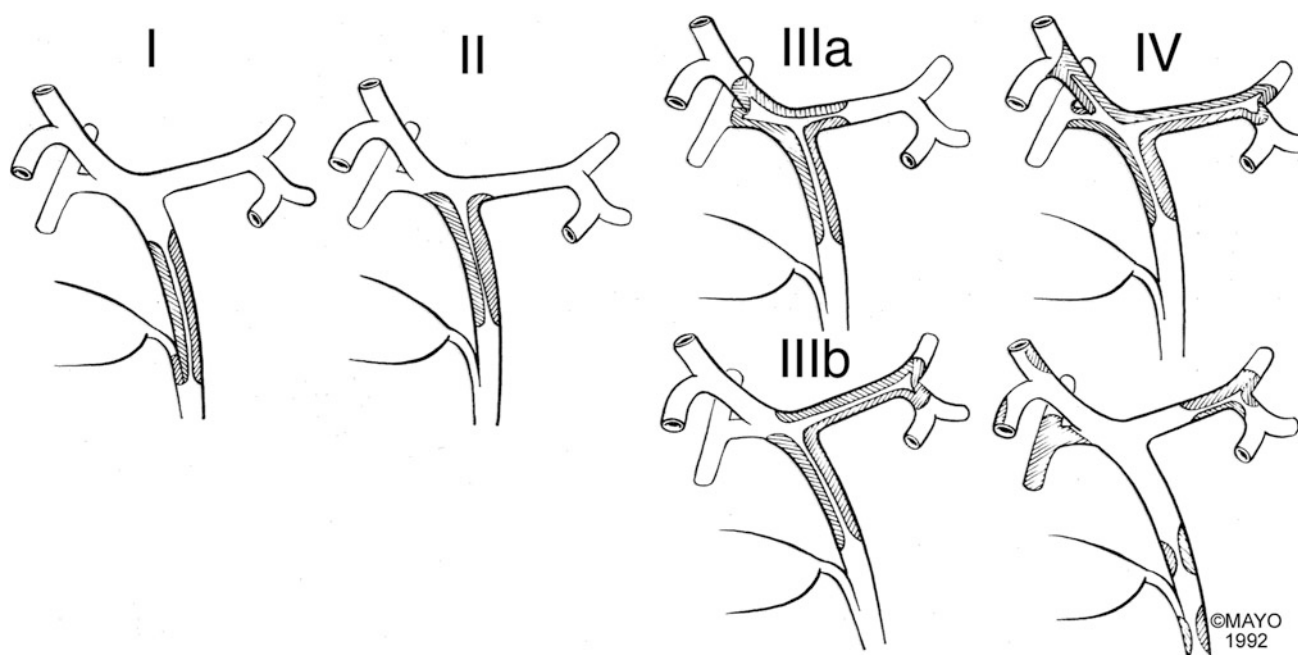


Fig. 1 Bismuth–Corlette classification of perihilar cholangiocarcinoma: *I* below the bifurcation of right and left hepatic ducts; *II* at the bifurcation of right and left hepatic ducts; *IIIa* at the bifurcation with

extension into the right hepatic duct; *IIIb* at the bifurcation with extension into the left hepatic duct; and *IV* extension into both right and left hepatic ducts or multicentric disease

bile ducts based on bile drainage (i.e., proximal ducts in the liver and distal duct draining bile into the duodenum). Patients with hilar cholangiocarcinoma below the bifurcation of the hepatic ducts (Bismuth–Corlette I) or just at the bifurcation (Bismuth–Corlette II) are best treated with resection of the extrahepatic bile duct and biliary reconstruction. Intraoperative pathological examination of the specimen is necessary to confirm a tumor-free margin. Types I and II hilar cholangiocarcinoma are rare; most patients prove to have extension of tumor into the biliary bifurcation and the right or left hepatic ducts or both. Cholangiocarcinoma arising in the common bile duct also often extends into the head of the pancreas.

Bismuth–Corlette classification of hilar cholangiocarcinoma involving one or both hepatic ducts is IIIa for right duct involvement, IIIb for left duct involvement, and IV for bilateral duct involvement or diffuse multifocal disease. In general, resection is possible for IIIa and IIIb tumors by right or left hepatectomy provided that the vasculature to the contralateral side (residual liver) is free from involvement.

Liver vasculature must be assessed to ensure that the remnant liver will have both arterial and portal venous inflow. Vascular involvement is best assessed by CT or MRI. Limited vascular involvement to the remnant liver can occasionally be overcome by vascular reconstruction of the hepatic artery and/or portal vein, more commonly for IIIa tumors involving the right duct than for IIIb tumors involving the left duct since the left portal vein is more amenable to reconstruction.

Generally accepted criteria for unresectability are as follows: portal and/or arterial involvement not amenable to reconstruction (all types); unilateral biliary involvement with contralateral vascular involvement not amenable to reconstruction (types IIIa and IIIb); bilateral biliary involvement of secondary ducts (type IV); and an inadequate future liver remnant. Underlying chronic liver disease—especially primary sclerosing cholangitis (PSC)—usually precludes resection [2, 3]. These patients are best treated by neoadjuvant therapy and liver transplantation.

The aim of this chapter is to discuss (1) preoperative imaging of patients with hilar cholangiocarcinoma to determine resectability, (2) evaluation of unresectable patients to determine candidacy for transplantation, and (3) utilization of post-transplant imaging to follow patients transplanted for hilar cholangiocarcinoma since they are prone to develop late vascular complications related to neoadjuvant radiotherapy.

1 Determination of Resectability

1.1 Cross-Sectional Imaging

Both CT and MRI are useful imaging techniques for evaluation of patients with hilar cholangiocarcinoma [4–6]. The choice between CT and MRI varies due to clinician preference and institutional experience. MRI combined with MRCP is the preferred preoperative test at many institutions

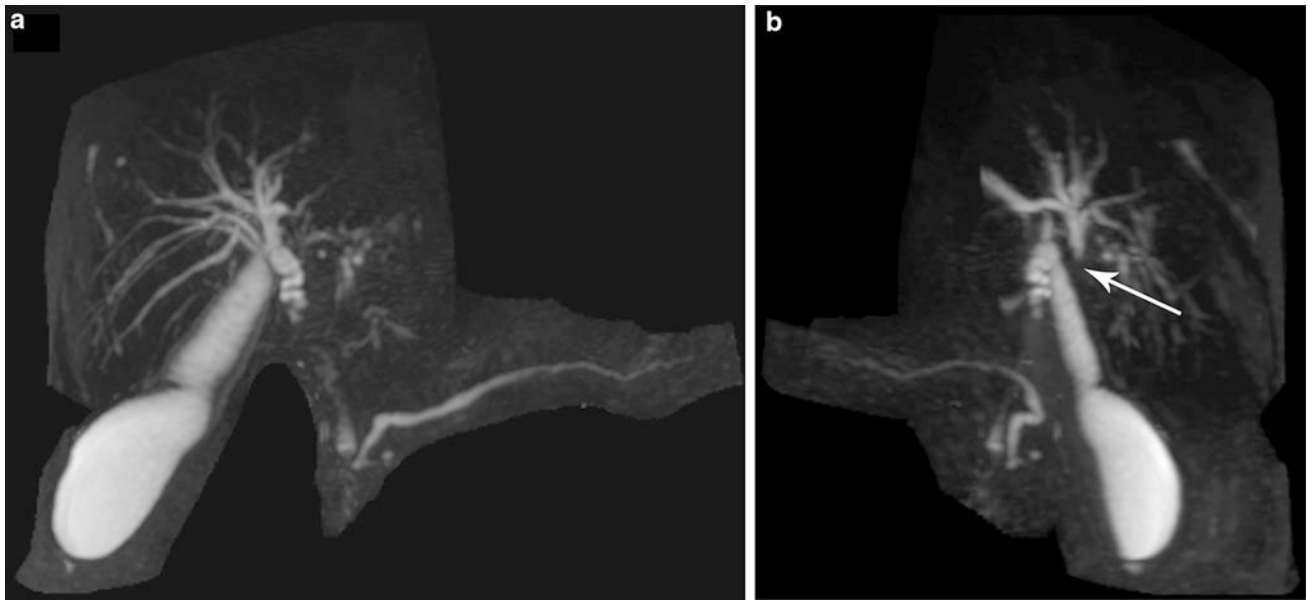


Fig. 2 Perihilar cholangiocarcinoma with the proximal extent obscured by the cystic duct (Panel **a**). Rotation of the MRCP reconstruction reveals proximal extent at the bifurcation (*arrow*)—a Type II cholangiocarcinoma (Panel **b**)

since it provides both cholangiography and cross-sectional imaging [7–9]. MRI allows for accurate assessment of both longitudinal and radial tumor extent in a single study. MRCP highlights biliary anatomy, delineates the level of biliary obstruction, and allows for visualization of the proximal and distal biliary ducts. Contrast MRI can detect a hilar mass (if present) and, most importantly, demonstrates vascular (arterial and venous) involvement.

Contrast multiphase CT has excellent diagnostic accuracy. CT can also demonstrate the location of the mass (if present) and determine vascular anatomy [6, 10]. Advances in CT imaging, particularly CT cholangiography and 3-dimensional reconstruction, have allowed for vast improvements in the assessment of anatomic relationships between the hepatic vasculature and biliary pathology [11, 12]. CT is somewhat better than MRI for detection of intra-abdominal and chest metastases.

ERCP is done to assess biliary anatomy, obtain an intraluminal specimen for histology and/or cytology, and to provide preoperative biliary decompression. Patients with suspected hilar cholangiocarcinoma require an experienced endoscopist. They require precise imaging, intraluminal biopsy and brush cytology for diagnosis, and biliary intubation for decompression. PTC is reserved for patients who have an inadequate ERCP (usually inadequate imaging to determine the extent of left or right duct involvement) or who are not amenable to biliary decompression with ERCP. We avoid PTC whenever possible. PTC and PTC-directed biopsy of cholangiocarcinoma can lead to tumor seeding and have been associated with higher rates of postoperative recurrence [13, 14].



Fig. 3 MRCP revealing involvement of the ductal bifurcation (*arrow*) by cholangiocarcinoma. Additional rotations of the images and dynamic interpretation in conjunction with cross-sectional imaging are required to distinguish proximal ductal extent (Type IIIA vs. Type IIIB) of the cholangiocarcinoma

Cross-sectional imaging studies should be carefully interpreted by a multidisciplinary team. Images cannot be interpreted in isolation, and a combination of cross-sectional imaging and dynamic biliary reconstructions is needed to appreciate tumor presence, location, and vascular involvement. As an example, the lesion in (Fig. 2a) can be

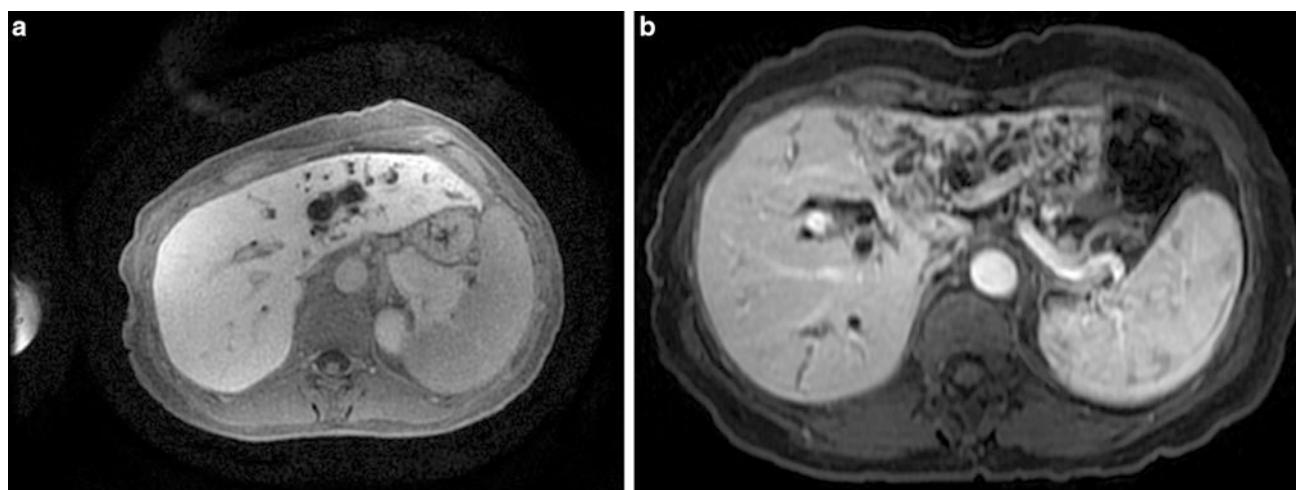


Fig. 4 Type IIIB hilar cholangiocarcinoma with significant intrahepatic ductal dilatation of the left ductal system (Panel **a**). With time, biliary and/or vascular obstruction leads to development of lobar atrophy (Panel **b**)

misinterpreted as Bismuth–Corlette I, if the MRCP is not rotated to unmask the cystic duct overlap and appreciate Bismuth–Corlette II extension (Fig. 2b). Similarly, Fig. 3 demonstrates a Bismuth–Corlette III lesion, but additional imaging interpretation is necessary to distinguish IIIA from IIIB—a critical step for operative preparation.

Both MRI and CT imaging modalities frequently fail to identify a discrete tumor mass. If visible, the mass is usually hypovascular compared to adjacent liver parenchyma and increases in intensity with delayed MRI imaging. MRCP and CT cholangiography can identify ductal irregularities and intraluminal infiltration in the absence of a discrete mass [8, 11]. Biliary stricture and corresponding proximal dilatation help delineate longitudinal extension of tumor along the duct. Viewing of both axial and coronal reconstructions helps to visualize the anatomical relationships between the tumor and vascular structures. Extension of a stricture to secondary biliary ducts in a contralateral lobe is a contraindication to resection.

1.2 Bismuth–Corlette Classification

Patients with Bismuth–Corlette IIIa and IIIb cholangiocarcinoma are best treated with right or left hepatectomy (respectively), radical common bile duct resection, hepatoduodenal ligament lymphadenectomy, and bilio-enteric reconstruction. Preoperative cross-sectional imaging is necessary to confirm patency of the main and contralateral branch of the hepatic artery and patency of the main and contralateral branch of the portal vein. Ipsilateral portal vein impingement or occlusion, or involvement of ipsilateral hepatic artery does not preclude resection. A hallmark feature of locally advanced resectable hilar cholangiocarcinoma is lobar atrophy. It is associated with ipsilateral portal vein

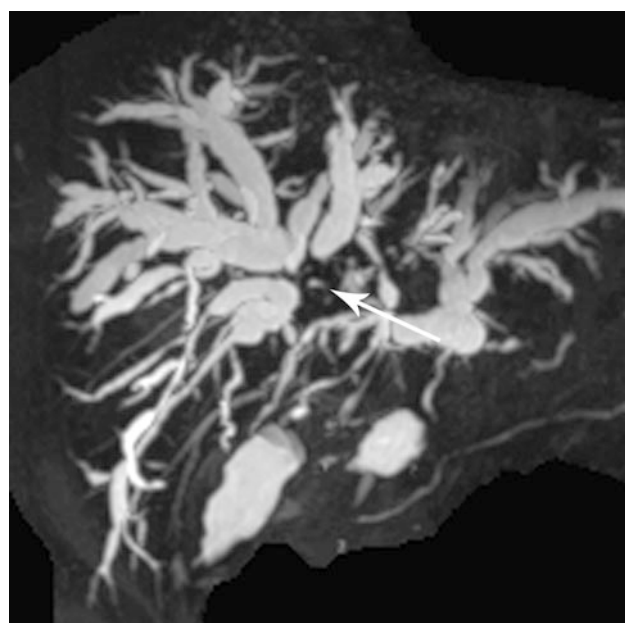


Fig. 5 Large Type IV hilar cholangiocarcinoma (arrow) obstructing biliary drainage from both right and left ductal systems with bilateral ductal dilatation. Tumor extends to secondary ducts in both right and left hepatic lobes

involvement and/or complete biliary obstruction [15, 16] and usually develops over time (Fig. 4a, b). While lobar atrophy has been associated with worse overall survival among patients with resectable hilar cholangiocarcinoma, it does not preclude a margin-negative resection [15, 17].

Patients with Bismuth–Corlette IV cholangiocarcinoma (Fig. 5) and those with contralateral vascular involvement are unresectable and should be evaluated for liver transplantation. Vascular encasement of the hepatic artery and/or portal vein and their branches is not a contraindication to liver

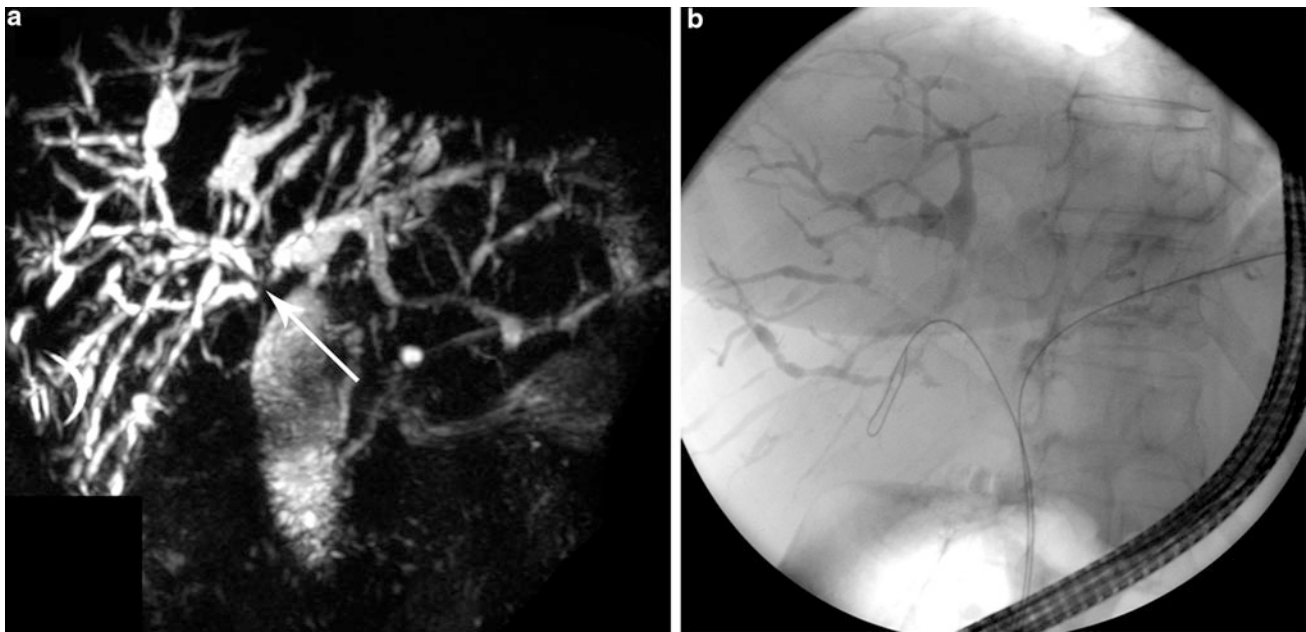


Fig. 6 MRCP demonstrating a Type IV cholangiocarcinoma (*arrow*) in a patient with PSC with multifocal intrahepatic strictures and dilatations (Panel **a**). A corresponding ERCP demonstrates classic beading of the intrahepatic bile ducts (Panel **b**)

transplantation. Liver transplantation is also a primary treatment option for cholangiocarcinoma in patients with PSC (Fig. 6a, b). PSC is an idiopathic chronic cholestatic liver disease with manifestations of progressive inflammatory destruction and biliary fibrosis of the entire bile duct system [18, 19]. These patients often have parenchymal disease precluding resection. Seven to 15 % of patients with PSC develop cholangiocarcinoma during their lifetime. The diagnoses of cholangiocarcinoma and PSC may be established at the same time, or cholangiocarcinoma may develop at a later time in patients with PSC. These patients are considered to have a “field defect” and are probably best treated by neoadjuvant therapy and liver transplantation rather than resection, even if they have otherwise potentially resectable tumors.

2 Evaluation for Transplantation

2.1 Inclusion/Exclusion Criteria for Liver Transplantation

Patients with unresectable hilar cholangiocarcinoma or hilar cholangiocarcinoma arising in the setting of PSC should be evaluated for liver transplantation. Transplantation and resection are mutually exclusive therapeutic pathways, without possibility for cross over. Patients found to have unresectable disease during exploration for resection do not do well with subsequent neoadjuvant therapy and liver transplantation. In our experience, operative exploration and subsequent neoadjuvant therapy increases the technical

difficulty with transplantation and increased the likelihood for recurrence after transplantation.

Patients who undergo pathologic confirmation of tumor by transperitoneal tumor biopsy or fine-needle aspiration (including endoscopic ultrasound (EUS)-directed aspiration of the tumor) should also be excluded from transplantation [20].

Conversely, patients who fall out of the neoadjuvant therapy transplantation protocol cannot undergo liver resection even if they were thought to have potentially resectable disease. Neoadjuvant therapy causes widespread hilar biliary necrosis that would make resection and subsequent biliary reconstruction hazardous.

The United Network for Organ Sharing/Organ Procurement and Transplantation Network (UNOS/OPTN) approved a model for end-stage liver disease (MELD) exception score in 2009 for patients enrolled in an approved neoadjuvant therapy protocol. The MELD score exception is similar to the exception model for patients with transplantable hepatocellular carcinoma [21, 22]. Neoadjuvant therapy and staging are critical to success [22–24]. Since histological and/or cytological confirmation of diagnosis is not always possible, diagnosis is often dependent on imaging. Definitive diagnosis for treatment requires presence of a malignant-appearing stricture and at least one of the following: (1) endoluminal biopsy or cytology positive for cholangiocarcinoma; (2) polysomy by fluorescent in situ hybridization (FISH); (3) mass lesion on cross-sectional imaging at the location of the malignant-appearing stricture; or (4) CA 19-9 >100.

EUS with fine-needle aspiration (FNA) of suspicious nodes is useful to rule out patients with regional lymph node

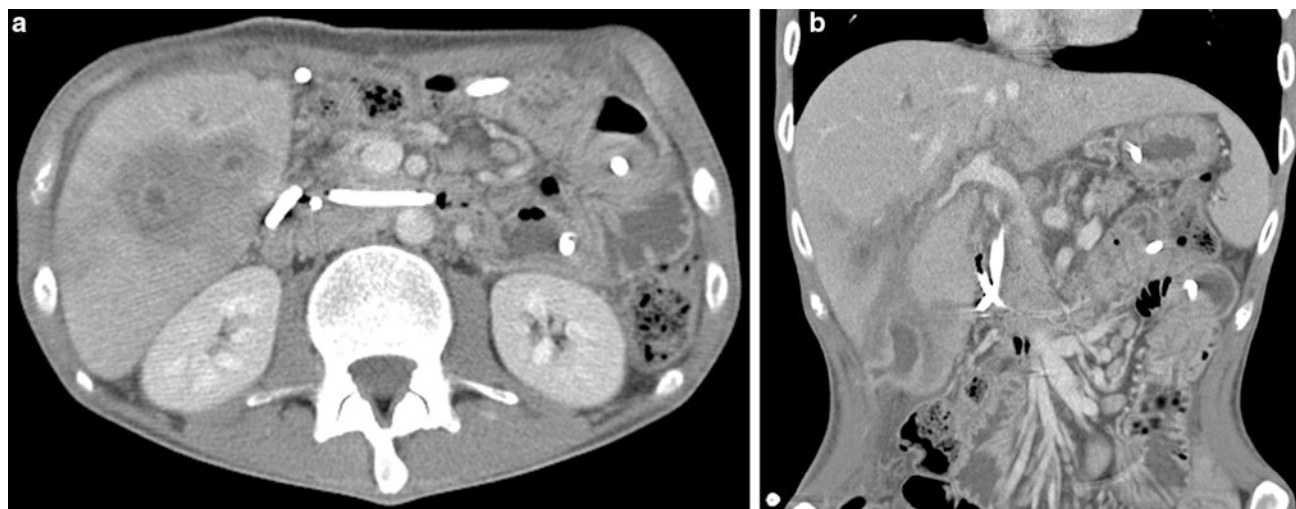


Fig. 7 Intrahepatic abscess (right lobe)—axial (Panel **a**) and coronal (Panel **b**) reconstructions—in a patient with hilar cholangiocarcinoma and PSC receiving neoadjuvant chemoradiation

involvement destined to fall out at operative staging. Neoadjuvant therapy is associated with a multitude of side effects and potentially lethal complications and should not be administered unless the patient is a candidate for transplantation. It is important to make sure that aspiration is only done on the regional nodes and not on the primary tumor since that will preclude transplantation. Nodal metastases in any location—hepatoduodenal ligament (N1) or celiac, aortocaval, or retropancreatic (N2) lymph node basins—are a contraindication to neoadjuvant therapy and transplantation. Cross-sectional imaging of the abdomen and chest is also necessary to rule out extrahepatic metastases. Approximately 25–35 % of patients evaluated for neoadjuvant therapy are not amenable to treatment due to distant or nodal metastases.

Patients with intrahepatic cholangiocarcinoma, distal cholangiocarcinoma (below the level of the cystic duct), and gallbladder cancer are best treated by resection. Even if tumors in these locations are unresectable, neoadjuvant therapy and transplantation have not been shown to have any efficacy. Other exclusion criteria include the following: primary tumor greater than 3 cm in radial diameter (perpendicular to the duct); uncontrolled infection; prior treatment with radiotherapy and/or chemotherapy that would preclude full-dose neoadjuvant therapy; and history of other malignancy within 5 years [23, 24]. Patients must also be a suitable candidate for transplantation.

Operative staging is essential prior to transplantation. When possible, operative staging is done by hand-assisted laparoscopy. It is best done prior to the actual transplant procedure to rule out locally extensive disease and presence of nodal, peritoneal, and extrahepatic metastases. Operative staging involves a thorough intra-abdominal exploration and biopsy of any suspicious lesions, a common hepatic



Fig. 8 Intrahepatic abscess(es) can be successfully managed with percutaneous interventional techniques under radiologic guidance. Pigtail catheter is located within the abscess cavity

artery lymph node overlying the hepatic artery at the takeoff of the gastroduodenal artery, and a pericholedochal lymph node. Occasionally, patients are too sick to undergo a separate operation, and staging is done at the time a donor liver becomes available.

2.2 Pre-Transplant Imaging

Pre-transplant cross-sectional imaging is performed every 3 months prior to transplantation as required by UNOS/OPTN policy for continuous surveillance of patients with malignancy awaiting liver transplantation. Evidence of disease progression or metastases while on the neoadjuvant protocol precludes transplantation. The patient dropout rate after starting neoadjuvant therapy is approximately 11.5 %

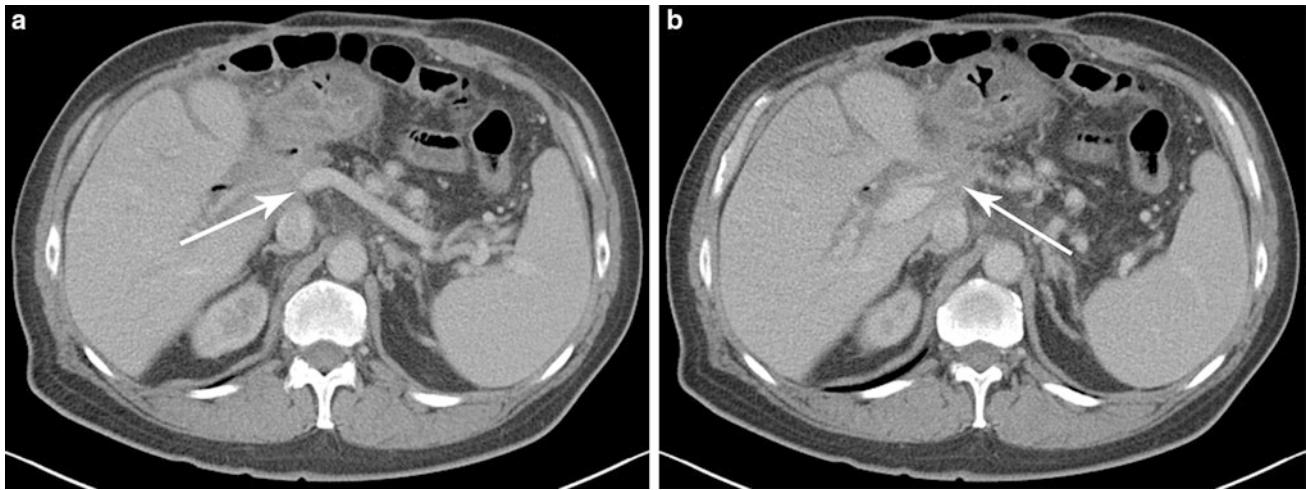


Fig. 9 Recurrent cholangiocarcinoma *arrow* presenting with hilar venous stenosis at the outflow of the splenomesenteric vein (Panel **a**) into the portal vein (Panel **b**)

per 3 months. Recurrent cholangitis occurs in the majority of the patients during neoadjuvant therapy [22]. Select patients, particularly those with PSC, can develop hepatic abscesses (Fig. 7a, b) requiring preoperative percutaneous drainage Fig. (8) and prolonged antibiotic therapy. Active patient surveillance and supervision by a multidisciplinary transplant team throughout the course of therapy is critical for successful management of treatment complications and achieving success.

2.3 Liver Transplantation

Liver transplantation for hilar cholangiocarcinoma is done in a similar fashion to transplantation for other acute and chronic liver diseases with several technical considerations [24, 25]. Hilar dissection is avoided to prevent dissemination of tumor. The portal vein is transected as proximal to its emergence from retropancreatic groove as possible to excise majority of native vein present in the irradiated field. Similarly, the bile duct is transected as close to the pancreas as possible, and the margin is checked by frozen section. Marginal involvement has been limited to patients with underlying PSC and has been observed in approximately 10 % of PSC patients. Options include re-excision and pancreatoduodenectomy. Hepatic artery thrombosis (HAT) due to irradiation of the artery is avoided by reconstruction with a donor iliac artery jump graft from infrarenal aorta to the donor hepatic artery during deceased donor liver transplantation. This technique has not been successful with living donor liver transplantation due to the size mismatch between an iliac graft and a donor left or right hepatic artery. We now perform reconstruction with the irradiated

recipient proper or common hepatic artery and monitor the recipient closely with Doppler ultrasound during the post-operative period for any change in hepatic arterial flow.

Portal vein reconstruction is done by a direct anastomosis between the donor and recipient portal veins during deceased donor transplantation. A direct anastomosis is not possible during living donor transplantation due to low division of the recipient portal vein. The gap is reconstructed with a segment of deceased donor iliac vein as an interposition graft.

Biliary reconstruction is performed with a Roux-en-Y choledochojejunostomy or hepaticojejunostomy. We prefer to use an external transjejunal cholangiocatheter to obtain a cholangiogram during the early postoperative period with deceased donor transplantation; biliary reconstruction in a living donor transplant recipient is performed with an internal biliary stent.

3 Post-Transplant Imaging

3.1 Routine Surveillance

Biliary complications occur with the same frequency after transplantation for cholangiocarcinoma as they do for other diseases. Vascular complications, however, are more common after transplantation for cholangiocarcinoma due to the preoperative neoadjuvant therapy. Patients are examined by Doppler ultrasound immediately after transplantation and the next day to confirm patency of all vessels with flows in the normal directions. In addition, Doppler ultrasonography is also routinely performed on days 7 and 21, at 4 months, and annually. Patients with external biliary

cholangiocatheters undergo tube cholangiography on days 7 and 21 or when laboratory tests indicate a potential biliary problem. Follow-up specific for patients with cholangiocarcinoma includes chest, abdomen, and pelvis CT and CA 19-9 testing at 4 month intervals for the 1st year and yearly afterward. Approximately 20 % of patients develop recurrent cholangiocarcinoma, and local recurrence is a frequent cause of vascular (Fig. 9a, b), biliary, or enteric occlusion.

3.2 Vascular Complications

Vascular complications are more common after transplantation for cholangiocarcinoma than for other liver diseases due to the high-dose neoadjuvant radiotherapy. These complications primarily occur with the reconstructed portal vein in deceased and living donor recipients, and the reconstructed hepatic artery in living donor recipients [26]. Hepatic artery complications are avoided in deceased donor recipients by abandoning the irradiated artery and using an iliac artery jump graft. Vascular complications occurring late after transplantation can also be due to recurrent cancer. We have observed vascular complications in up to 40 % of our recipients attributable to neoadjuvant therapy: 20 % for the hepatic artery (living donor recipients) and 20 % for the portal vein (living and deceased donor recipients). Hepatic venous or caval outflow complications are rare and are comparable to non-cholangiocarcinoma transplant recipients [26]. Doppler ultrasound and contrast-enhanced ultrasound technology have formed the foundation of vascular evaluation after liver transplantation with very high sensitivity and specificity [27–29]. Suspicious but inconclusive findings can be confirmed by cross-sectional imaging.

3.3 Hepatic Artery Complications

An iliac artery jump graft from donor hepatic artery to recipient infrarenal aorta is routinely used in deceased donor liver recipients. This approach was used in our early living donor recipients, but high rates of early hepatic artery complications led to a change in our practice. Currently, we perform donor hepatic artery to recipient-irradiated hepatic artery reconstructions with acceptable morbidity. In contrast to non-transplant patients, compromise in the hepatic arterial flow must be promptly corrected to avoid biliary ischemia, cholangiopathy, and subsequent biliary and infectious complications.

Arterial complications are approximately twice as common (approximately 20 %) after transplantation for hilar cholangiocarcinoma compared to non-cholangiocarcinoma recipients; rates can be even higher among living donor recipients. Hepatic artery thrombosis (HAT) is more severe



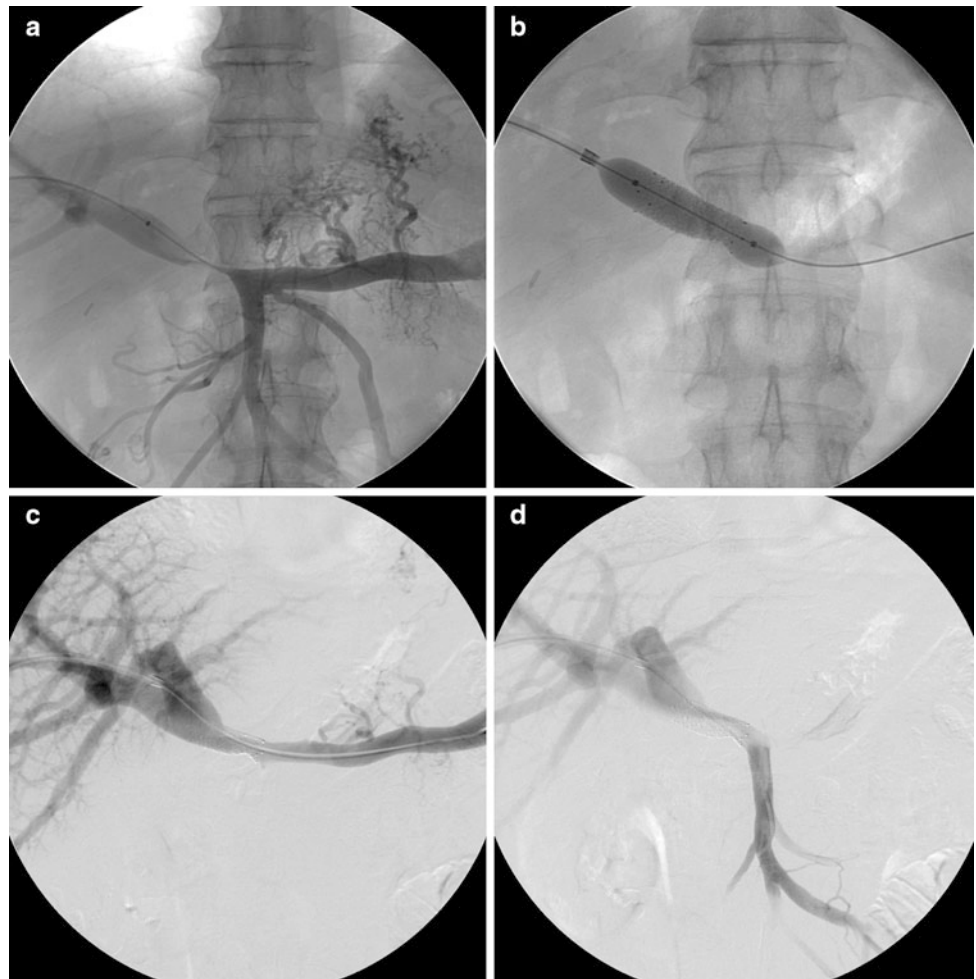
Fig. 10 Gastroduodenal artery stump pseudoaneurysm (arrow) in a patient with stented biliary anastomotic leak

than hepatic artery stenosis (HAS) and is associated with higher morbidity and mortality. Early HAT is best managed with emergency operative thrombectomy and thrombolysis. Operative intervention for late HAT is largely futile. Re-transplantation is necessary for up to 75 % of patients with early HAT, usually due to development of cholangiopathy and hepatic abscesses [30].

HAS may be managed with percutaneous transluminal angioplasty [31, 32]. Percutaneous stents have been used with increasing success for HAS, but they have also been associated with rare post-procedural thrombosis requiring retransplantation. Depending on the location of HAS, operative revision is also a possible treatment. We routinely use aspirin for HTA prophylaxis, and all cholangiocarcinoma patients receive short-term prophylactic low molecular weight heparin during the immediate postoperative period.

Mycotic pseudoaneurysms from either HA anastomosis or gastroduodenal artery stump (Fig. 10) are rare (approximately 1 %), but formidable complications of liver transplantation. The most frequent cause is an intra-abdominal infection or bile leak. We have also observed them after a leak from the pancreatic anastomosis for patients that required pancreatoduodenectomy. Unlike pancreatoduodenectomy only patients, these complications in liver transplant recipients require hepatic artery revision and occasionally retransplantation. Percutaneous embolization and/or intraluminal stent placement has been described, but both can lead to thrombosis, dissection, or arterial rupture with subsequent need for retransplantation [33].

Fig. 11 Late portal vein stenosis managed with transvenous balloon angioplasty and stent placement (Panels a through d)



3.4 Portal Vein Complications

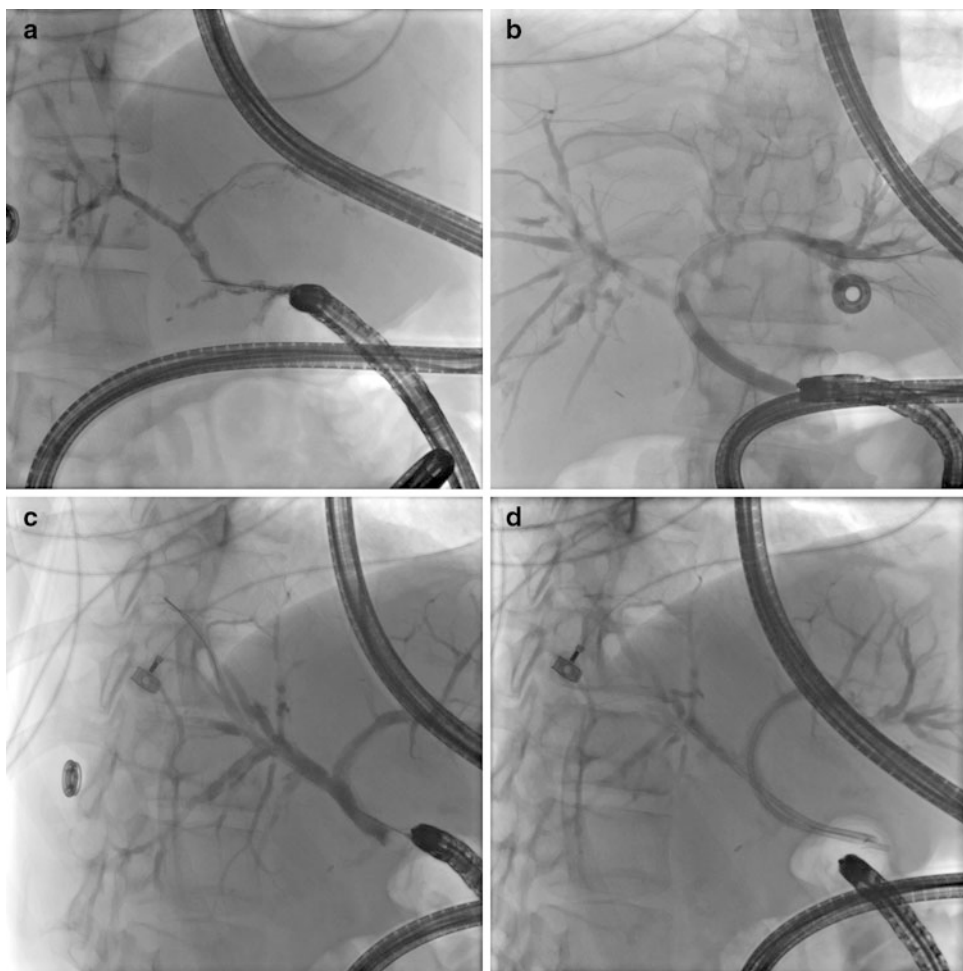
Portal vein reconstruction is performed with primary end-to-end anastomosis during deceased donor transplantation and with an iliac vein jump graft during living donor transplantation. Regardless of donor type, the native portal vein is divided as close as possible to the pancreas to achieve better tumor clearance and avoid the irradiated vein. Portal vein thrombosis (PVT) and portal vein stenosis (PVS) are significantly more common (approximately 20 %) after transplantation for hilar cholangiocarcinoma than for other indications [26]. Early PVT is managed by operative thrombectomy and anastomotic revision. Late PVT is rare, and recurrent cholangiocarcinoma must be excluded as a cause. PVS due to neoadjuvant therapy is most often detected by surveillance ultrasound and CT 4 months after transplantation. We have had excellent success and very few complications with percutaneous transhepatic portal angioplasty and stent placement (Fig. 11a–d) [34, 35]. When detected late, we have observed progressive portal stenosis

and thrombosis with observation, and we now prefer to intervene with endovascular angioplasty and stent placement for most patients who develop PVS [26].

3.5 Vascular Outflow Complications

Hepatic vein and inferior vena cava complications after liver transplantation for hilar cholangiocarcinoma are rare and do not differ in prevalence compared to non-cholangiocarcinoma liver recipients. Outflow complications have been more commonly described after living donor graft implantation, but we have not noticed a difference in our experience [26]. The hepatic veins are not affected by neoadjuvant radiotherapy. Outflow complications are thus purely technical and require intervention with either an endovascular approach or reoperation. Angioplasty and stent placement is the preferred strategy for management of venous outflow complications [36, 37]. Reoperation is possible only during the immediate postoperative period.

Fig. 12 Hepaticojejunostomy stricture as well as cholangiopathy in a recipient of donation after cardiac death donor liver. Anastomotic stricture treated endoscopically with balloon dilatation and stent placement (Panels a through d)



3.6 Biliary Complications

Biliary complications after transplantation for hilar cholangiocarcinoma occur in approximately 10 % of deceased donor liver recipients and 30 % of living donor liver recipients. Leaks with Roux-en-Y choledochojejunostomy after deceased donor transplantation are best managed by reoperation and revision. Leaks with Roux-en-Y hepaticojejunostomy after living donor transplantation are best managed by biliary intubation and drainage. Biliary strictures and cholangiopathy occur in approximately 15–20 % of liver recipients. Strictures can develop after resolution of a bile leak. Cholangiopathy can result from HAT (even with prompt revascularization) and donation after cardiac death (Fig. 12a–d) [22]. Biliary strictures are predominantly managed by endoscopic intervention and may require multiple procedures over a prolonged period of time. Cholangiopathy usually requires prolonged intervention and may eventually require retransplantation.

4 Summary

Interpretation of relevant imaging is essential for accurate preoperative diagnosis, operative planning, management of complications during neoadjuvant therapy, and post-transplant surveillance of patients with hilar cholangiocarcinoma. Treatment decisions and management of complications warrant a multidisciplinary team approach to patient care in order to achieve success.

References

1. Bismuth H, Corlette MB (1975) Intrahepatic cholangioenteric anastomosis in carcinoma of the hilus of the liver. *Surg Gynecol Obstet* 140:170–178
2. Rosen CB, Nagorney DM, Wiesner RH, Coffey RJ Jr, LaRusso NF (1991) Cholangiocarcinoma complicating primary sclerosing cholangitis. *Ann Surg* 213:21–25

3. Rosen CB, Heimbach JK, Gores GJ (2008) Surgery for cholangiocarcinoma: the role of liver transplantation. *HPB (Oxford)* 10:186–189
4. Ruys AT, van Beem BE, Engelbrecht MR, Bipat S, Stoker J, van Gulik TM (2012) Radiological staging in patients with hilar cholangiocarcinoma: a systematic review and meta-analysis. *Br J Radiol* 85:1255–1262
5. Hyodo T, Kumano S, Kushihata F et al (2012) CT and MR cholangiography: advantages and pitfalls in perioperative evaluation of biliary tree. *Br J Radiol* 85:887–896
6. Khan SA, Davidson BR, Goldin RD et al (2012) Guidelines for the diagnosis and treatment of cholangiocarcinoma: an update. *Gut* 61:1657–1669
7. Maccioni F, Martinelli M, Al Ansari N et al (2010) Magnetic resonance cholangiography: past, present and future: a review. *Eur Rev Med Pharmacol Sci* 14:721–725
8. Manfredi R, Masselli G, Maresca G, Brizi MG, Vecchioli A, Marano P (2003) MR imaging and MRCP of hilar cholangiocarcinoma. *Abdom Imaging* 28:319–325
9. Masselli G, Gualdi G (2008) Hilar cholangiocarcinoma: MRI/MRCP in staging and treatment planning. *Abdom Imaging* 33:444–451
10. Ariff B, Lloyd CR, Khan S et al (2009) Imaging of liver cancer. *World J Gastroenterol* 15:1289–1300
11. Kim HJ, Kim AY, Hong SS et al (2006) Biliary ductal evaluation of hilar cholangiocarcinoma: three-dimensional direct multi-detector row CT cholangiographic findings versus surgical and pathologic results—feasibility study. *Radiology* 238:300–308
12. Endo I, Shimada H, Sugita M et al (2007) Role of three-dimensional imaging in operative planning for hilar cholangiocarcinoma. *Surgery* 142:666–675
13. Chapman WC, Sharp KW, Weaver F, Sawyers JL (1989) Tumor seeding from percutaneous biliary catheters. *Ann Surg* 209:708–713
14. Sakata J, Shirai Y, Wakai T, Nomura T, Sakata E, Hatakeyama K (2005) Catheter tract implantation metastases associated with percutaneous biliary drainage for extrahepatic cholangiocarcinoma. *World J Gastroenterol* 11:7024–7027
15. Matsuo K, Rocha FG, Ito K et al (2012) The Blumgart preoperative staging system for hilar cholangiocarcinoma: analysis of resectability and outcomes in 380 patients. *J Am Coll Surg* 215:343–355
16. Friesen BR, Gibson RN, Speer T, Vincent JM, Stella D, Collier NA (2011) Lobar and segmental liver atrophy associated with hilar cholangiocarcinoma and the impact of hilar biliary anatomical variants: a pictorial essay. *Insights Imaging* 2:525–531
17. Zaydfudim VM, Clark CJ, Kendrick ML et al (2013) Correlation of staging systems to survival in patients with resected hilar cholangiocarcinoma. *Am J Surg* 206:159–165
18. Burak K, Angulo P, Pasha TM, Egan K, Petz J, Lindor KD (2004) Incidence and risk factors for cholangiocarcinoma in primary sclerosing cholangitis. *Am J Gastroenterol* 99:523–526
19. Lazaridis KN, Gores GJ (2006) Primary sclerosing cholangitis and cholangiocarcinoma. *Semin Liver Dis* 26:42–51
20. Heimbach JK, Sanchez W, Rosen CB, Gores GJ (2011) Transperitoneal fine needle aspiration biopsy of hilar cholangiocarcinoma is associated with disease dissemination. *HPB (Oxford)* 13:356–360
21. Gores GJ, Gish RG, Sudan D, Rosen CB (2006) Model for end-stage liver disease (MELD) exception for cholangiocarcinoma or biliary dysplasia. *Liver Transpl* 12:S95–S97
22. Darwish Murad S, Kim WR, Harnois DM et al (2012) Efficacy of neoadjuvant chemoradiation, followed by liver transplantation, for perihilar cholangiocarcinoma at 12 US centers. *Gastroenterology* 143:88–98
23. Rosen CB, Heimbach JK, Gores GJ (2010) Liver transplantation for cholangiocarcinoma. *Transpl Int* 23:692–697
24. Rea DJ, Rosen CB, Nagorney DM, Heimbach JK, Gores GJ (2009) Transplantation for cholangiocarcinoma: when and for whom? *Surg Oncol Clin N Am* 18:325–337
25. Heimbach JK, Gores GJ, Haddock MG et al (2004) Liver transplantation for unresectable perihilar cholangiocarcinoma. *Semin Liver Dis* 24:201–207
26. Mantel HT, Rosen CB, Heimbach JK et al (2007) Vascular complications after orthotopic liver transplantation after neoadjuvant therapy for hilar cholangiocarcinoma. *Liver Transpl* 13:1372–1381
27. Dodd GD 3rd, Memel DS, Zajko AB, Baron RL, Santaguida LA (1994) Hepatic artery stenosis and thrombosis in transplant recipients: Doppler diagnosis with resistive index and systolic acceleration time. *Radiology* 192:657–661
28. Hom BK, Shrestha R, Palmer SL et al (2006) Prospective evaluation of vascular complications after liver transplantation: comparison of conventional and microbubble contrast-enhanced US. *Radiology* 241:267–274
29. Huang TL, Cheng YF, Chen TY et al (2010) Doppler ultrasound evaluation of postoperative portal vein stenosis in adult living donor liver transplantation. *Transplant Proc* 42:879–881
30. Duffy JP, Hong JC, Farmer DG et al (2009) Vascular complications of orthotopic liver transplantation: experience in more than 4,200 patients. *J Am Coll Surg* 208:896–903
31. Sommacale D, Aoyagi T, Dondero F et al (2013) Repeat endovascular treatment of recurring hepatic artery stenoses in orthotopic liver transplantation. *Transpl Int* 26:608–615
32. Rostambeigi N, Hunter D, Duval S, Chinnakotla S, Golzarian J (2013) Stent placement versus angioplasty for hepatic artery stenosis after liver transplant: a meta-analysis of case series. *Eur Radiol* 23:1323–1334
33. Saad WE, Dasgupta N, Lippert AJ et al (2013) Extrahepatic pseudoaneurysms and ruptures of the hepatic artery in liver transplant recipients: endovascular management and a new iatrogenic etiology. *Cardiovasc Intervent Radiol* 36:118–127
34. Ko GY, Sung KB, Yoon HK, Lee S (2007) Early posttransplantation portal vein stenosis following living donor liver transplantation: percutaneous transhepatic primary stent placement. *Liver Transpl* 13:530–536
35. Cheng YF, Ou HY, Tsang LL et al (2010) Vascular stents in the management of portal venous complications in living donor liver transplantation. *Am J Transplant* 10:1276–1283
36. Ko GY, Sung KB, Yoon HK et al (2002) Endovascular treatment of hepatic venous outflow obstruction after living-donor liver transplantation. *J Vasc Interv Radiol* 13:591–599
37. Darcy MD (2007) Management of venous outflow complications after liver transplantation. *Tech Vasc Interv Radiol* 10:240–245

Magnetic Resonance Imaging (Including MR Cholangiopancreatography)

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Abstract

Imaging features of biliary tree and primary liver tumors are extremely diverse. Magnetic resonance imaging (MRI) of the liver and MR cholangiopancreatography (MRCP) provide a solid understanding of imaging manifestations useful for accurate detection, characterization, and tumor assessment. Knowledge of tumor characteristics and mimickers is essential for tumor diagnosis and appropriate management. In this chapter, we will discuss the imaging features of biliary tree, gallbladder, and primary liver tumors.

1 Introduction

Magnetic resonance imaging (MRI) is a comprehensive imaging modality with multiplanar capability to assess the liver parenchyma, gallbladder, and biliary tree. MRI provides a comprehensive assessment of the tissue characteristics and vascularity of different pathologies with excellent soft tissue contrast resolution. The addition of magnetic resonance cholangiopancreatography (MRCP) to the MR protocol can help delineate the fluid-filled lumen of the biliary tree and the gallbladder. The diagnostic accuracy of MRCP is comparable to endoscopic retrograde cholangiopancreatography (ERCP) in the diagnosis of biliary pathologies [1–7].

2 Magnetic Resonance Imaging Techniques

Liver MRI protocol typically includes a T1-weighted turbo field-echo in-phase and opposed sequence or a multi-echo Dixon sequence to separate water and fat as well as tissue iron, a breath-hold or respiratory-triggered multishot T2-weighted sequence, diffusion-weighted imaging either respiratory-triggered or breath-held with at least two *b* values, and unenhanced and contrast-enhanced gradient-echo

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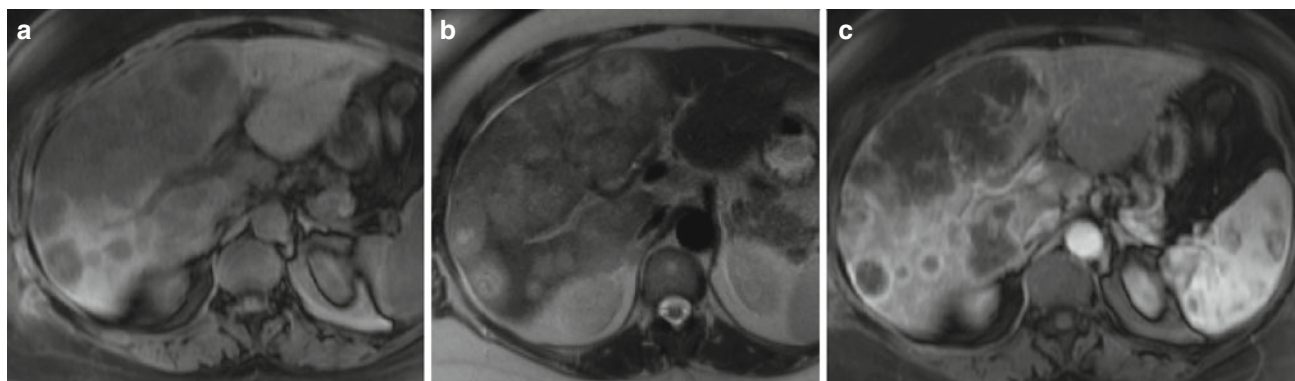


Fig. 1 **a–b** T1- and T2-weighted images show multiple hepatic masses throughout the liver, with a more confluent area of tumor within the inferior right hepatic lobe consistent with metastatic cholangiocarcinoma. These lesions demonstrate rim enhancement on **c** arterial phase

(GRE) T1-weighted imaging sequences, which can be either acquired at predetermined time points, i.e., arterial phase (20–25 s), portal venous phase (60 s), delayed phase (3 min), or using a bolus-tracking technique to determine the patient-specific timing of the different post-contrast phases. This protocol provides comprehensive overview, which allows the detection of hepatic or biliary malignancies, with diffusion and contrast-enhanced sequences providing a noninvasive assessment of the vascularity, viability, and cell density of the tissue and suspected malignancy.

MRCP is performed using breath-hold (using a single-shot approach) or non-breath-hold techniques (with respiratory triggering) two-dimensional (2D) or three-dimensional (3D) T2-weighted sequences. A 3D technique provides a higher signal-to-noise ratio (SNR), which is traded off for thinner contiguous slices. Acquiring images with near isotropic voxels allows improved post-processing manipulation of the images with multiplanar reconstruction, maximum intensity projection (MIP), and volume rendering. The introduction of faster gradients and a parallel acquisition technique has resulted in even greater spatial resolution and faster acquisition times. Often, patients fast for at least 4 h in order to reduce fluid secretions within the stomach and duodenum, reduce bowel peristalsis, and promote gallbladder distension. Sometimes, a negative oral contrast agent (e.g., iron oxide or blueberry juice) is used to reduce the signal intensity of overlapping fluid within the stomach and duodenum. Recently, functional assessment of biliary excretion has become possible with the use of hepatobiliary contrast media by T1-weighted sequences for MRCP.

Hepatobiliary contrast agents include, historically, mangafodipir trisodium (Teslascan; Nycomed Amersham), gadobenate dimeglumine (Gd-BOPTA, MultiHance; Bracco Imaging), and gadolinium ethoxybenzyl-diethylenetriamine penta-acetic acid (Gd-EOB-DTPA, Eovist; Bayer Healthcare). These contrast agents can provide standard arterial, portal venous, and equilibrium phase images with an added

hepatobiliary phase 10–20 min after contrast injection for Gd-EOB-DTPA and 1 h after contrast injection for Gd-BOPTA. Furthermore, delayed imaging in the axial and coronal planes, 10–120 min after contrast administration, results in hyperintense bile on T1-weighted fat-saturated images. The advantages of functional MRCP compared to classical T2-weighted MRCP are effective in evaluation of biliary anatomy [8], improved visibility communications between cystic lesions and draining bile ducts in the diagnosis of congenital biliary disorders [9, 10], differentiation of biliary from extrabiliary lesions [8], improved diagnostic accuracy of true obstruction in a dilated biliary system compared pseudo-obstruction [11], and better detection of post-operative complications including depiction of active extravasation of contrast in suspected bile leaks [10, 12].

3 Cholangiocarcinoma

Cholangiocarcinomas are a distinct type of tumors that originate from the biliary epithelium either within the liver or within the biliary tract (Fig. 1). Grossly, cholangiocarcinoma is a firm hypovascular tumor with predominantly fibrous stroma, and, histologically, it is a well-differentiated adenocarcinoma with desmoplasia. There are various recognized risk factors for cholangiocarcinoma; infections with liver flukes and hepatolithiasis are considered common in endemic areas. Endogenous and dietary nitrosamine compounds associated with parasitic infections act as cofactors in carcinogenesis owing to the carcinogenic effect of nitrosamine compounds on the proliferation of epithelial cells of the bile duct [13, 14]. In the western world, most common risk factors for cholangiocarcinoma include primary sclerosing cholangitis, hepatic cirrhosis, chronic hepatitis C infection, alcoholic liver disease, chronic inflammatory bowel disease, and diabetes [15, 16]. MR imaging and MRCP are very useful in the diagnosis and

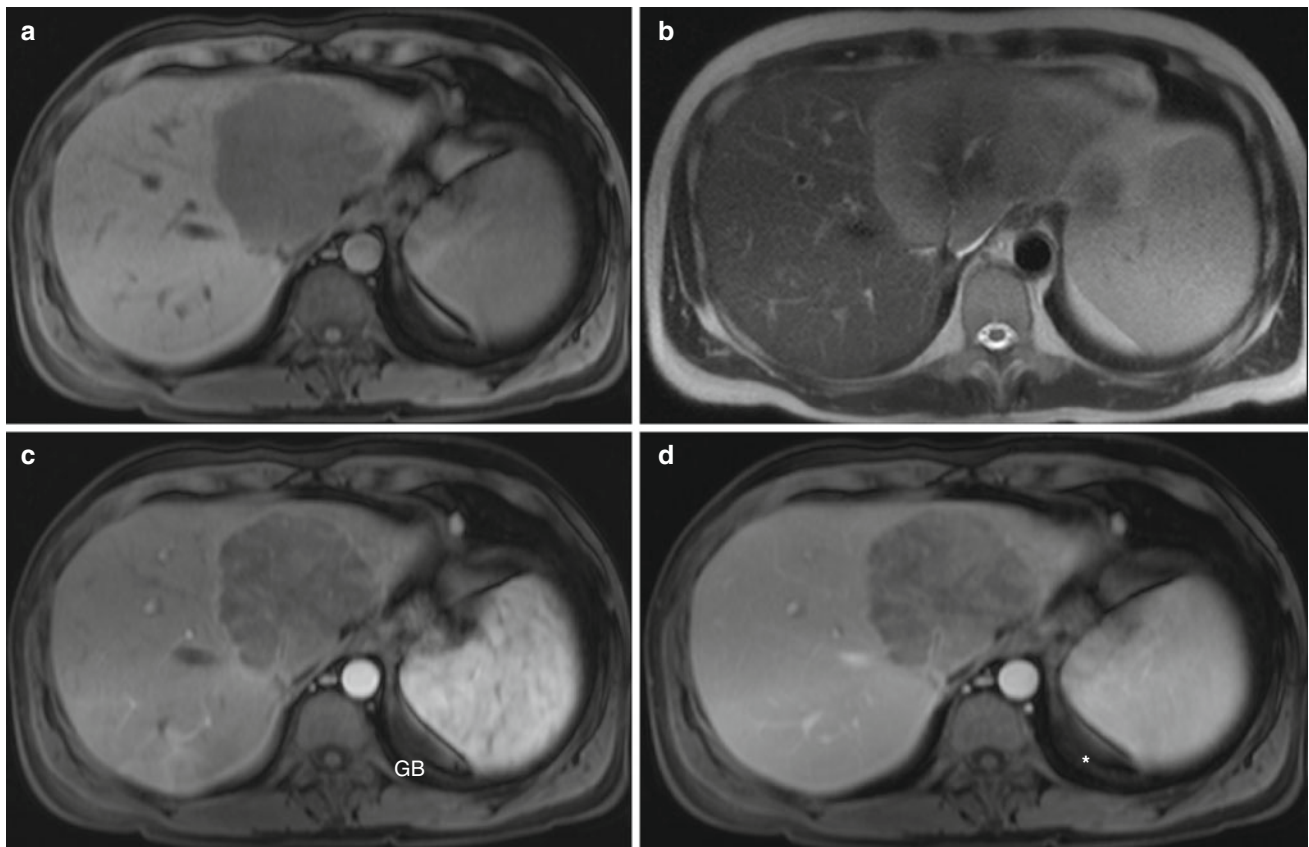


Fig. 2 A 73-year-old male diagnosed with cholangiocarcinoma. There is a heterogeneous mass of approximately 8×5.5 cm to the left of the falciform ligament involving the left and anterior right hepatic lobes. The left and middle hepatic veins are occluded. The

mass demonstrates irregular borders predominantly hypointense in **a** T1- and **b** T2-weighted images. There is rim irregular enhancement of the mass after contrast administration in **c** arterial and **d** venous phases with presence

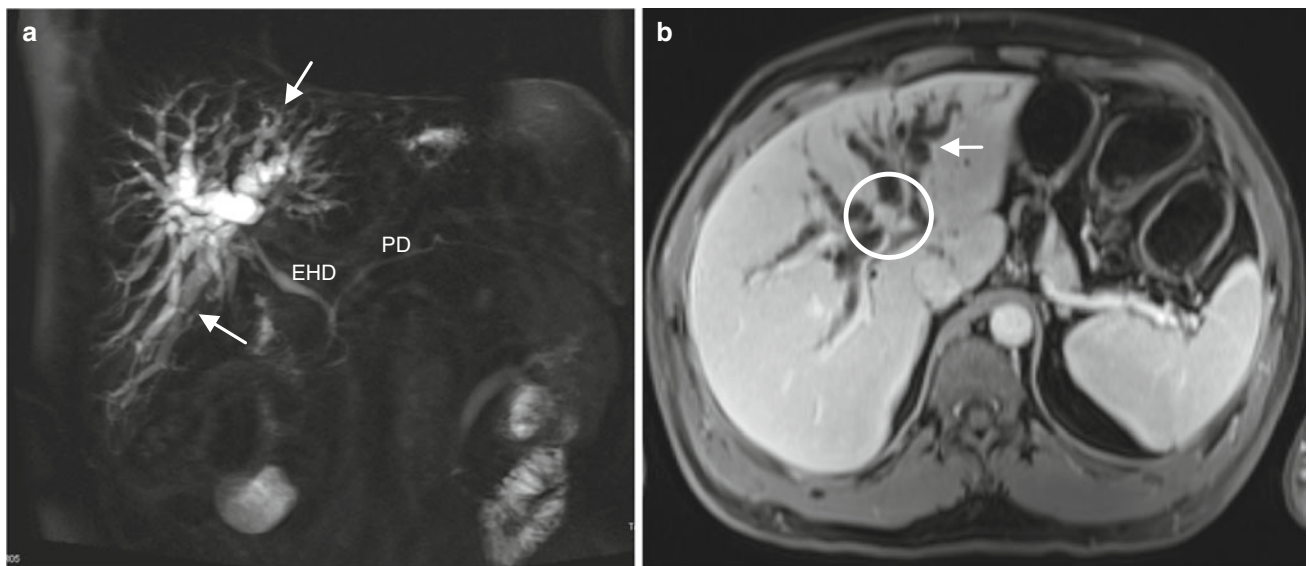


Fig. 3 **a** MRCP shows marked intrahepatic biliary dilatation on the right and left ducts (arrows); the pancreatic duct (PD) and the extrahepatic biliary duct (EHD) are normal. **b** Delayed contrast-

enhanced image depicts an abrupt transition point at the porta hepatis showing a nodular area of enhancement (circle) due to a small tumor demonstrated by brushing

assessment of resectability in cholangiocarcinoma and to visualize surrounding bile ducts, vessels, and hepatic parenchyma owing to its intrinsic high tissue contrast and multiplanar capability [17, 18]. MR imaging consists of axial and coronal T2-weighted images, fat-sat T1-weighted images, dynamic contrast-enhanced T1-weighted images (DCE-MR), and MRCP. T2-weighted images and T1-weighted MR images are useful in detection and characterization of the tumor (Fig. 1), DCE-MR is useful in differentiating benign from malignant strictures, and MRCP is useful in evaluating periductal-infiltrating or intraductal-growing-type cholangiocarcinoma (Figs. 2 and 3).

Cholangiocarcinoma is broadly classified into (1) extrahepatic and (2) intrahepatic cholangiocarcinoma (ICC).

3.1 Intrahepatic Cholangiocarcinoma

ICC is the second most common primary malignancy of the liver behind hepatocellular carcinoma (HCC). Based on the morphology and growth patterns, cholangiocarcinoma has been classified into three types: (1) mass-forming (2) intraductal-growing, and (3) periductal-infiltrating types [19].

Dynamic contrast-enhanced MR imaging findings in cholangiocarcinoma, in general, include early rim enhancement, characteristic delayed and persistent enhancement of the tumor. These findings reflect the characteristic fibrous content in the tumor and delayed diffusion of the contrast through the tumor interstitium [20]. On Gd-EOB-DTPA-enhanced MRI (Eovist), ICC presents as a hypointense lesion in delayed phase. Images in the hepatobiliary phase demonstrate highest lesion conspicuity with high contrast-to-noise ratio (CNR), and there will be no liver-specific contrast uptake since there will be negligible increase in SNR from late venous to hepatobiliary phases [21].

3.2 Intrahepatic Cholangiocarcinoma: Mass-Forming Type

Mass-forming cholangiocarcinoma presents as a homogeneous mass with irregular, well-defined margins often associated with dilatation of the biliary trees in the periphery (Fig. 4). On MR imaging, the mass demonstrates irregular margins with high signal intensity on T2-weighted imaging and low signal intensity on T1-weighted imaging. On T2-weighted images, there may be a central hypointense area, which probably reflects severe fibrosis. On post-contrast MR images, there will be irregular peripheral enhancement with concentric filling of contrast material [22]. Significant central enhancement can be seen in delayed phase MR imaging, and this may be, again, due to the fibrous stroma

of the tumor. Atypical presentations such as homogenous hypervascular enhancement, strong hyperintensity, and centripetal enhancement on T2-weighted MR images can be seen in mucinous carcinoma, but it presents with continuous ragged rim enhancement, which can help differentiate it from a hemangioma.

3.3 Intrahepatic Cholangiocarcinoma: Periductal-Infiltrating Type

Periductal-infiltrating type of cholangiocarcinoma usually presents as a growth along a dilated or narrowed bile duct without mass formation and as an elongated or branchlike abnormality. Early diagnosis of periductal-infiltrating type of cholangiocarcinoma may be difficult since it may appear to be a benign looking stricture in the early stages. It is important to differentiate a benign from a malignant stricture and findings such as stricture with an irregular margin, asymmetric narrowing, lymph node enlargement, enhancing ducts, and periductal soft tissue lesion should raise strong suspicion for a malignant stricture [6]. On MR imaging, periductal-infiltrating type of cholangiocarcinoma presents with diffuse periductal thickening and increased enhancement due to tumor infiltration with abnormally dilated or irregularly narrowed duct and peripheral ductal dilatation [23] (Figs. 5 and 6).

3.4 Intrahepatic Cholangiocarcinoma: Intraductal-Growing Type

Intraductal-growing-type cholangiocarcinoma has a significantly better prognosis than mass-forming-type or periductal-infiltrating-type cholangiocarcinoma (Fig. 7). MR imaging features suggestive of intraductal-growing-type cholangiocarcinoma include (1) papillary or irregular polypoid shape, (2) lack of constriction of the tumor-bearing segment, (3) hypoenhancement of the tumor to the liver during the equilibrium phase, (4) tumor multiplicity, (5) upstream and downstream bile duct dilatation, and (6) no bile duct wall thickening adjacent to the tumor. Kim et al. suggested that the presence of at least two of these six imaging features, when used in combination, has a sensitivity and specificity in the diagnosis intraductal-growing-type cholangiocarcinoma of 95 and 70 %, respectively. Intraductal-growing-type cholangiocarcinoma has a tendency to spread superficially along the mucosal surface, resulting in multiplication. Intraductal-growing-type cholangiocarcinoma more often showed washout, whereas mass-forming cholangiocarcinoma more often showed gradual persistent or progressive enhancement, which will help in differentiating between the two [24] (Figs. 8 and 9).

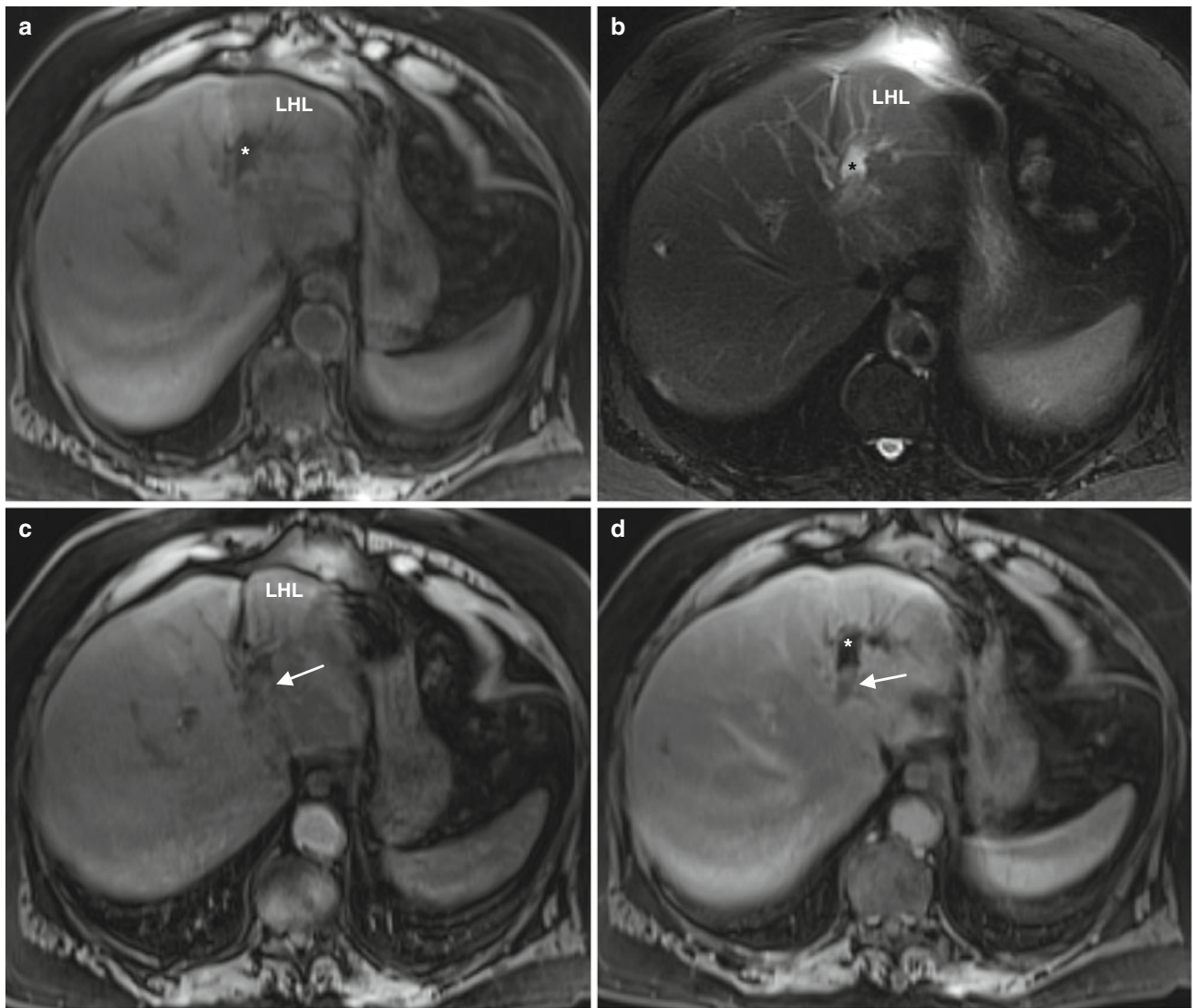


Fig. 4 **a** T1-weighted and **b** T2-weighted images demonstrate atrophy of the left hepatic lobe (LHL) with intrahepatic biliary dilatation (*). The biliary dilatation terminates at the level of an enhancing mass

(arrows) in the left lobe as shown in the arterial **c** and venous phases **d** consistent with cholangiocarcinoma

3.5 Extrahepatic Cholangiocarcinoma

Extrahepatic cholangiocarcinoma arises from the ductal epithelium of the extrahepatic bile duct. The most important factors in evaluating patients with extrahepatic cholangiocarcinoma are to determine tumor location and its longitudinal extent since these factors have greatest influence on surgical method and survival [13]. MRI is one of the most important diagnostic imaging modalities of choice used in assessing the longitudinal and lateral spread of a tumor when determining resectability. Perihilar cholangiocarcinomas have been categorized into four types by the modified Bismuth–Corlette classification adapted from the original classification [25]. On MR imaging, the enhancement pattern of

extrahepatic cholangiocarcinomas is similar to that of ICCs. The tumors are hypovascular and enhance slowly and gradually to a peak on delayed imaging. These tumors are less heterogeneous than ICC since they present as small infiltrating tumors. Satellite nodules and central scars are unusual compared to ICC. Extrahepatic cholangiocarcinoma typically presents as abnormal circumferential extrahepatic bile duct wall thickening and enhancement best visualized 1–5 min after gadolinium administration [26].

On diffusion-weighted imaging, extrahepatic cholangiocarcinoma demonstrates differential levels of high signal intensity and low signal intensity in apparent diffusion coefficient maps and has great sensitivity in detection of extrahepatic cholangiocarcinoma comparable to MRCP [27].

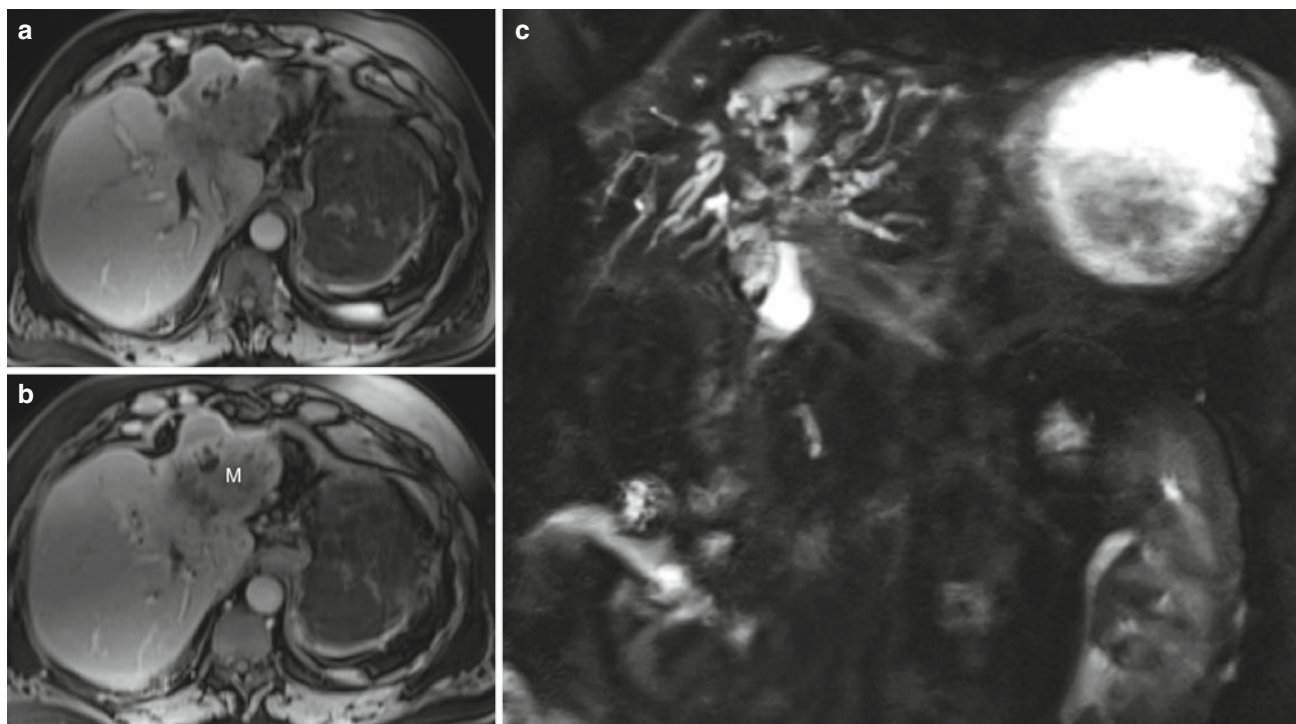


Fig. 5 There is a hypointense ill-defined 9.4×6.2 cm mass within the left hepatic lobe associated with atrophy of the left hepatic lobe. After contrast administration, there is heterogeneously rim

enhancement in **a** portal and **b** delayed phases. **c** MRCP shows intrahepatic biliary ductal dilatation in the right and left hepatic lobes

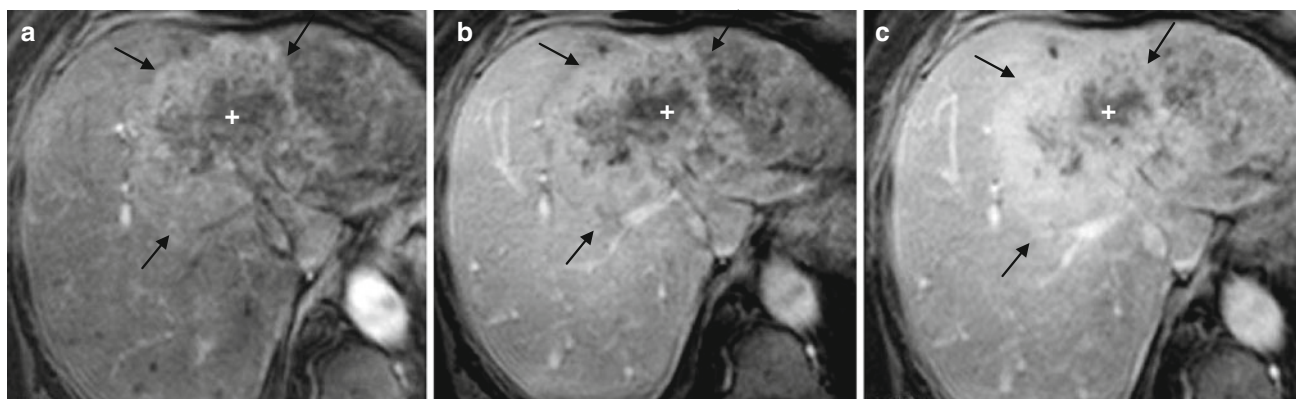


Fig. 6 A 66-year-old female diagnosed with intrahepatic cholangiocarcinoma. There is a large heterogeneously infiltrating mass involving the right and left hepatic lobes. **a–c** after contrast administration

enhanced images showed peripheral enhancement (*arrows*) and central hypointense region probably associated with necrosis (+)

3.6 Hilar Cholangiocarcinoma/Klatskin Tumors

Hilar cholangiocarcinoma, also known as Klatskin tumors, are adenocarcinomas that arise at the confluence of the right and the left hepatic bile ducts. MRI and MRCP can provide accurate preoperative staging of biliary tree, liver, and vascular involvement, and this is crucial in choosing the

most appropriate treatment option in patients with cholangiocarcinoma.

Hilar cholangiocarcinoma demonstrates circumferential growth and spreads along the bile ducts with poor conspicuity on non-contrast MR images [28]. Hilar cholangiocarcinoma presents with the same signal intensity as peripheral tumors on both T1- and T2-weighted images. On post-contrast images, hilar cholangiocarcinomas do not

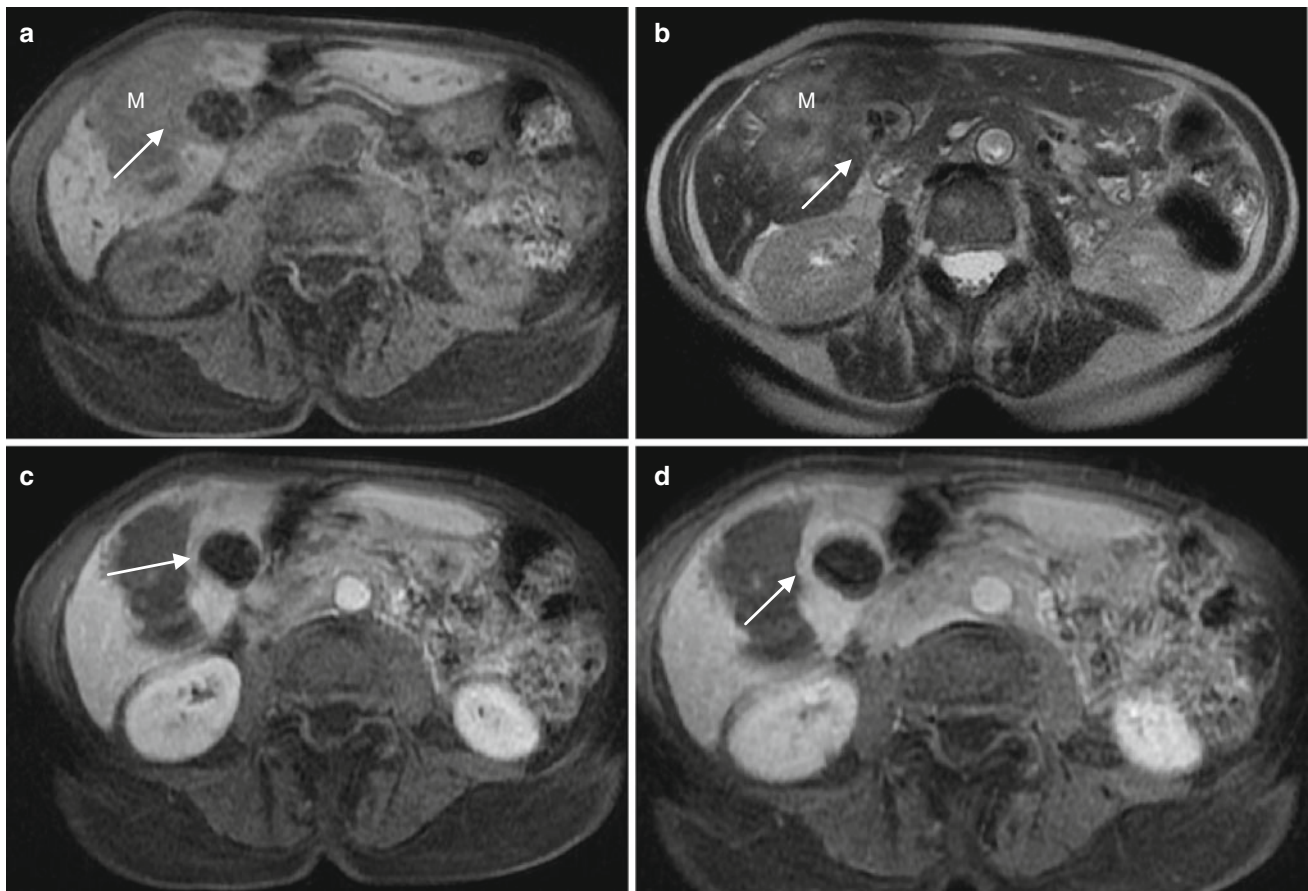


Fig. 7 A 73-year-old female with history of metastatic cholangiocarcinoma. **a** T1 image shows a dark infiltrative mass with irregular border on the right hepatic lobe. On **b** T2-weighted image, the mass looks heterogeneously hyperintense. After contrast administration,

c portal and **d** delayed images showed peripheral enhancement. The mass appears contiguous with the gallbladder fundus with associated thickening of the fundus (arrows) and gallbladder stones

always show a unique enhancement pattern. Most of the lesions are hypovascular compared to the adjacent liver parenchyma, with a heterogeneous enhancement that gradually peaks on delayed phase images, which is due to the fibrous nature of the tumor [29]. Some lesions show periductal enhancement, whereas very few hilar cholangiocarcinoma are hypervascular, but they do not demonstrate immediate diffuse enhancement unlike other hypervascular lesions.

3.7 Mixed Hepatocellular carcinoma–Cholangiocarcinoma

Mixed hepatocellular carcinoma–cholangiocarcinoma (HCC-CC) contributes to a small but significant proportion of primary liver malignancies, and they are comprised of cells with histopathological features of both cholangiocarcinoma and HCC [30].

On MRI, mixed hepatocellular carcinoma–cholangiocarcinoma usually presents with a single mass, moderately

high signal intensity on T2, tumor demonstrating progressive enhancement or contrast retention, and frequent lack of capsule. Enhancement patterns include early rim enhancement and diffuse heterogeneous enhancement [31]. Hwang et al. demonstrated that on Gadoxetic acid-enhanced MRI, irregular shape, strong rim enhancement during early dynamic phase MRI, and absence of target appearance on hepatobiliary phase were more suggestive of hepatocellular carcinoma–cholangiocarcinoma (HCC-CC), whereas the findings of a lobulated shape, weak peripheral rim enhancement, and presence of complete target appearance on the hepatobiliary phase were more of suggestive ICC [32].

• Differential Diagnosis:

Variety of neoplastic and non-neoplastic conditions can mimic the findings of cholangiocarcinoma and, thus, poses significant challenges in the diagnosis and management of these patients.

• Neoplastic Conditions:

A tumors that should be considered in the differential diagnosis of cholangiocarcinoma includes HCCs. Patients

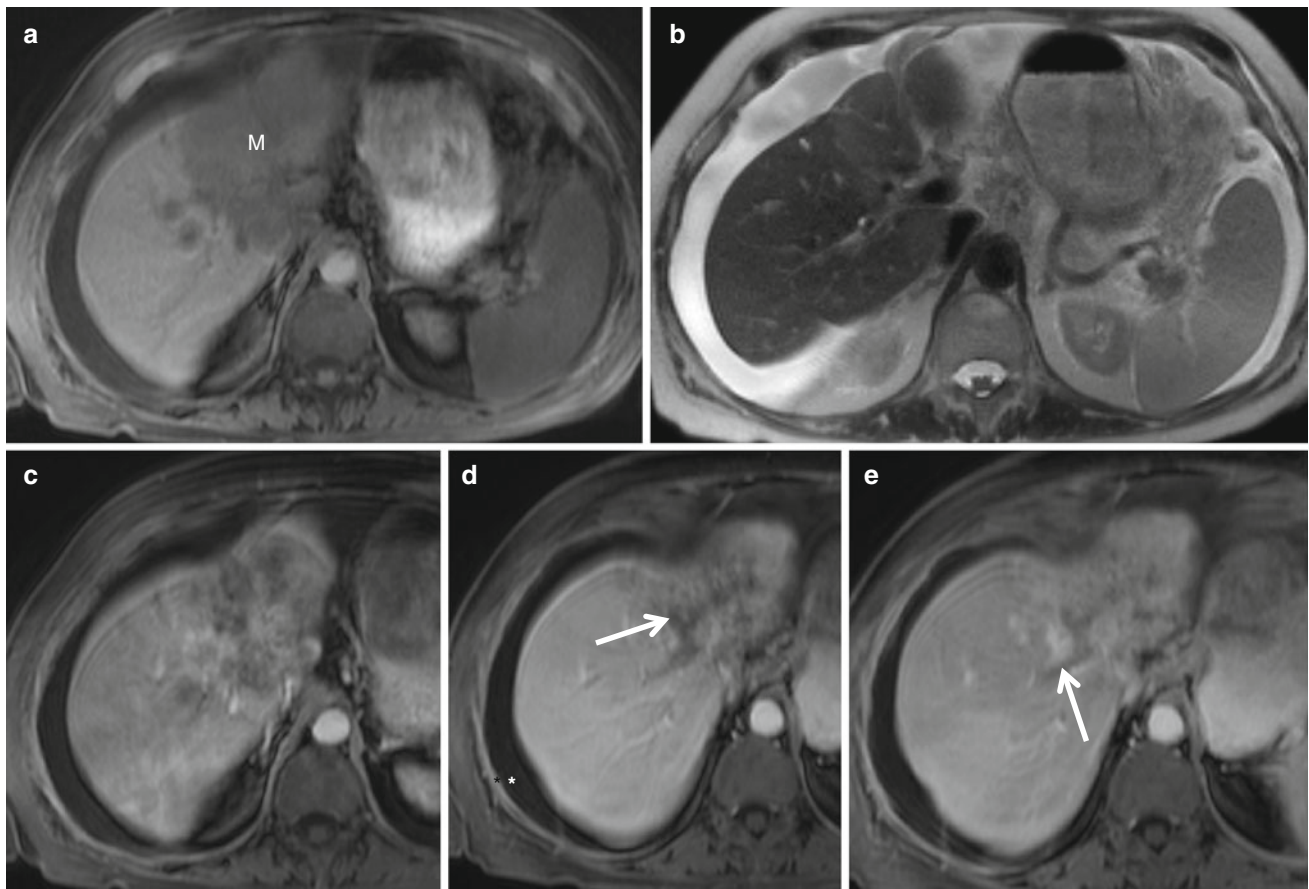


Fig. 8 Shrinkage of left hepatic lobe is noticed. **a** T1-weighted image shows a hypointense mass (*M*), which nearly replaces the entire left lobe of the liver, and it measures 8×7 cm. There is mild intrahepatic

biliary duct dilatation (arrows). After contrast administration (**c–e**), there is late and heterogeneously enhancement of the mass. There is ascites seen in abdomen (*)

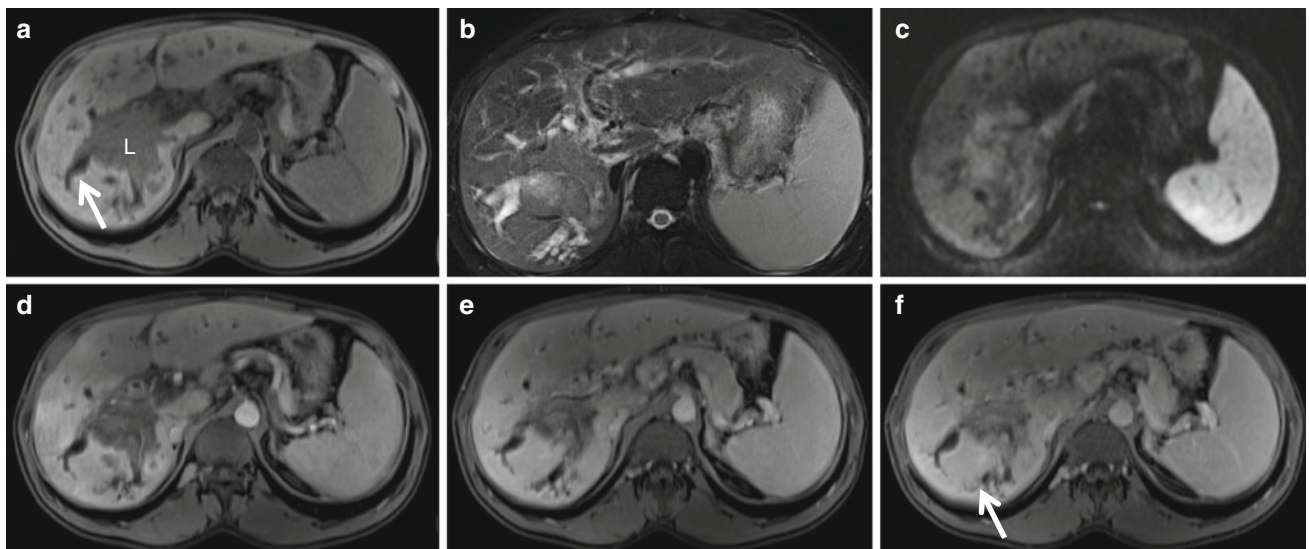


Fig. 9 A 33-year-old male with diagnosis of cholangiocarcinoma. There is a **(a)** T1 hypointense **(b)** T2, and **(c)** diffusion hyperintense infiltrative lesion (*L*) with **(d–f)** delayed enhancement near to the porta

hepatis extending to the right hepatic lobe. There is severe intrahepatic biliary ductal dilatation proximal to the lesion involving the entire right hepatic lobe (arrows)

where clinical background of cirrhosis, hepatitis B/C positive serology, and/or high levels of alpha feto protein (AFP) should alert toward the suspicion of HCC. According to the American Association for the Study of Liver Diseases (AASLD), diagnosis of HCC is to be considered in a mass larger than 2 cm with typical features of hypervascularity in the arterial phase and wash-out in the venous phase on a contrast-enhanced computed tomography or magnetic resonance (MR) imaging. A mass measures 1–2 cm is also considered suspicious for HCC if it shows these features at both CT and MRI. Park et al. demonstrated that a target appearance, with central enhancement and a hypointense rim on diffusion-weighted imaging (DWI), proved to be a reliable imaging marker indistinguishing small mass-forming an ICC from a small HCC [33].

Other neoplastic conditions to be considered in the differential diagnosis include intrabiliary metastases. Although rare, if suspected, metastasis from colonic adenocarcinoma tops the list since it shows increased predilection for biliary ducts [34]. Biliary tract melanoma can either be a primary melanoma arising from the biliary epithelium or metastasis from elsewhere. Owing to its own melanin content, this mass may demonstrate high signal intensity on T1-weighted images and low signal intensity on T2-weighted images [35]. Lymphoma of the bile ducts is very rare and usually a secondary manifestation of systemic disease. Most biliary lymphomas are non-Hodgkin's lymphomas. Carcinoid tumors of the bile ducts are rare and account for less than 2 % of gastrointestinal carcinoid tumors. Imaging findings vary and are non-specific, including biliary strictures with associated wall thickening or a large exophytic mass, thus mimicking the periductal-infiltrating or mass-forming types of cholangiocarcinoma.

- **Non-neoplastic Conditions:**

Various conditions that mimic cholangiocarcinoma on imaging include primary and secondary sclerosing cholangitis (SSC) and Mirizzi syndrome. MR cholangiopancreatography (MRCP) is considered the best initial approach in the diagnosis of primary sclerosing cholangitis (PSC) and characteristic imaging findings suggestive of PSC include multifocal strictures, irregular beading of the intra- and extrahepatic bile ducts, segmental ectasia, and ductal wall thickening [36]. SSC represents various disorders that are similar to PSC resulting from distinct pathologic process and include recurrent pyogenic cholangitis, which presents in the setting of biliary obstruction by stones or biliary strictures with recurrent episodes of acute pyogenic cholangitis and usually affects the extrahepatic duct, lateral segment of the left lobe, and posterior segment of the right lobe. MR imaging findings characteristic of recurrent pyogenic cholangitis include biliary strictures,

intraductal pigmented stones, and ductal wall thickening due to fibrosis [37]. Mirizzi syndrome occurs due to the obstruction of the common hepatic duct due to compression by a gallstone impacted at the gallbladder neck or cystic duct, and it is considered that a low insertion of the cystic duct into the common hepatic duct is a predisposing factor for Mirizzi syndrome [38]. MRCP is a useful imaging modality in detecting gall stones and bile duct stenosis. Although imaging findings may not be specific, MRCP imaging findings suggestive of Mirizzi syndrome include presence of gallstone in the cystic duct, extrinsic narrowing of the common hepatic duct, dilatation of the intrahepatic and common hepatic ducts, and a normal common bile duct. In some cases, there will be strictures secondary to inflammation around the common bile duct and, thus, can resemble cholangiocarcinoma of the periductal-infiltrating type [39].

Another condition that resembles periductal-infiltrating-type cholangiocarcinoma is autoimmune pancreatitis–cholangitis. It presents with focal or diffuse strictures of the pancreatic ducts and the bile ducts. Narrowing of the intrapancreatic bile duct and bile duct strictures with upstream ductal dilatation can be seen resembling the periductal-infiltrating-type cholangiocarcinoma on MRI. The presence of pancreatic abnormalities, which include focal/diffuse/sausage-shaped diffuse enlargement of the pancreas with a peripheral hypoattenuating halo, should favor the diagnosis of autoimmune pancreatitis [40].

MRCP has been demonstrated to be a useful noninvasive imaging modality comparable to ERCP in differentiating extrahepatic bile duct cholangiocarcinoma from benign stricture. Based on cholangiographic criteria described by Park et al. [6] for malignant biliary strictures, irregular margins, and asymmetric narrowing were more commonly found in cholangiocarcinomas than in biliary strictures.

4 Periapillary Tumors

Periapillary tumors are neoplasms that arise within 2 cm of the major duodenal papilla and include pancreatic head carcinoma, intrapancreatic bile duct carcinoma, and periapillary duodenal carcinoma. While they share an anatomic location and clinical presentation, each malignancy has a different prevalence and outcome. While obstructive jaundice is the most common clinical symptom, the major changes seen on cross-sectional imaging are pancreaticobiliary duct dilatation, double duct sign, three-segment sign, four-segment sign, and shape and wall thickness of the distal margins of the common bile duct and the main pancreatic duct [41–43]. Periapillary tumors appear as low signal intensity masses in the region of the ampulla on T1-weighted fat-suppressed MRI. Most

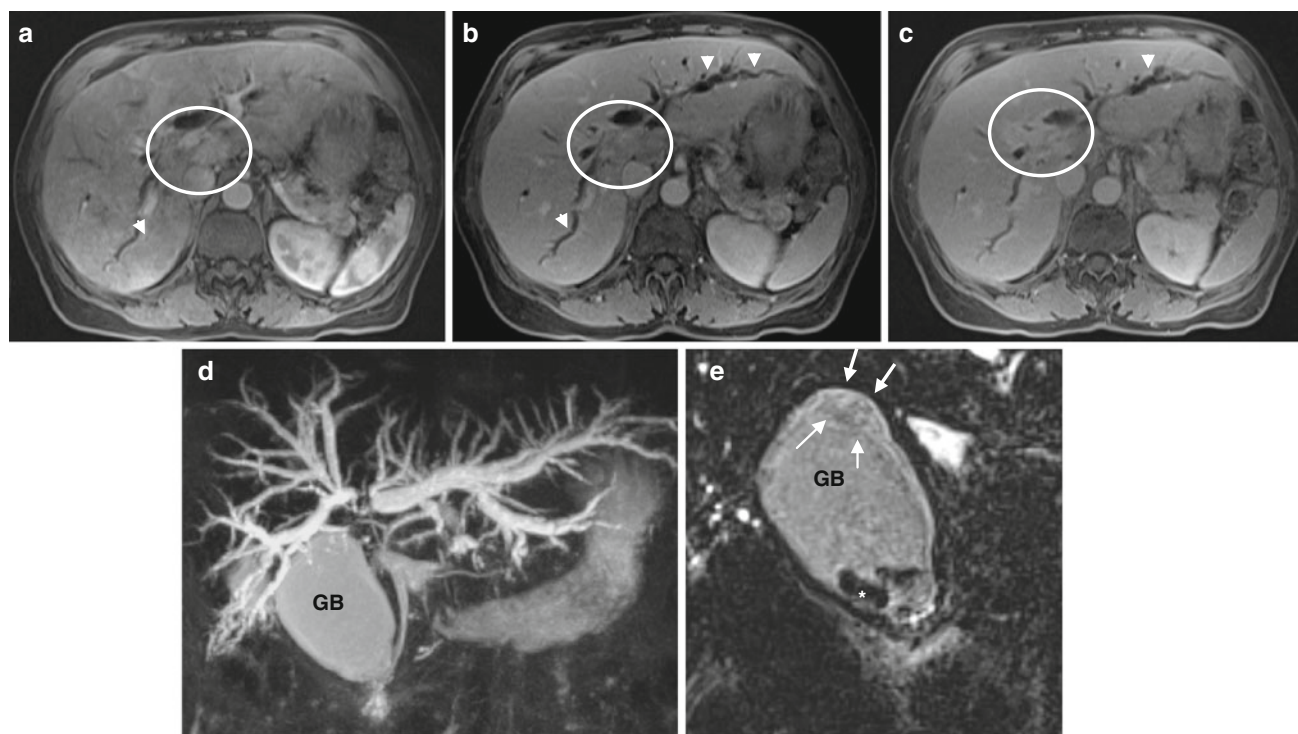


Fig. 10 A 69-year-old male with diagnosis of metastatic gallbladder adenocarcinoma. Contrast-enhanced MRI demonstrates (a–c) extensive intrahepatic ductal dilatation (arrowheads) with abrupt truncation at the hilar region related to an infiltrating mass measuring 4.2×2.6 cm (circle). On the delayed image (c), there is enhancement of the mass at

the hilar level. MRCP (d) shows a distended gallbladder (GB) with intrahepatic ductal dilatation of the biliary tree. e There is diffuse wall thickening more pronounced at the fundus (arrows) and presence of cholelithiasis (*)

lesions are hypovascular with low signal intensity relative to adjacent normal tissue on contrast-enhanced T1-weighted images; however, a thin rim of peripheral enhancement can often be found on these fat-suppressed images. MRCP helps determine the precise location and organ of origin of these tumors. MR imaging can not only be used to differentiate these lesions, but also to assess resectability [44].

5 Ampullary Carcinoma

Ampullary carcinoma is often considered part of the group of periampullary tumors. They are small tumors arising in the ampulla of Vater that often cause biliary outflow obstruction. Diagnosis can be difficult because these small tumors mimic the appearance of benign causes of biliary outflow obstruction such as papillitis, papillary stenosis, and sphincter of Oddi dysfunction [41, 45–47]. Classical signs of ampullary carcinoma seen on MRI with MRCP are the presence of an ampullary mass, papillary bulging, irregular and asymmetric common bile duct narrowing, and proportional biliary dilatation [41, 46, 47]. MRCP can detect

ampullary carcinoma with a high sensitivity (100 %), but limited specificity (59.1–63.6 %) [45]. The addition of diffusion-weighted MRI has been shown to improve detection in a recent study [48].

6 Gallbladder Carcinoma

Primary carcinoma of the gallbladder is the fifth most common tumor of the gastrointestinal tract [49]. Gallbladder carcinoma is a highly malignant tumor with a poor 5-year survival rate of less than 5 % [50]. Gallbladder carcinoma is more common in women than in men; this predilection is thought to be associated with a higher incidence of cholelithiasis. Up to 80 % of gallbladder carcinomas are associated with gallstones, and the risk seems to be associated with stone size [51]. Stones with diameter greater than 3 cm are detected more frequently in gallbladder carcinoma, suggesting a pathogenic role in the gallbladder epithelium carcinogenesis (Fig. 10) [50]. Chronic inflammation of the gallbladder by biliary components such as bile acids, bilirubin, and cholesterol also plays a pathogenic role in gallbladder malignant transformation [52].

Other risk factors are polypoid lesions (>10 mm), an anomalous junction of pancreaticobiliary ducts (AJPBD), especially without choledochal cyst, and aa porcelain gallbladder in up to 25 % of cases [51]. Gallbladder polyps are predisposing factors for gallbladder carcinoma with a prevalence of 3–6 %. This prevalence increases with polyp size (>15 mm, 46–70 %), number (solitary, 80–100 %), shape (sessile, 30 %), and echogenicity (isoechoic or echopenic) of the polypoid lesion [50]. AJPBD is a congenital defect in the union of pancreatic and biliary ducts; this condition is associated with gallbladder cancer in approximately 10 % of patients, particularly in patients without cystic dilatation of the common bile duct [53]. Calcification of the gallbladder wall, known as “porcelain gallbladder,” is associated with approximately 20 % of gallbladder carcinomas. Patients with incomplete calcification of the gallbladder will be at higher risk than those with complete mucosal calcifications. However, the relationship of calcification to malignancy has not been well established.

Less important associated conditions are chronic bacterial infections (*Escherichia coli*, *Opisthorchis viverrini*), typhoid carrier state (*Salmonella typhi* or *paratyphi*), occupational environmental carcinogens, hormonal changes in women, and familial factors. Clinical presentation in patients with gallbladder carcinoma is non-specific including abdominal pain, weight loss, jaundice, and fever [54]. Carcinoembryogenic antigen values higher than 4 ng/mL in the appropriate clinical setting are 93 % specific but 50 % sensitive for diagnosis [55]. Clinical and radiological diagnosis of gallbladder carcinoma is essential in patients with increased risk of developing tumors and in surgical planning. Gallbladder carcinoma is typically classified according to its appearance as (1) intramural polypoid mass, (2) focal or diffuse asymmetrical gallbladder wall thickening, and (3) occupying the gallbladder fossa [56].

6.1 Intramural Polypoid Mass

Intramural polypoid mass is the least common form, representing 15–25 % of gallbladder carcinomas [54]. Usually well-differentiated and confined to the muscular layer, this variety tends to expand into the lumen of the gallbladder before invading the wall. Lesions are usually ≥ 1 cm in size and can be mistakenly considered adenomatous or hyperplastic cholesterol polyps, adenomas, non-shadowing stones, or metastases from melanoma. T1-weighted images demonstrate polypoid mass with intermediate signal intensity arising from the thickened wall of the gallbladder. On T2-weighted images, the mass demonstrates high signal intensity. Polypoid lesions show moderately early enhancement, which persist through the portal phase, while benign lesions usually wash out [57].

6.2 Focal or Diffuse Asymmetric Gallbladder Wall Thickening

Focal or diffuse asymmetric gallbladder wall thickening represents 20–30 % of the gallbladder carcinomas. Focal or diffuse thickening of more than 10 mm is highly suspicious. This variant is difficult to differentiate from acute or chronic cholecystitis, xanthogranulomatous cholecystitis, adenomyomatosis, hepatitis, portal hypertension, and congestive heart failure [58]. The tumor is usually seen on MRI as a diffuse asymmetric, extensive irregular thickened wall heterogeneously hyperintense relative to the liver on T2-weighted and iso- or hypointense in T1-weighted images. All gallbladder tumors show conspicuous arterial enhancement after contrast administration, which is irregular in the early phase [59] and persists or becomes isointense to the liver during portal venous phase [60]. However, these characteristics may overlap with benign conditions.

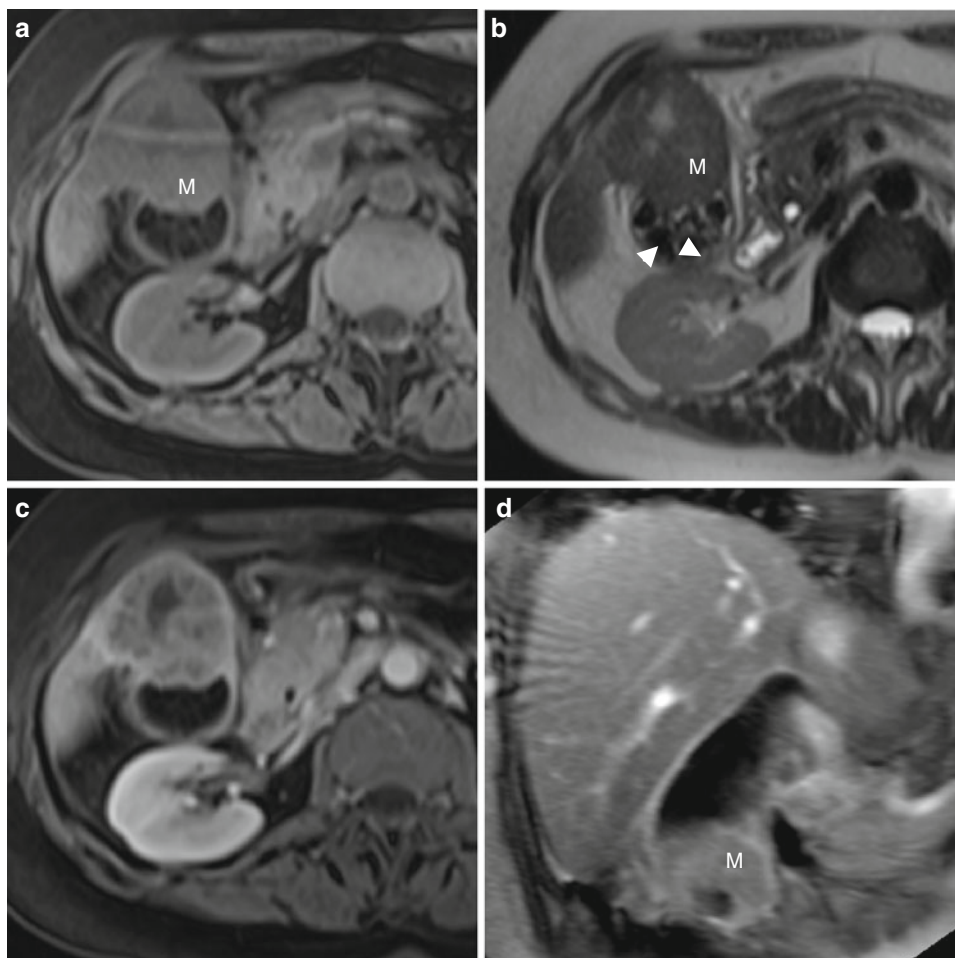
6.3 Subhepatic Mass Occupying the Gallbladder Fossa

Subhepatic mass occupying the gallbladder fossa is the most common form, representing 40–65 % of gallbladder carcinomas [56]. This variant tends to occupy nearly the entire lumen of the gallbladder, often invading the surrounding liver parenchyma, which is highly suggestive of gallbladder carcinoma. MRI usually shows hypo- to isointense signal intensity on T1-weighted and heterogeneously hyperintense signal intensity on T2-weighted images [56] (Fig. 11). Tumors show avid irregular enhancement on the periphery of the lesion during arterial phase and tend to maintain the enhancement throughout the portal and delayed phase, which facilitates differentiation from HCCs. Post-contrast fat-suppressed T1-weighted images are useful for tumor extent and vascular invasion.

6.4 Lymphoma of the Gallbladder

Lymphomas of the gallbladder are extremely rare. To date, there are only 50 cases of primary lymphoma of the gallbladder reported in the literature [61]. Of these, most reported cases of gallbladder lymphoma are diffuse large B cell or mucosa-associated lymphoid tissue types. Chronic lymphocytic leukemia, small lymphocytic lymphoma, and follicular lymphoma are exceedingly rare. Cases are reported in elder patients, and the majority of patients present clinical symptoms of cholecystitis or cholelithiasis. Radiological findings in previous reports have shown wall thickening associated with intramural mass formation [62]. Differentiation between lymphoma of the gallbladder and

Fig. 11 A 73-year-old female with primary gallbladder carcinoma. **a** T1 weighted demonstrates an isointense mass-like (M) thickening of the gallbladder fundus with a central hypointense area probably related to central necrosis. **b** The mass is hypointense in T2-weighted images with a central hyperintense area. There are innumerable gallstones (arrowheads). **c** There is enhancement of the mass after contrast administration consistent with gallbladder carcinoma. **d** Oblique coronal image depicts the mass-like thickening of the gallbladder fundus



gallbladder cancer is difficult. T1-weighted images showed low signal intensity on fat-suppression images and high signal intensity on T2-weighted fat-suppression sequence. T2-weighted images show homogenous signal slightly hypointense compared to gallbladder carcinoma with presence of enlarged lymph nodes.

7 Conclusion and Future Directions

The role of MRCP for the diagnosis of biliary malignancies will further expand due to technological advances both in acquisition and in post-processing software. Functional MRCP using hepatobiliary contrast agents and MRI-based assessment of tumor angiogenesis will further progress. Image resolution and SNR will increase with the development of dedicated MR coils. These current developments will provide a unique opportunity for excellent depiction of anatomic and pathophysiological information using MRI.

References

1. Domagk D et al (2004) Endoscopic retrograde cholangiopancreatography, intraductal ultrasonography, and magnetic resonance cholangiopancreatography in bile duct strictures: a prospective comparison of imaging diagnostics with histopathological correlation. *Am J Gastroenterol* 99:1684–1689
2. Fernandez-Esparrach G et al (2007) Comparison of endoscopic ultrasonography and magnetic resonance cholangiopancreatography in the diagnosis of pancreatobiliary diseases: a prospective study. *Am J Gastroenterol* 102:1632–1639
3. Hekimoglu K et al (2008) MRCP vs. ERCP in the evaluation of biliary pathologies: review of current literature. *J Dig Dis* 9:162–169
4. Hintze RE et al (1997) Clinical significance of magnetic resonance cholangiopancreatography (MRCP) compared to endoscopic retrograde cholangiopancreatography (ERCP). *Endoscopy* 29:182–187
5. Kaltenthaler EC et al (2006) MRCP compared to diagnostic ERCP for diagnosis when biliary obstruction is suspected: a systematic review. *BMC Med Imaging* 6:9
6. Park MS et al (2004) Differentiation of extrahepatic bile duct cholangiocarcinoma from benign stricture: findings at MRCP versus ERCP. *Radiology* 233:234–240

7. Schulte B, Beyer D, Wedekind G, Meuser W (1998) Single shot MRI cholangiopancreatography (MRCP) with a "fast acquisition spin echo" sequence (FASE). Replacement of ERCP? *Aktuelle Radiol* 8:18–24
8. Lee NK et al (2009) Biliary MR imaging with Gd-EOB-DTPA and its clinical applications. *Radiographics* 29:1707–1724
9. Park MS, Yu JS, Lee JH, Kim KW (2007) Value of manganese-enhanced T1- and T2-weighted MR cholangiography for differentiating cystic parenchymal lesions from cystic abnormalities which communicate with bile ducts. *Yonsei Med J* 48:1072–1074
10. Holzapfel K, Breitwieser C, Prinz C, Rummeny EJ, Gaa J (2007) Contrast-enhanced magnetic resonance cholangiography using gadolinium-EOB-DTPA. Preliminary experience and clinical applications. *Radiologe* 47:536–544
11. Fayad LM, Kamel IR, Mitchell DG, Bluemke DA (2005) Functional MR cholangiography: diagnosis of functional abnormalities of the gallbladder and biliary tree. *AJR Am J Roentgenol* 184:1563–1571
12. Aduna M et al (2005) Bile duct leaks after laparoscopic cholecystectomy: value of contrast-enhanced MRCP. *Abdom Imaging* 30:480–487
13. Khan SA, Thomas HC, Davidson BR, Taylor-Robinson SD (2005) Cholangiocarcinoma. *Lancet* 366:1303–1314
14. Soyer P, Lacheheb D, Levesque M (1993) False-positive CT portography: correlation with pathologic findings. *AJR Am J Roentgenol* 160:285–289
15. Shaib YH, El-Serag HB, Davila JA, Morgan R, McGlynn KA (2005) Risk factors of intrahepatic cholangiocarcinoma in the United States: a case-control study. *Gastroenterology* 128:620–626
16. Welzel TM et al (2007) Risk factors for intrahepatic and extrahepatic cholangiocarcinoma in the United States: a population-based case-control study. *Clin Gastroenterol Hepatol* 5:1221–1228
17. Paulson EK et al (1992) CT arterial portography: causes of technical failure and variable liver enhancement. *AJR Am J Roentgenol* 159:745–749
18. Kuhn M, Neufang KF, Gross-Fengels W, Zieren U (1992) Liver angiography—technique, indications and significance in focal liver processes. *Aktuelle Radiol* 2:285–292
19. Yamasaki S (2003) Intrahepatic cholangiocarcinoma: macroscopic type and stage classification. *J Hepatobiliary Pancreat Surg* 10:288–291
20. Menias CO et al (2008) Mimics of cholangiocarcinoma: spectrum of disease. *Radiographics* 28:1115–1129
21. Peporte AR, Sommer WH, Nikolaou K, Reiser MF, Zech CJ (2012) Imaging features of intrahepatic cholangiocarcinoma in Gd-EOB-DTPA-enhanced MRI. *Eur J Radiol*
22. Maetani Y et al (2001) MR imaging of intrahepatic cholangiocarcinoma with pathologic correlation. *AJR Am J Roentgenol* 176:1499–1507
23. Han JK, Lee JM (2004) Intrahepatic intraductal cholangiocarcinoma. *Abdom Imaging* 29:558–564
24. Kim JE et al (2010) Differentiation of intraductal growing-type cholangiocarcinomas from nodular-type cholangiocarcinomas at biliary MR imaging with MR cholangiography. *Radiology* 257:364–372
25. Bismuth H, Nakache R, Diamond T (1992) Management strategies in resection for hilar cholangiocarcinoma. *Ann Surg* 215:31–38
26. Vanderveen KA, Hussain HK (2004) Magnetic resonance imaging of cholangiocarcinoma. *Cancer Imaging* 4:104–115
27. Cui XY, Chen HW (2010) Role of diffusion-weighted magnetic resonance imaging in the diagnosis of extrahepatic cholangiocarcinoma. *World J Gastroenterol* 16:3196–3201
28. Manfredi R et al (2003) MR imaging and MRCP of hilar cholangiocarcinoma. *Abdom Imaging* 28:319–325
29. Guthrie JA, Ward J, Robinson PJ (1996) Hilar cholangiocarcinomas: T2-weighted spin-echo and gadolinium-enhanced FLASH MR imaging. *Radiology* 201:347–351
30. Kassahun WT, Hauss J (2008) Management of combined hepatocellular and cholangiocarcinoma. *Int J Clin Pract* 62:1271–1278
31. de Campos RO et al (2012) Combined hepatocellular carcinoma-cholangiocarcinoma: report of MR appearance in eleven patients. *J Magn Reson Imaging* 36:1139–1147
32. Hwang J et al (2012) Differentiating combined hepatocellular and cholangiocarcinoma from mass-forming intrahepatic cholangiocarcinoma using gadoxetic acid-enhanced MRI. *J Magn Reson Imaging* 36:881–889
33. Park HJ, Kim YK, Park MJ, Lee WJ (2012) Small intrahepatic mass-forming cholangiocarcinoma: target sign on diffusion-weighted imaging for differentiation from hepatocellular carcinoma. *Abdom Imaging*
34. Riopel MA, Klimstra DS, Godellas CV, Blumgart LH, Westra WH (1997) Intrahepatic growth of metastatic colonic adenocarcinoma: a pattern of intrahepatic spread easily confused with primary neoplasia of the biliary tract. *Am J Surg Pathol* 21:1030–1036
35. Medina V et al (2003) Primary biliary tract malignant melanoma: US, CT, and MR findings. *Abdom Imaging* 28:842–846
36. Vitellas KM et al (2000) Radiologic manifestations of sclerosing cholangitis with emphasis on MR cholangiopancreatography. *Radiographics* 20:959–975; quiz 1108–1109, 1112
37. Park MS et al (2001) Recurrent pyogenic cholangitis: comparison between MR cholangiography and direct cholangiography. *Radiology* 220:677–682
38. Lai EC, Lau WY (2006) Mirizzi syndrome: history, present and future development. *ANZ J Surg* 76:251–257
39. Choi BW et al (2000) Radiologic findings of Mirizzi syndrome with emphasis on MRI. *Yonsei Med J* 41:144–146
40. Finkelberg DL, Sahani D, Deshpande V, Brugge WR (2006) Autoimmune pancreatitis. *N Engl J Med* 355:2670–2676
41. Kim JH et al (2002) Differential diagnosis of periampullary carcinomas at MR imaging. *Radiographics* 22:1335–1352
42. Kim JY et al (2007) Contrast-enhanced MRI combined with MR cholangiopancreatography for the evaluation of patients with biliary strictures: differentiation of malignant from benign bile duct strictures. *J Magn Reson Imaging* 26:304–312
43. Soto JA et al (2000) Biliary obstruction: findings at MR cholangiography and cross-sectional MR imaging. *Radiographics* 20:353–366
44. Wu DS, Chen WX, Wang XD, Acharya R, Jiang XH (2012) Pancreaticobiliary duct changes of periampullary carcinomas: quantitative analysis at MR imaging. *Eur J Radiol* 81:2112–2117
45. Chung YE et al (2011) Differentiation of benign and malignant ampullary obstructions on MR imaging. *Eur J Radiol* 80:198–203
46. Chung YE et al (2010) Differential features of pancreatobiliary- and intestinal-type ampullary carcinomas at MR imaging. *Radiology* 257:384–393
47. Irie H et al (2002) MR imaging of ampullary carcinomas. *J Comput Assist Tomogr* 26:711–717
48. Jang KM et al (2012) Added value of diffusion-weighted MR imaging in the diagnosis of ampullary carcinoma. *Radiology*
49. Roberts JW, Daugherty SF (1986) Primary carcinoma of the gallbladder. *Surg Clin North Am* 66:743–749
50. Tazuma S, Kajiyama G (2001) Carcinogenesis of malignant lesions of the gall bladder. The impact of chronic inflammation and gallstones. *Langenbecks Arch Surg* 386:224–229

51. Sheth S, Bedford A, Chopra S (2000) Primary gallbladder cancer: recognition of risk factors and the role of prophylactic cholecystectomy. *Am J Gastroenterol* 95:1402–1410
52. Weiss KM, Ferrell RE, Hanis CL, Styne PN (1984) Genetics and epidemiology of gallbladder disease in New World native peoples. *Am J Hum Genet* 36:1259–1278
53. Sameshima Y, Uchimura M, Muto Y, Maeda J, Tsuchiyama H (1987) Coexistent carcinoma in congenital dilatation of the bile duct and anomalous arrangement of the pancreatobiliary duct. Carcinogenesis of coexistent gall bladder carcinoma. *Cancer* 60:1883–1890
54. Reid KM, Ramos-De la Medina A, Donohue JH (2007) Diagnosis and surgical management of gallbladder cancer: a review. *J Gastrointest Surg* 11:671–681
55. Bartlett DL (2000) Gallbladder cancer. *Semin Surg Oncol* 19:145–155
56. Levy AD, Murakata LA, Rohrmann CA Jr (2001) Gallbladder carcinoma: radiologic-pathologic correlation. *Radiographics* 21:295–314; questionnaire, 549–555
57. Gore RM, Yaghami V, Newmark GM, Berlin JW, Miller FH (2002) Imaging benign and malignant disease of the gallbladder. *Radiol Clin North Am* 40(6):1307–23
58. van Breda Vriesman AC, Engelbrecht MR, Smithuis RH, Puylaert JB (2007) Diffuse gallbladder wall thickening: differential diagnosis. *AJR Am J Roentgenol* 188, 495–501
59. Catalano OA et al (2008) MR imaging of the gallbladder: a pictorial essay. *Radiographics* 28, 135–155; quiz 324
60. Yoshimitsu K et al (1997) Dynamic MRI of the gallbladder lesions: differentiation of benign from malignant. *J Magn Reson Imaging* 7:696–701
61. Mani H et al (2010) Gall bladder and extrahepatic bile duct lymphomas: clinicopathological observations and biological implications. *Am J Surg Pathol* 34:1277–1286
62. Ono A et al (2009) Primary malignant lymphoma of the gallbladder: a case report and literature review. *Br J Radiol* 82:e15–e19

Functional Imaging

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Abstract

Positron Emission Tomography (PET) has been in clinical practice since early 1990s; however, widespread use was hampered due to non-availability of anatomical reference. In the late 1990s and early 2000s, with the advent of hybrid imaging, PET/computed tomography (CT), the oncological utilization has increased significantly. PET/CT has been shown to have high sensitivity and negative predictive value for the detection of tumors, with many studies reporting superior utility of PET/CT over conventional anatomical imaging such as CT, magnetic resonance imaging (MRI), and ultrasound. Despite limited literature regarding utilization of PET/CT in gall bladder and biliary tract cancer, the available literature studies provide evidence for the potential advantage of PET/CT in staging, restaging, and detecting recurrence of gallbladder and biliary tract cancer.

1 Overview of PET and PET/CT

1.1 History

Positron emission tomography (PET) has been in clinical use since early 1990s; however, widespread use was hampered due to non-availability of anatomical reference. In the late 1990s and early 2000s, with the advent of hybrid imaging, PET/CT, the oncological utilization has increased significantly. Hybrid imaging has been helpful in improving diagnostic certainty.

PET is based on coincidence detection, where two photons are released when a positron annihilates an electron. These photons travel at 180° to each other. A ring of detectors is present around the patient, collecting this information. This information is then, utilizing various software, transformed into images to be visually and qualitatively interpreted by detecting metabolic differences between malignant and benign processes.

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1.2 National Oncologic PET Registry

The Centers for Medicare and Medicaid Services (CMS) included the use of 18F-FDG PET/CT in their National Coverage Determination for several solid tumors, which was eventually reviewed to provide coverage for initial and subsequent treatment strategies of most cancer types. The National Oncologic PET Registry (NOPR) was then developed in 2006 in response to the CMS proposal to expand coverage for 18F-FDG PET/CT to include cancers and indications not currently eligible for Medicare reimbursement. For these cancers, the PET/CT scan may be obtained under the coverage of an evidence-based development program, provided that the referring physician and PET provider submit data to a clinical registry assessing the impact of PET on patient management.

To date, over 150,000 patients have undergone 18F-FDG PET scans under the NOPR program, and, due to the documented value of PET in oncologic patient management, approximately 30 % of patients would have had a different management strategy based on PET/CT scan. In 2008, CMS was asked to reconsider its coverage policy for PET. In 2009, CMS released a decision memo for FDG-PET for solid tumors which included expanded coverage as per the results of the NOPR.

2 PET/CT Imaging Technique

PET provides whole-body three-dimensional images of metabolic processes of cells and tissue in the body by detecting gamma rays emitted by positron-emitting radionuclides [1]. Various radiotracers are available with the ability to measure cell metabolism, hypoxia, proliferation, angiogenesis, and apoptosis [2]. 2-deoxy-2-[18F]fluoro-D-glucose (18F-FDG) is the most frequently used radiopharmaceutical in oncologic PET/CT imaging [3]. Since tumor cells typically display increased metabolism [4], 18F-FDG accumulates to a greater degree in malignant tissue when compared to benign processes. As such, lesions may be identified and differentiated from normal tissue by various visual and quantitative processes that measure the degree of FDG uptake. However, a few tumors exhibit low FDG avidity due to their small size and a different metabolic profile. As such, these tumors (typically less than 10 mm) may be missed by functional PET imaging, leading to false-negative readings [2]. Furthermore, FDG is a non-specific tumor tracer as many have reported increased uptake in benign processes such as inflammation, infection, and abscesses [5]. 18F-FDG uptake may also be increased following radiotherapy and/or chemotherapy due to the body's own defense mechanisms, leading to an increased chance of

false-positive images. This is because tissues exposed to therapy may exhibit benign reactive changes that are FDG-avid [2]. Nonetheless, research is ongoing regarding the optimal timing of functional PET imaging after therapy, the use of tumor-specific radiotracers, and the development of software that increases image resolution.

2.1 18-Fluorine Fluorodeoxyglucose

As mentioned previously, ¹⁸F-FDG is the most commonly used radiotracer. Once the radiotracer is injected into the patient's bloodstream, it will lead to phosphorylation by hexokinase to 2-deoxy-2-[¹⁸F]fluoro-D-glucose-6-phosphate [3]. The tracer will then be distributed throughout the body with a short physical half-life of approximately 110 min [3]. Native glucose will undergo further metabolism, while ¹⁸F-FDG will accumulate over time in most malignant cells, allowing for the differentiation between benign and malignant processes after, ideally, 60 min from when the radiotracer was injected [3].

The technique of measuring glucose utilization was first applied to mapping local cerebral glucose metabolism, in vivo, in humans [6]. Preclinical studies then suggested the use of ¹⁸F-FDG for tumor metabolism in line with Warburg's observation of increased glucose uptake by malignant cells [6]. The value of ¹⁸F-FDG for oncological management has been demonstrated in localization of the primary tumor, detection of local and distant metastasis, evaluation of response to therapy, and detection of residual disease and recurrence [3].

2.2 Quantification of Tumor Glucose Metabolism

To quantify the difference between normal glucose metabolism and that of malignant cells, standardized uptake value (SUV) is typically utilized [7]. SUV is a semi-quantitative method providing the radioactivity concentration from the region of interest (ROI), compared to uptake in the whole body. The SUV may be normalized to body mass, lean body mass, or body surface area [8]. SUVs based on body weight may vary greatly from one patient to the next; for example, the SUV of a heavy-weighted patient may be twice that of an average-weighted patient [9]. SUV corrected for lean body mass (SUL) and body surface area, however, is less dependent on body weight [9]. As such, SUL is more widely used, especially when monitoring response to therapy where the patient's weight may change significantly due to treatment. Various methods of standardizing quantitative assessment have been proposed, most recent being PET

Table 1 Diagnosis of the primary tumor in gallbladder and cholangiocarcinoma by PET/CT: comparison with conventional imaging

Study	Type	No. of patients	PET					Conventional imaging				
			Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Lee et al. [10]	P	99	84	70	93	48	82	90	71	94	60	87
Rodriguez et al. [11]	P	16	80	82	67	90	–	–	–	–	–	–
Petrowsky et al. [12]	P	61	100	33	90	6	53	71	33	90	7	56
Yamada et al. [13]	R	14	69	–	90	–	64	–	–	–	–	–

R retrospective study, *P* prospective study, *PET* positron emission tomography or fused PET/CT, conventional imaging includes contrast-enhanced CT and MDCT, *PPV* positive predictive value, *NPV* negative predictive value

response criteria in solid tumors (PERCIST) 1.0. It is along the lines of RECIST (Response Evaluation Criteria in Solid Tumors [8]).

3 Diagnostic Functional Imaging of Gallbladder and Biliary Tract Cancer

PET/CT has been shown to have high sensitivity and negative predictive value for the detection of tumors, with many studies reporting superior utility of PET/CT over conventional anatomical imaging such as CT, MRI, and ultrasound [5]. The role of PET/CT has been studied in various oncologic diseases for staging, evaluating treatment response, restaging, and follow-up [5]. However, limited data are available for the role of PET/CT in gallbladder and biliary tract cancers due to the low prevalence of these cancers and poor prognosis associated with them. Nonetheless, the available literature provides evidence for the potential advantage of PET/CT in staging, restaging, and detecting recurrence of gallbladder and biliary tract cancer.

4 Utilizing 18F-FDG PET/CT for Initial Treatment Strategy in Gallbladder Cancer

4.1 Staging

Patients with gallbladder carcinoma (GBC) typically present in advanced stages of the disease, rendering the staging process a crucial element to successful treatment planning early during the course of treatment. Very few studies have examined the role of PET/CT specific to GBC, but initial studies pooling the efficacy of PET/CT in both gallbladder and cholangiocarcinoma show promising results when compared to conventional imaging [such as contrast-enhanced CT (ceCT) and Multidetector Computed Tomography (MDCT)]. Sensitivity of PET/CT for the detection of the primary tumor ranges from 80 to 90 % [10–12] (see

Table 1), allowing PET/CT to accurately differentiate between malignancy and benign conditions such as chronic cholecystitis and biliary colic, especially in cases of equivocal conventional imaging.

In addition to the importance of accurately localizing the primary tumor, the detection of regional and distant metastasis is crucial to the determination of initial treatment strategies. Lymph node involvement and presence of metastatic lesions may preclude surgical resection. With respect to the detection of regional lymph nodes, PET/CT does not seem to offer a significant advantage over conventional imaging with reported sensitivities ranging from 12–82 to 24–80 % for PET/CT and conventional imaging, respectively [10, 12] (see Table 2). There is a clear and significant advantage, however, of PET/CT for the detection of suspected and unsuspected distant lesions, with reported sensitivities ranging from 95–100 to 25–63 % for PET/CT and conventional imaging, respectively [10, 12] (see Table 3).

As a result of staging by PET/CT, treatment plans that were based on previous conventional staging were modified in up to 17 % of patients, whereby the detection of unsuspected metastasis by PET/CT led to non-surgical treatment, sparing unnecessary resection in patients deemed resectable following conventional work-up [10, 12] (see Figs. 1, 2, 3).

Much more research is warranted before definitive conclusions can be made; however, although limited, the data depict superiority of PET/CT for the staging of gallbladder cancer, especially in cases of equivocal conventional imaging, for evaluating metastatic disease, and for deciding on the initial treatment strategy.

4.2 Restaging

Very little data are available in the restaging setting, as such; the utility of PET/CT cannot be evaluated to a great extent. One prospective study compared PET/CT with MDCT for evaluating residual disease in patients with incidental gallbladder cancer [14]. Twenty-four patients with incidental gallbladder cancer who were suitable for

Table 2 Detection of nodal metastasis in gallbladder and cholangiocarcinoma by PET/CT: comparison with conventional imaging

Study	Type	No. of patients	PET					Conventional imaging				
			Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Lee et al. [10]	P	99	82	95	94	85	89	80	79	78	81	79
Petrowsky et al. [12]	P	61	12	96	67	64	64	24	86	50	65	62

P prospective study, PET positron emission tomography or fused PET/CT, conventional imaging includes contrast-enhanced CT and MDCT, PPV positive predictive value, NPV negative predictive value

Table 3 Detection of distant metastasis in gallbladder and cholangiocarcinoma by PET/CT: comparison with conventional imaging in prospective studies

Study	Type	No. of patients	PET					Conventional imaging				
			Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Lee et al. [10]	P	99	95	95	86	98	95	63	94	75	89	87
Petrowsky et al. [12]	P	61	100	100	100	100	100	25	100	100	85	85

P prospective study, PET positron emission tomography or fused PET/CT, conventional imaging includes contrast-enhanced T and MDCT, PPV positive predictive value, NPV negative predictive value

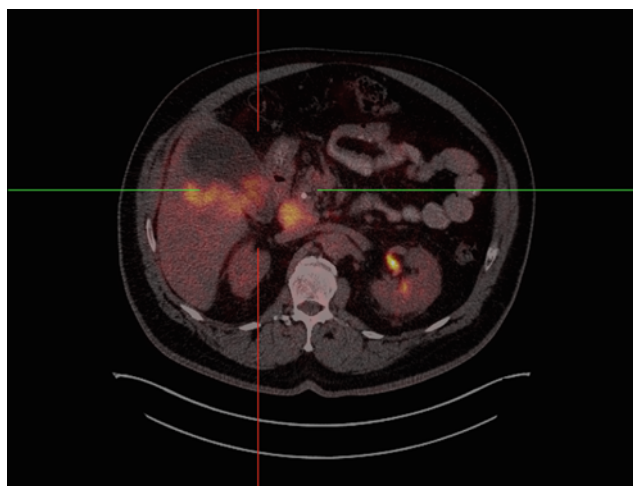


Fig. 1 54 year-old male with a newly found gallbladder mass. Axial fused staging 18F-FDG PET/CT image demonstrates intensely FDG-avid gallbladder wall thickening involving the posterior wall (SUV-max 10.1) and the hepatic parenchyma

surgery were recruited for the study [14]. For detecting residual disease, the authors reported a sensitivity and positive predictive value (PPV) of 28.5 and 20 % for PET/CT and 42.8 % each for MDCT, respectively [14]. Despite the low values, PET/CT was able to detect occult metastatic and loco-regional disease missed on MDCT, emphasizing the importance of using multimodality imaging in a complementary fashion as concluded by the authors as well [14].

5 Utilizing 18F-FDG PET/CT for Initial Treatment Strategy in Extra-Hepatic Cholangiocarcinoma

5.1 Staging

Similar to gallbladder cancer, malignancies of the biliary tract are also rare and have poor prognosis. There have been more studies, however, evaluating the role of PET/CT in biliary tract cancer, with the greatest advantages reported to be in detecting metastatic disease and selecting candidates for surgery [15]. Preoperative imaging is, thus, an integral part of initial assessment.

With respect to staging of the primary tumor, PET/CT seems to have limited or no clear advantage over conventional imaging techniques such as CT and MRI. This may be due to the overlap in FDG uptake between biliary tract malignancies and benign inflammatory lesions, especially in patients with primary sclerosing cholangitis (see Table 4).

The detection of metastatic lymph nodes by PET/CT has been reported to be limited; nonetheless, the sensitivity is significantly higher than that of conventional imaging, with sensitivity measures ranging from 37–76 to 33–60 % for PET/CT and CT, respectively [18, 20, 21] (see Table 5). As is the case in gallbladder cancer, the role of PET/CT in metastatic staging is undisputed, with many studies reporting significant advantage of PET/CT for detecting unsuspected metastatic disease (see Table 6).

Fig. 2 Coronal (*left*) and axial (*right*) fused PET/CT images demonstrate FDG-avid lymphadenopathy involving the aortocaval nodes with the highest SUVmax of 6.6

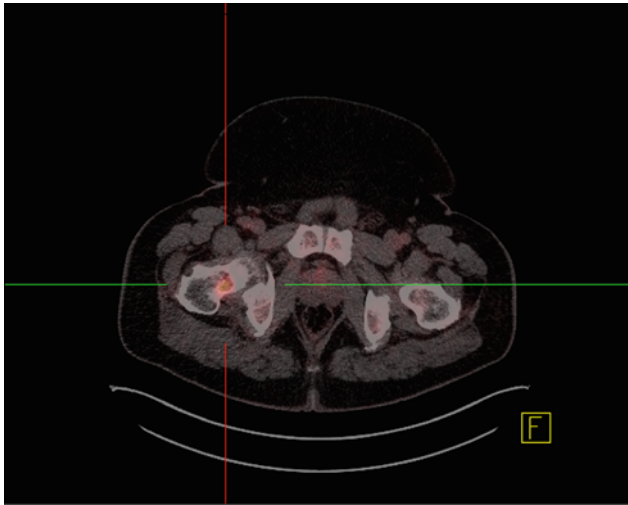
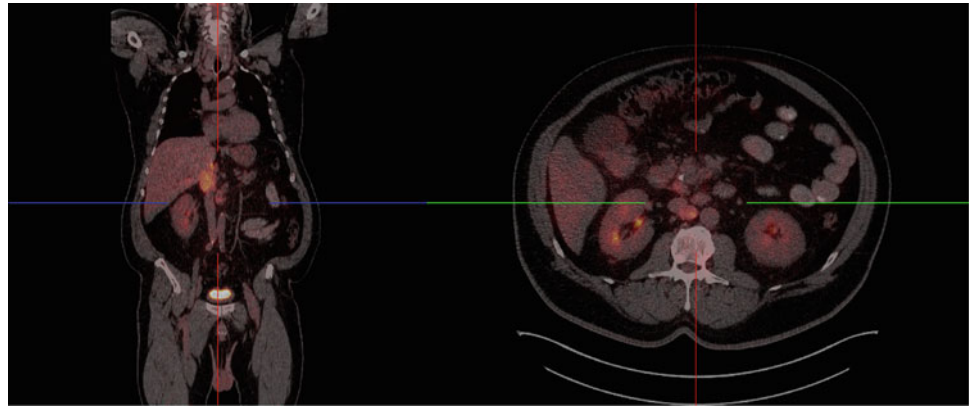


Fig. 3 Intense focal FDG uptake is noted in a lucent lesion involving the right femoral neck and is highly suspicious for malignant involvement with a SUVmax of 7.2

Moreover, due to the limitations of anatomical imaging, additional preoperative information provided by functional PET/CT may lead to a change in management in up to 16 % of patients [18], typically by detecting metastasis not seen on conventional imaging and consequently changing the patient's status from resectable to unresectable. Interestingly, one study also reported that while there was only a trend toward higher SUVs being associated with malignancy compared to benign biliary disease, the SUV of the primary tumor was significantly higher in patients with metastasis than in those without [20]; these results imply that resectability may be quantified, although more prospective trials are needed to evaluate this possibility.

In summary, it seems that the role of PET/CT in staging gallbladder and biliary tract cancers offers the greatest advantage in detecting metastatic disease and subsequently influencing patient management and treatment planning;

much more research is needed, however, to determine the utility of PET/CT in primary and nodal staging.

6 Utilizing 18F-FDG PET/CT for the Detection of Recurrence in Gallbladder and Biliary Tract Cancers

Curative surgical resection is the ultimate goal of treatment for patients with gallbladder and biliary tract cancers; however, recurrence rates are still high even with resection [22]. The limited data available are heterogeneous, with some studies reporting superiority of PET/CT over conventional imaging for the detection of recurrence and others reporting no significant differences between the imaging modalities (see Table 7). However, most studies have recruited a small number of patients and the lack of statistical significance may be due to the small sample sizes. Furthermore, PET/CT findings, in light of clinical suspicion of recurrence, were reported to change subsequent treatment management in up to 20 % of patients [23] (see Fig. 4).

7 Future Direction and Conclusion

With PET/CT's introduction to clinical practice almost two decades ago, much data have accumulated regarding its utility in various oncologic diseases. Current research studies, however, have begun to search for alternate PET radiotracers that are tumor specific, unlike FDG. While this field of study has yet to target gallbladder and biliary tract cancers, several non-FDG radiotracers have been proposed to be useful in several other types of cancer. For example, angiogenesis, a process critical to tumor growth and survival, may be visualized by radiolabeled arginine–glycine–aspartic acid (RGD) peptides [27]. Apoptosis, which has been linked to unsuccessful therapy, may be quantified by

Table 4 Detection of primary cholangiocarcinoma tumor by 18F-FDG PET/CT

Study	Type	No. of patients	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Alkhalwaldeh et al. [16]	R	65	94	83	94	83	91
Corvera et al. [17]	P	93	69	67	–	–	–
Kim et al. [18]	P	123	81	79	95	44	81
Li et al. [19]	P	17	59	–	100	0	–
Ruys et al. [20]	R	30	88	0	85	0	77
Yamada et al. [13]	R	20	84	–	94	–	80

R retrospective study, *P* prospective study, *PET* positron emission tomography or fused PET/CT, *PPV* positive predictive value, *NPV* negative predictive value

Table 5 Detection of nodal metastasis in cholangiocarcinoma by PET/CT: comparison with CT

Study	Type	No. of patients	PET					Conventional imaging				
			Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Corvera et al. [17]	P	93	93	86	–	–	–	–	–	–	–	–
Kim et al. [18]	P	123	32	88	43	82	76	47	65	27	82	61
Kobayashi et al. [21]	R	36	37	97	86	72	87	49	81	75	36	87
Li et al. [19]	P	17	42	80	39	36	–	–	–	–	–	–
Ruys et al. [20]	R	30	67	67	40	86	67	33	67	–	–	–

R retrospective study, *P* prospective study, *PET* positron emission tomography or fused PET/CT, *CT* computed tomography, *PPV* positive predictive value, *NPV* negative predictive value

Table 6 Detection of distant metastasis in cholangiocarcinoma by 18F-FDG PET/CT

Study	Type	No. of patients	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Corvera et al. [17]	P	93	93	86	–	–	–
Kim et al. [18]	P	123	58	93	54	94	88
Li et al. [19]	P	17	56	88	83	64	–
Ruys et al. [20]	R	30	33	96	66	85	83

R retrospective study, *P* prospective study, *PET* positron emission tomography or fused PET/CT, *PPV* positive predictive value, *NPV* negative predictive value

Table 7 Detection of recurrent disease by PET/CT: comparison with conventional imaging

Study	Type	No. of patients	PET					Conventional imaging				
			Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Kumar et al. [24]	R	49	94	100	100	89	96	88	50	78	67	75
Corvera et al. [17]	P	33	89	100	–	–	–	–	–	–	–	–
Jadvar et al. [25]	R	24	94	100	–	–	–	82	43	–	–	–
Kitajima et al. [23]	R	50	86	91	–	–	88	–	–	–	–	–
Lee et al. [26]	R	50	88	69	86	73	82	76	44	74	47	66

R retrospective study; *P* prospective study; *PET* positron emission tomography or fused PET/CT; conventional imaging includes computed tomography, magnetic resonance imaging, and MDCT; *PPV* positive predictive value; *NPV* negative predictive value

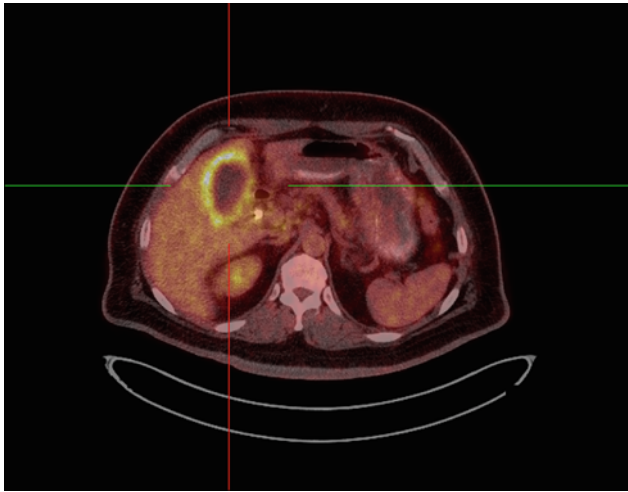


Fig. 4 54 year-old male with a history of cholangiocarcinoma with liver metastases. PET/CT scan was indicated for the evaluation of residual disease following completion of chemotherapy. Fused axial ^{18}F -FDG PET/CT image demonstrates intensely FDG-avid thickening noted along the gallbladder with the highest SUVmax of 9.7. The wall thickening measuring up to 1 cm is consistent with persistent disease presence

^{18}F -Annexin V [27], allowing for the possibility of early identification of non-responders and changing their treatment accordingly. Tumors that exhibit hypoxia are also associated with chemoresistance and poor response to therapy, and 18-fluoride-fluoromisonidazole (^{18}F -FMISO) is currently the most widely studied radiotracer for measuring hypoxia [27]. Finally, proliferation, one of the biological hallmarks of cancer, may be imaged by 3-deoxy-3-18-fluoride-fluorothymidine (^{18}F -FLT) [27].

Recent research is also focusing on advanced software and techniques for optimized anatomical and functional imaging. The main area of interest is currently in fused PET/MR imaging. Several aspects may be considered when analyzing the utility of PET/MR. First, since PET and CT images are not acquired simultaneously, PET/CT imaging still faces the issue of misregistration, leading to reportedly up to 10 % of error in SUV calculations [28]. This is especially important in abdominal imaging where breathing and motion artifacts are a concern. More accurate attenuation correction may be achieved by using simultaneous and hybrid PET/MR scanners, eliminating the issue of misregistration. Second, due to the superior soft tissue contrast of MRI, fused PET/MR will benefit from increased accuracy of tumor and nodal localization. Third, the combination of quantitative MRI biomarkers and PET radiotracers may significantly improve the sensitivity, specificity, and accuracy of tumor detection and response to therapy. These concepts and advances in technology, however, have yet to be studied in a large pool of cancers, including gallbladder and biliary tract cancers.

To conclude, the use of ^{18}F -FDG PET/CT in gallbladder and biliary tract cancers is much less studied in comparison with more common malignancies, for example, lung and breast cancers. This is due to the rare nature of the disease, as well as the poor prognosis associated with it. However, despite the limited data, ^{18}F -FDG PET/CT seems to offer an advantage over conventional imaging for the detection of metastatic disease, recurrence, and subsequently changing management in a number of patients. As such, the use of FDG/non-FDG PET/CT and, subsequently, PET/MR for the management of gallbladder and biliary tract cancers should be assessed to a much greater degree in current clinical practice, allowing physicians a better anatomical and functional understanding of the patient's extent of disease.

References

1. Bailey D, Townsend PE, Valk MN (2005) Positron emission tomography: basic sciences 2005. Springer, Secaucus
2. Wood K, Hoskin PJ, Saunders MI (2007) Positron emission tomography in oncology: a review. *Clin Oncol* 19:237–255
3. Beuthien-Baumann B, Hamacher K, Oberdorfer F et al (2000) Preparation of fluorine-18 labelled sugars and derivatives and their application as tracer for positron-emission-tomography. *Carbohydr Res* 327(1–2):107–118
4. Warburg O, Wind F, Negelein E (1927) The metabolism of tumors in the body. *J Gen Physiol* 8(6):519–530
5. Kumar K, Halanaik D, Malhotra A (2010) Clinical applications of positron emission tomography-computed tomography in oncology. *Indian J Cancer* 47:100–119
6. Anderson H, Price P (2000) What does positron emission tomography offer oncology? *Eur J Cancer* 36:2028–2035
7. Lucignani G, Paganelli G, Bombardieri E (2004) The use of standardized uptake values for assessing FDG uptake with PET in oncology: a clinical perspective. *Nucl Med Commun* 25:651–656
8. Wahl R, Jacene H, Kasamon Y et al (2009) From RECIST to PERCIST: evolving considerations for PET response criteria in solid tumors. *J Nucl Med* 50(Suppl 1):122S–150S
9. Vriens D, Visser EP, Geus-Oei LF et al (2010) Methodological considerations in quantification of oncological FDG PET studies. *Eur J Nucl Med Mol Imaging* 37(7):1408–1425
10. Lee S, Kim HJ, Park JH (2010) Clinical usefulness of ^{18}F -FDG PET-CT for patients with gallbladder cancer and cholangiocarcinoma. *J Gastroenterol* 45:560–566
11. Rodrigues-Fernandez A, Gomez-Rio M, Llamas-Elvira JM et al (2004) Positron-emission tomography with fluorine- ^{18}F -FDG for gallbladder cancer diagnosis. *Am J Surg* 188:171–175
12. Petrowsky H, Wildbrett P, Husarik DB et al (2006) Impact of integrated positron emission tomography and computed tomography on staging and management of gallbladder cancer and cholangiocarcinoma. *J Hepatol* 45:43–50
13. Yamada I, Ajiki T, Ueno K et al (2012) Feasibility of ^{18}F -FDG PET for preoperative evaluation of biliary tract cancer. *Anticancer Res* 32(11):5105–5110
14. Shukla P, Barreto SG, Arya S et al (2008) Does PET-CT scan have a role prior to radical re-resection for incidental gallbladder cancer? *HPB* 10:439–445
15. Breitenstein S, Aperstegui C, Clavien PA (2008) Positron emission tomography (PET) for cholangiocarcinoma. *HPB* 10:120–121

16. Alkhalwaleh K, Falten S, Biersack HJ et al (2011) The value of F-18 FDG PET in patients with primary sclerosing cholangitis and cholangiocarcinoma using visual and semiquantitative analysis. *Clin Nucl Med* 36(10):879–883
17. Corvera C, Blumgart LH, Akhurst T et al (2008) 18F-FDG PET influences management decisions in patients with biliary cancer. *J Am Coll Surg* 206:57–65
18. Kim J, Kim MH, Lee TY et al (2008) Clinical role of 18F-FDG PET-CT in suspected and potentially operable cholangiocarcinoma: a prospective study compared with conventional imaging. *Am J Gastroenterol* 103:1145–1151
19. Li J, Kuehl H, Grabellus F et al (2008) Preoperative assessment of hilar cholangiocarcinoma by dual-modality PET/CT. *J Surg Oncol* 98:438–443
20. Ruys A, Bennink RJ, van Westreenen HL et al (2011) FDG-positron emission tomography/computed tomography and standardized uptake value in the primary diagnosis and staging of hilar cholangiocarcinoma. *HPB* 134:256–262
21. Kobayashi S, Nagano H, Hoshino H et al (2011) Diagnostic value of FDG-PET for lymph node metastasis and outcome of surgery for biliary cancer. *J Surg Oncol* 103(3):223–229
22. Cameron K, Golan S, Simpson W et al (2011) Recurrent pancreatic carcinoma and cholangiocarcinoma: 18F-FDG PET/CT. *Abdom Imaging* 36(4):463–471
23. Kitajima K, Murakami K, Kanagae K (2009) Clinical impact of whole body FDG-PET for recurrent biliary cancer: a multicenter study. *Ann Nucl Med* 23:709–715
24. Kumar R, Sharma P, Kumari A et al (2012) Role of 18F-FDG PET/CT in detecting recurrent gallbladder carcinoma. *Clin Nucl Med* 37(5):431–435
25. Jadvar H, Henderson RW, Conti PS (2007) 18F-FDG PET and PET/CT in recurrent and metastatic cholangiocarcinoma. *J Comput Assist Tomogr* 31(2):223–228
26. Lee Y, Han SW, Oh DY et al (2011) Diagnostic performance of contrast enhanced CT and 18F-FDG PET/CT in suspicious recurrence of biliary tract cancer after curative resection. *BMC* 11(1):188–195
27. Smith G, Carroll L, Aboagye EO (2012) New frontiers in the design and synthesis of imaging probes for PET oncology: current challenges and future directions. *Mol Imaging Biol* 14(6):653–666
28. Yankeelov T, Pererson TE, Abramson RG et al (2012) Simultaneous PET-MRI in oncology: a solution looking for a problem? *Magn Reson Imaging* 30:1342–1356

Computed Tomography

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Abstract

Cross-sectional imaging with multi-slice computed tomography (MSCT) is an essential tool in the evaluation of patients with gallbladder and biliary tract cancers. The biliary tract cancers are a combination of three distinct classes of tumors: (i) intrahepatic cholangiocarcinoma, (ii) proximal intrahepatic cholangiocarcinoma, and (iii) distal cholangiocarcinoma. These cancers share the unfortunate feature of having a poor prognosis unless surgical intervention is possible. The pre-operative evaluation of these tumors with MSCT with high quality imaging provides required information for the assessment of therapeutic options. The imaging varies depending on the type of tumor. The image interpretation will assist in patient stratification and tumor staging. This chapter presents a brief summary of the epidemiology and clinical presentation of the various biliary cancers. In addition, the specific imaging protocol for the various biliary cancers is presented. The imaging findings for correct interpretation and the role of imaging in staging is described. For each tumor, the imaging characteristics

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of tumor and tumor growth are described as well as the findings for the detection of nodal and distant metastatic disease. Finally, the role of MSCT in treatment response and surveillance is presented.

1 Gallbladder Cancer

1.1 Epidemiology

Gallbladder carcinoma (GB CA) is the most common malignancy of the biliary tree and the fifth most common gastrointestinal cancer following malignant tumors of the colon, pancreas, stomach, liver, and esophagus. The incidence of gallbladder cancer is approximately 1–3 cases per 100,000 persons; it represents approximately 6,000 new diagnoses per year in the United States alone [1]. The anatomy of the gallbladder can be divided into the fundus, the body, and the neck. Most of the tumors are located in the fundus (60 %) while the body (30 %) and neck (10 %) account for the rest of the tumors [2]. Women are two to six times more commonly affected than men, and the incidence increases with age. GB CAs tend to present in an older population, with the greatest incidence in patients older than 65 years. There is a geographic bias with the highest number of GB CAs found in Chile and Bolivia. Other locations with a higher incidence of GB CA are Japan, India, and Israel.

Cholelithiasis, in particular cholesterol stones, is the most common risk factor. The incidence of gallbladder cancer is also increased with obesity, high-carbohydrate diet, alcohol use, and smoking [3]. Other reported risk factors include congenital biliary cysts, infectious factors (*Salmonella typhi*), primary sclerosing cholangitis (PSC), and genetic factors [3]. For the last 6 decades, the incidence and mortality of GB CA have decreased. A possible explanation is the increased frequency of cholecystectomy with a subsequent decrease in the population at risk.

Although the exact etiology is unclear, chronic irritation of the gallbladder mucosa by stones is believed to play a major role. Gallstones are present in 74–92 % of patients with GB CA [4]. The proposed pathophysiology consists of stepwise progression from dysplasia to carcinoma: a normal GB affected with chronic inflammation and eventually repetitive epithelial repair result in epithelial dysplasia with later progression to carcinoma in situ and, ultimately, to invasive carcinoma. The rate of progression from dysplasia to invasive carcinoma is estimated at 5–15 years [4]. Most tumors are adenocarcinomas (85 %).

1.2 Clinical Presentation

The clinical presentation is usually insidious, and early stage is typically diagnosed incidentally on pathologic review of cholecystectomy specimens. The early clinical presentation of GB CA could be right upper quadrant pain and symptoms indistinguishable from those of cholecystitis [5]. Other presenting symptoms include chronic abdominal pain, anorexia, unintentional weight loss, jaundice, hepatomegaly, and palpable mass. These are poor prognostic signs and usually indicate advanced disease. In cases of invasion, bowel obstruction and fistulous communication could also be observed. On physical exam, signs of advanced disease may also include left supraclavicular and periumbilical adenopathy.

Laboratory studies rarely show any abnormalities in patients with early disease. Elevated levels of serum bilirubin, transaminase, and γ -glutamyl transpeptidase may be seen in advanced cases that present with obstructive jaundice [3]. Several serum markers can be elevated with GB CA, with the most common being CA19-9 and CA-125 [6]. However, these values should not be interpreted in isolation and are not recommended for screening. The mean survival rate is 6 months and the 5-year survival rate is approximately 5 % for nonresected GB CA [7].

1.3 Imaging

The main role of imaging in GB CA is to provide accurate information regarding the extent of disease, particularly the relationship of the infiltrative process to adjacent organs. The size and location of the primary mass, the depth of hepatic parenchymal invasion and liver metastases, regional and distant nodal metastases, vascular anatomy (hepatic artery, portal vein and hepatic vein variants), and the presence of distant metastases constitute the crucial elements to be addressed by imaging [3].

1.4 Imaging Protocol and Technique

Decision making on imaging protocol for the evaluation of GB CA depends on the potential for surgery. The preoperative imaging for GB CA is performed in a selected population. In the setting of surgical staging of gallbladder cancer, a multiphasic liver protocol is recommended, including pre-contrast, late arterial, portal venous, and delayed phases. 150 ml of intravenous iodinated contrast is injected at a rate of 5 ml/s. SmartPrep® (GE Healthcare,



Fig. 1 Normal gallbladder. Axial MSCT contrast-enhanced image obtained during the portal phase shows a normal gallbladder. Its walls are sharply demarcated from the adjacent liver parenchyma and peritoneal fat. Thin homogeneous wall enhancement (*arrow*) during the arterial or portal phase is a normal finding

Milwaukee) is used for contrast bolus tracking, monitoring the aorta at the level of the celiac artery until 100 Hounsfield units is obtained; this takes roughly 20 s. Dynamic imaging through the liver in the late arterial phase is then obtained at 13 s postthreshold. Portal venous phase imaging is then obtained after 60 s, and delayed images through the liver are obtained at 90 s.

The computed tomography (CT) evaluation of non-surgical GB CA does not require a multiphase study after contrast administration. In a patient with clinical suspicion of advanced GB CA, we initially perform a non-enhanced scan through the liver and kidneys with a 5-mm collimation acquisition reconstructed at 2.5-mm intervals. Images are routinely reconstructed at 2.5-mm intervals for better spatial resolution, providing optimal source images for multiplanar reconstruction. Nonenhanced images are evaluated to detect fatty infiltration of the liver, calcifications, and calcified gallstones. A single-phase scan after intravenous injection of 125–150 cc of nonionic iodinated contrast at a rate of 3 ml/s with an acquisition performed from the diaphragm to the ischial tuberosities after a 60 s delay. This routine protocol results in imaging of the liver during the portal venous phase of contrast enhancement and the kidneys in the early nephrographic phase, ensuring a comprehensive evaluation of the entire abdomen.

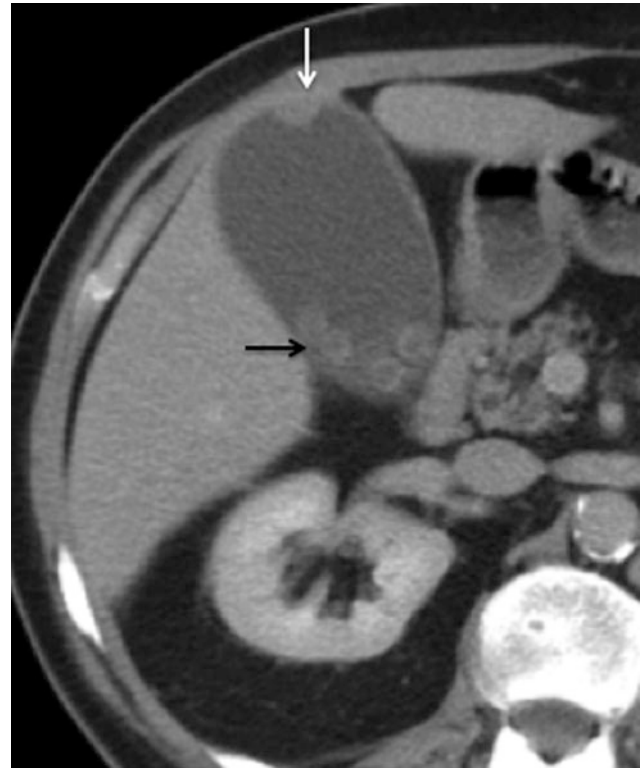


Fig. 2 Papillary gallbladder cancer: 68-year-old male patient with gallbladder cancer incidentally found during evaluation for abdominal pain. Contrast-enhanced MSCT axial image shows a soft tissue mass protruding from the wall (*white arrow*) into the homogeneous low-attenuation lumen of the gallbladder. Cholelithiasis (*black arrow*) is also present in this patient

1.5 Imaging Findings

The imaging analysis of GB CA should focus on the parameters used to judge the chances of achieving a complete surgical resection. Familiarity with the normal appearance of the gallbladder, cystic fossa, and adjacent structures on CT is essential for the accurate interpretation of CT findings. Knowing the imaging features and patterns of spread of disease is crucial for proper diagnosis, staging, and early detection as well.

1.6 Normal Gallbladder

The gallbladder lies within the cystic fossa. CT cannot distinguish between the four histologic layers of the gallbladder: mucosa, lamina propria, muscle, and connective tissue. On CT, the GB appears as an elongated tubular structure of homogenous low attenuation, with thin wall (up to 3 mm) and homogeneous mucosal enhancement (Fig. 1) when adequately distended. The size and shape of the normal gallbladder are highly variable and depend on the fasting state of the patient.



Fig. 3 Flat gallbladder cancer. Axial MSCT contrast-enhanced image of a 72-year-old male patient showing irregular thickening of the gallbladder wall (*arrows*), representing gallbladder cancer

2 Staging

2.1 Primary Tumor

The appearance of GB CA on CT depends upon the morphology of the tumor and extent of disease at the time of imaging [8]. GB CA can be divided into five subtypes based on gross morphologic appearance: papillary (best prognosis), nodular, flat, filling, and massive (most common) [7]. Papillary, nodular, and filling tumors share the same imaging feature of an enhancing soft tissue mass protruding into the normally low-density fluid attenuation lumen of the gallbladder (Fig. 2). Flat tumors may present as irregular thickening of the gallbladder wall without a discrete soft tissue mass or nodule (Fig. 3). Detection of wall thickening (>1 cm) with mural irregularity on CT raises the suspicion for malignancy [9]. A large mass that replaces the GB and adjacent liver parenchyma is an imaging feature of the massive type (Figs. 4 and 5). The mass may be iso- to hypoattenuating relative to the liver on the pre-contrast CT examination. The mass attenuation after intravenous contrast increases but may be heterogeneous owing to necrosis.

According to the American Joint Committee on Cancer (AJCC) for GB CA staging criteria, T1 tumor invades the lamina propria of muscle layer. T2 tumor invades the

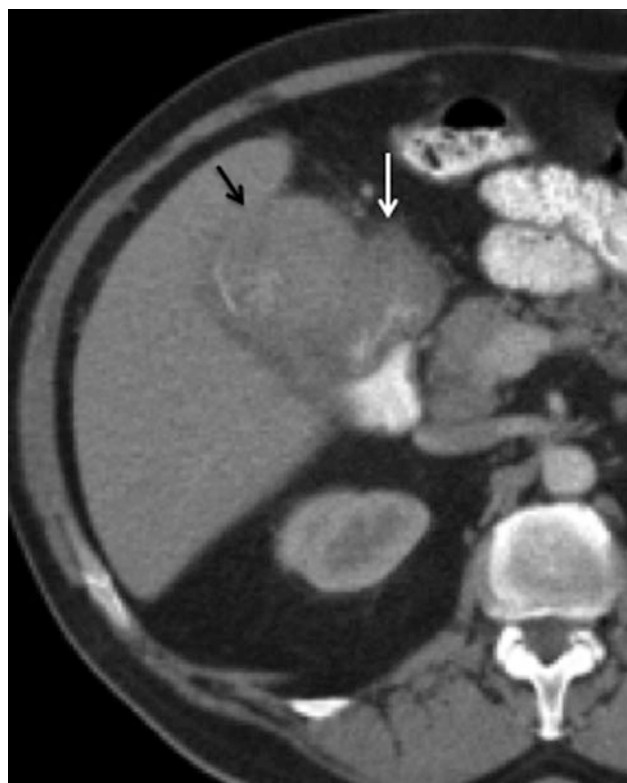


Fig. 4 Massive gallbladder cancer. 59-year-old female patient presenting with anorexia and weight loss. Contrast-enhanced MSCT axial image shows a large soft tissue mass (*black arrow*) occupying most of the lumen of the gallbladder. Note is also made for irregular thickening of the proximal duodenum (*white arrow*), representing neoplastic invasion

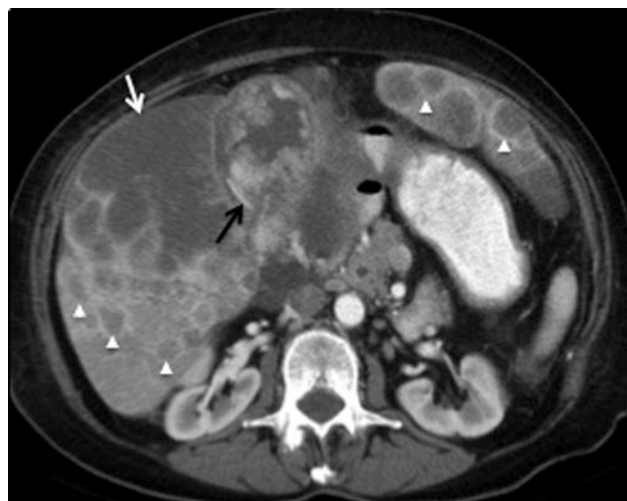


Fig. 5 Advanced gallbladder cancer. 60-year-old female patient presenting with jaundice and hepatomegaly. Axial MSCT contrast-enhanced image shows a large heterogeneous mass centered in the gallbladder (*black arrow*). Associated invasion of the liver with irregular peripheral enhancement and central hypodensity (*white arrow*), compatible with necrosis. Multiple hypodense masses with peripheral rim enhancement (*arrowheads*) are appreciated throughout both hepatic lobes, compatible with hematogenous metastases

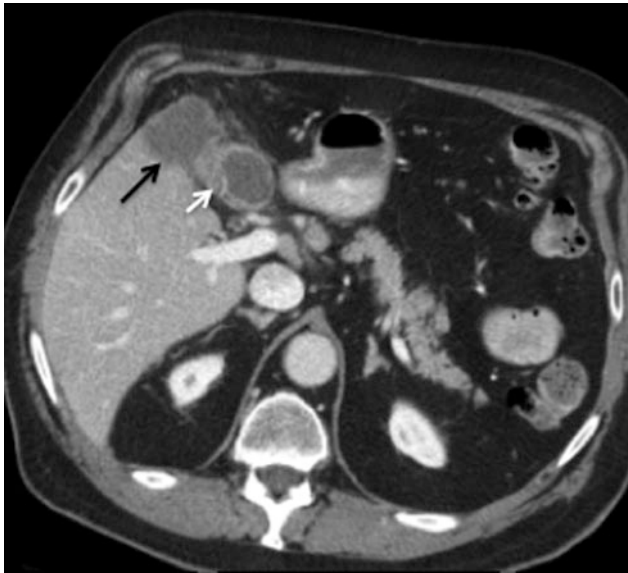


Fig. 6 Invasive gallbladder cancer. Contrast-enhanced MSCT axial image showing irregular gallbladder wall thickening (white arrow), with more than 2 cm of invasion into segment V (black arrow) of the right liver lobe (T4 tumor). Tumor in the gallbladder fundus has propensity for early invasion of segments IVb and V



Fig. 7 Vascular involvement of tumor. 68-year-old male with gallbladder cancer. Axial MSCT image in arterial phase shows soft tissue infiltration and encasement (arrows) of the hepatic artery at the hepatic hilum



Fig. 8 Adjacent organ invasion. 44-year-old female patient with gallbladder cancer. Contrast-enhanced MSCT axial image demonstrates a large mass invading the second portion of the duodenum (black arrow). It shows few air bubbles (white arrow) that correlate with fistulous communication

perimuscular connective tissue but does not extend beyond the serosa. T3 tumor perforates the serosa and directly invades the liver (<2 cm) or one other adjacent organ or structure, such as the bile duct, colon, duodenum, or pancreas. T4 tumor invades the liver (>2 cm), the main portal vein or hepatic artery, or multiple (two or more) adjacent organs and structures. Due to spatial resolution limitations, CT is not very useful for T-staging except to discriminate between T3 and T4 tumors. The sensitivity of CT to detect tumor extension is better when there is advanced disease (T4), approaching 100 % [10]. When the stage is T3, the sensitivity decreases to 65–79 % [10]. The addition of multiplanar reconstructions will improve the accuracy of T-staging of GB CA.

2.2 Radial Tumor Growth and Adjacent Organ Invasion

Direct extension to adjacent organs is the most common method of tumor spread. The gallbladder wall has a thin single muscle layer and a narrow lamina propria. The violation of the thin layers of the GB wall will result in liver extension, and lymphatic and vascular spread of disease. The liver is the organ most commonly involved by direct extension (65 % of cases), and tumors of the fundus and body of the gallbladder have a propensity to invade segments IVb and V at an early stage [4, 11, 12]. Hepatic invasion is demonstrated on contrast-enhanced CT as an irregular soft tissue mass disrupting the adjacent liver parenchyma, usually in segments IVb and V (Figs. 5, 6). Radical surgical resection (usually including excision of segments V and IVb) has been shown to improve survival in

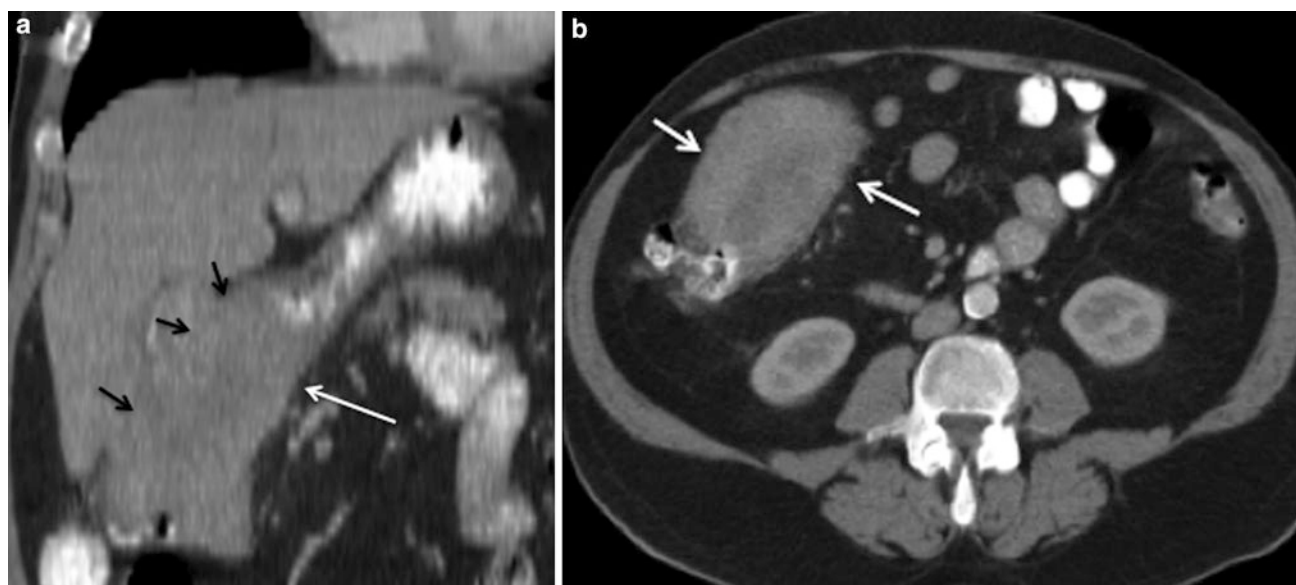


Fig. 9 Adjacent organ invasion. 59-year-old female patient with advanced gallbladder cancer. **a** Contrast-enhanced MSCT coronal image demonstrates a large mass (*black arrows*) invading the hepatic

flexure (*white arrow*). **b** Contrast-enhanced MSCT axial image shows irregular thickening and luminal narrowing (*arrows*) of the colonic hepatic flexure

patients whose tumors are locally confined to the cystic fossa and adjacent hepatic parenchyma [7, 10].

The criterion for tumor extension to adjacent organs, bile ducts, or vessels is the disruption of fat planes between the tumor and the adjacent structures [13, 14] (Fig. 7). Anatomic structures in the hepatic hilum and in close proximity to the gallbladder can also be involved by direct tumor extension: the bile duct and the portal vein are commonly involved by direct tumor extension of GB CA [15]. Biliary dilatation secondary to GB CA can be seen by CT. This may be a result of tumor spread along the cystic duct or extrinsic mass effect from tumor infiltration or enlarged nodes. The colon and duodenum (Fig. 8) are also frequently involved, followed by the pancreas [15]. Extension into the hepatic flexure of the colon is shown on CT as infiltration of the normal low-density pericolonic fat by soft tissue with obliteration of vessels. Wall thickening with possible luminal narrowing and eventual obstruction may also occur (Fig. 9).

2.3 Nodal Disease

The assessment for nodal disease is important for accurate staging. The prevalence of lymphatic metastases in GB CA exceeds 70 % in some series [4, 13]. Lymphatic spread in GB CA occurs first to the cystic, pericholedochal, periportal, hepatic artery, and hepatoduodenal nodes (N1 nodes) (Figs. 10, 11). Disease can then spread to the celiac, superior mesenteric, and peripancreatic nodes, which comprise the N2 nodes (Fig. 12). Attention to the presence of



Fig. 10 Lymphatic spread of gallbladder cancer. Contrast-enhanced MSCT axial image shows a lymph node in the hepatic artery nodal station (*arrow*), consistent with N1 node. Although this node does not exceed 1 cm in short axis diameter, because it is larger than the adjacent nodes and is located along the lymphatic path of spread of gallbladder carcinoma, it is considered suspicious for malignancy

suspicious interaortocaval, left para-aortic, and retropancreatic nodes is important because they may be regarded as distant nodal metastases. The CT detection of metastatic adenopathy will assist in the staging. Detection of nodal involvement on CT is based on size and internal imaging



Fig. 11 Lymphatic spread of gallbladder cancer. Contrast-enhanced MSCT axial image shows an enlarged lymph node in the portocaval nodal station (*black arrow*), consistent with N1 node. Nodes larger than 1 cm in short axis or with central low density (necrosis) are more likely metastatic. There is also nodularity and increased attenuation of the mesocolon adjacent to the hepatic flexure (*white arrow*), representing peritoneal infiltration

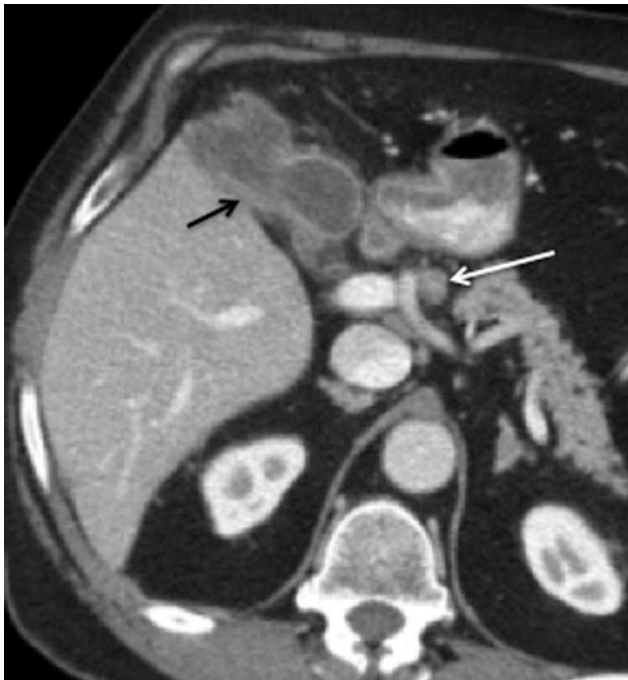


Fig. 12 Lymphatic spread of gallbladder cancer. Axial MSCT contrast-enhanced image shows irregular thickening (*black arrow*) of the gallbladder wall, compatible with tumor. There is also an enlarged lymph node in the celiac nodal station (*white arrow*), consistent with a metastatic N2 node



Fig. 13 Hematogenous metastasis in gallbladder cancer; 44-year-old female with advanced gallbladder carcinoma. Contrast-enhanced MSCT axial image shows several masses with central low-attenuation and peripheral rim enhancement (*arrows*) in the right hepatic lobe, representing hematogenous metastases

features of the nodes. Nodes >1 cm in short axis are likely malignant [15]. Nodes with a low-attenuation center indicating central necrosis are also likely to harbor metastatic disease [15]. Using the CT criteria for nodes larger than 1 cm, for the presence of malignancy, the sensitivity of CT in the detection of positive nodes in gallbladder cancer is 36 and 47 % for N1 and N2 nodes, respectively [16].

2.4 Metastatic Disease

Hematogenous metastases of GB CA occur most commonly to the liver. They appear as multifocal areas of low attenuation in relation to the adjacent hepatic parenchyma, usually with a peripheral rim of contrast enhancement (Fig. 13). Metastases to other organs, such as the lungs, osseous structures, kidneys, adrenals, and brain, occur less frequently.

GB CA spread into the peritoneum is also common. The imaging features of peritoneal deposits are discrete nodules and fat stranding of the low-attenuation peritoneal fat (Figs. 11, 14). The detection of peritoneal disease may be challenging [11].

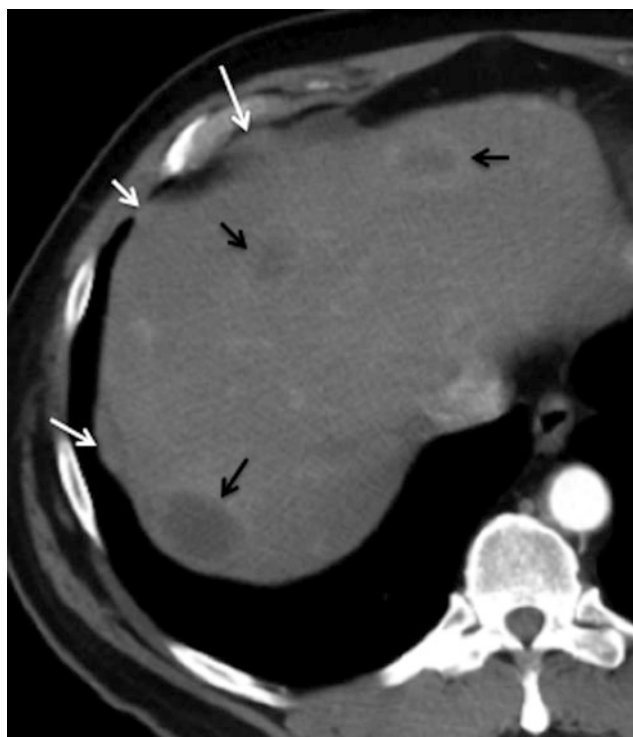


Fig. 14 Peritoneal metastasis; 70-year-old male with advanced gallbladder carcinoma. Axial contrast-enhanced MSCT image shows few areas of nodular soft tissue thickening in the perihepatic sub diaphragmatic wall (*white arrows*), consistent with metastatic deposits. Multiple rim-enhancing liver lesions (*black arrows*) are also present, representing hematogenous metastases

Resectability of GB CA depends on multiple factors. In the assessment of resectability, MSCT has been found to be an accurate technique to determine resectability of GB CA when factors such as vascular invasion, adjacent organ invasion, and metastases are considered [17].

2.5 Differential Diagnosis

There are several conditions that may mimic GB CA on imaging studies, such as acute and chronic cholecystitis, adenomyomatosis, and polyps. Gallstones and GB CA may coexist, making differentiation more problematic (Fig. 15). Enhancement of the liver parenchyma adjacent to the cystic fossa has been reported in acute cholecystitis and should not be misjudged as a focal hepatic lesion. Also, tumors arising in the neck of the gallbladder not uncommonly may cause obstruction of the cystic duct and present clinically as acute cholecystitis.

Imaging features of adenomyomatosis on CT include focal or diffuse cystic-appearing thickening of the gallbladder wall (Fig. 16) [18]. Although it is not possible to reliably differentiate GB CA from this condition in all cases, the presence of cystic-appearing spaces in the thickened

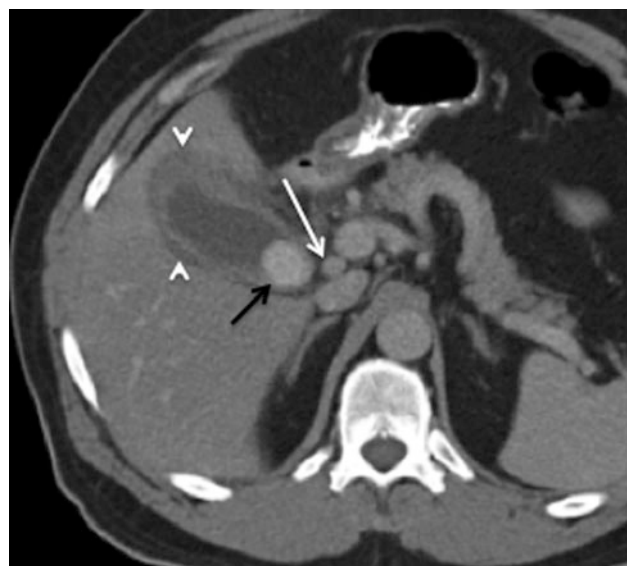


Fig. 15 Cholelithiasis and gallbladder cancer; 65-year-old female with gallbladder carcinoma presenting with chronic right upper quadrant pain. Contrast-enhanced MSCT axial image shows a large intraluminal calcified stone (*black arrow*), compatible with cholelithiasis. Irregular thickening (>1 cm) of the gallbladder wall seen as well, consistent with tumor. Associated hypodensity in the adjacent liver parenchyma (*arrowhead*), representing liver invasion (T3, <2 cm extension). A lymph node in the portocaval nodal station (*white arrow*), representing metastatic N1 node

gallbladder wall allows the diagnosis of adenomyomatosis to be made with reasonable confidence [18].

Cholesterol polyps may be single or multiple and represent approximately 50 % of all polypoid lesions in the gallbladder; they have no malignant potential [19]. Cholesterol polyps may be rarely apparent on contrast-enhanced scans due to vascularity within the polyp (Fig. 17).

There are other benign tumors that are reported in the literature that may involve the gallbladder and biliary tract. For example, adenoma of the gallbladder is found in approximately 0.5 % of cholecystectomy specimens. Only a small proportion of gallbladder adenomas progress to carcinomas. With contrast-enhanced CT, gallbladder adenoma presents as an enhancing intraluminal soft tissue mass or nodule that may be iso- or hypoattenuating relative to the liver [12].

2.6 Treatment Response and Recurrence

CT is used to monitor patients following treatment for GB CA. After therapy, careful assessment should be made not only for evidence of residual disease, but also for any evidence of complications from therapy. A nonenhancing fluid collection may be seen at the resection margin after surgery (Fig. 18). This fluid collection usually decreases in size after 3–6 months but may never completely disappear. If a

Fig. 16 Gallbladder adenomyomatosis. Sequential contrast-enhanced axial MSCT images show focal thickening with cystic spaces in the gallbladder fundus (arrows). Note the thin homogeneous enhancement of the remaining gallbladder wall

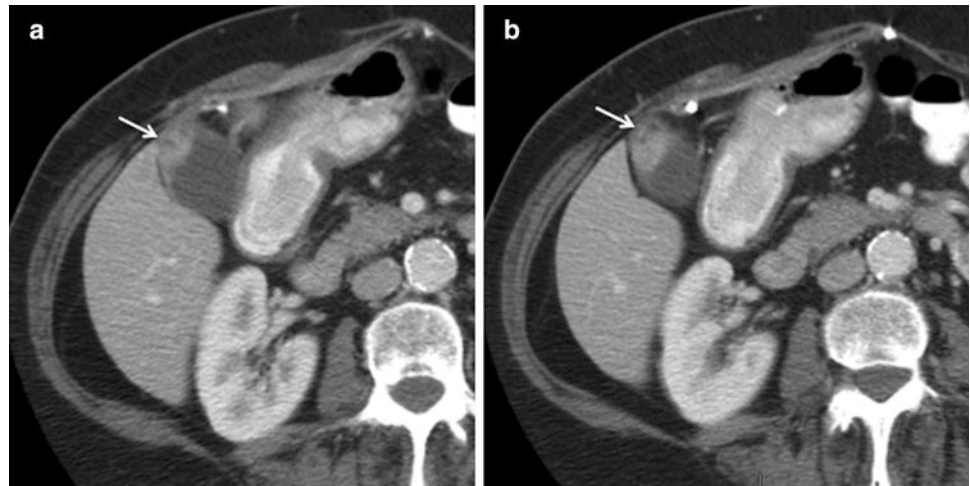


Fig. 17 Gallbladder polyps. Axial contrast-enhanced MSCT image shows two subcentimeter nodular-enhancing lesions in the gallbladder wall (arrows), protruding into the lumen. Note the thin homogeneous enhancement of the remaining gallbladder wall

catheter is left in the surgical bed, small pockets of air may be present and not necessarily imply infection. Increasing amounts of fluid and gas, development of a thick rim of peripheral enhancement, and progressive stranding of the adjacent fat are signs of abscess formation (Fig. 19). Differentiating an abscess from a postoperative seroma or hematoma requires correlation with clinical suspicion and can be challenging during the perioperative period.

Normal postsurgical findings such as infiltration of the peritoneal fat in the operative bed and focal areas of fat necrosis should not be misinterpreted as recurrent disease. The degree of soft tissue attenuation that increases in size 3–6 months following surgery is concerning for peritoneal metastasis and needs to be followed closely on subsequent imaging studies (Fig. 20). Careful attention to the detection of soft tissue nodules at the resection margin in the liver, at the tract of previous drainage catheters, at the laparoscopic ports tracts, and at the abdominal wall wound is essential for the detection of residual disease and peritoneal metastasis (Fig. 21) [13].

3 Intrahepatic Cholangiocarcinoma

3.1 Epidemiology

Intrahepatic cholangiocarcinoma (ICC), also called peripheral cholangiocellular carcinoma, is a primary malignant tumor arising from intrahepatic bile duct epithelium. It is thought to arise from the secondary bile duct or proximal branches of the intrahepatic bile ducts. ICC accounts for 8 % of all cholangiocarcinomas [20]. The estimated annual incidence in the United States is 1 per 100,000 persons. ICC is the second most common intrahepatic primary liver cancer (7–10 %), with HCC being first [21]. Mixed tumors of cholangiocarcinoma and hepatocellular carcinoma components may be seen in approximately 2–6 % of primary malignant liver tumors, called combined HCC-ICC (cHCC-ICC) [22]. ICC occurs most commonly in the 6th and 7th decades with male-to-female predominance of 3:2. The incidence and age-adjusted mortality have increased in the last 3 decades [23]. The 5-year survival is <10 % and

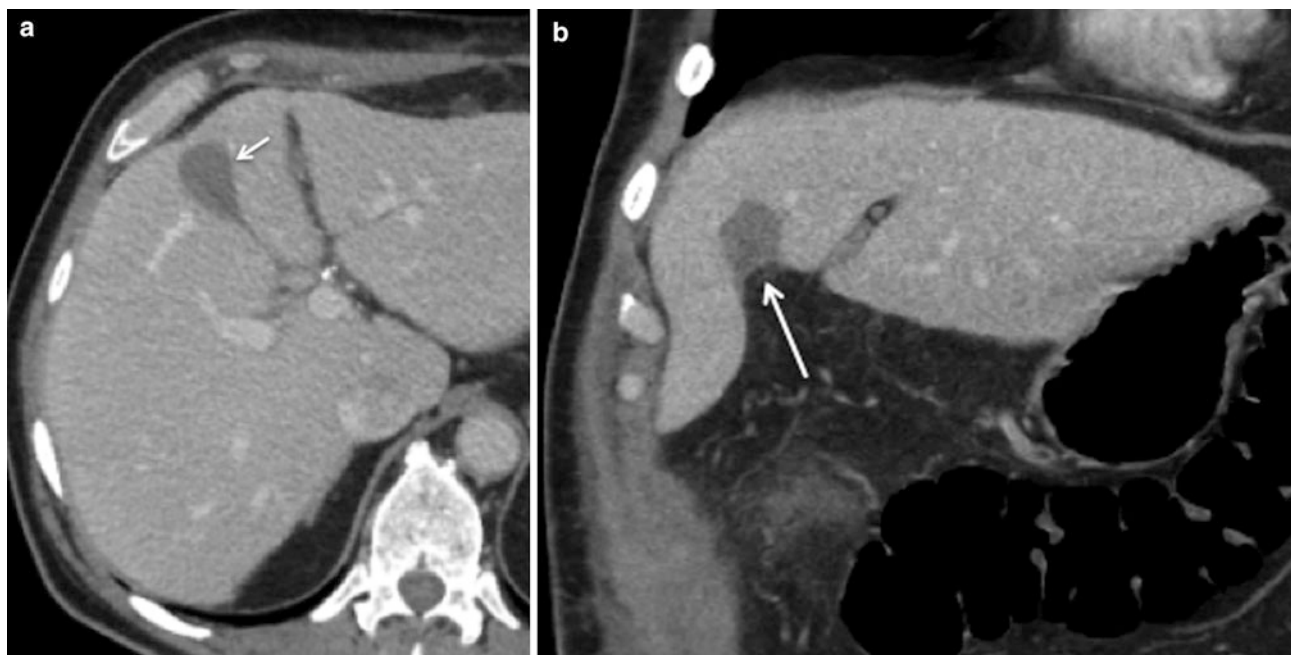


Fig. 18 Postoperative seroma following surgery for gallbladder cancer. 68-year-old male's status postsurgery for gallbladder cancer on routine follow-up. **a** Axial and **b** coronal images from contrast-enhanced MSCT reveals a small fluid collection without peripheral

enhancement in the surgical bed (*arrows*), compatible with seroma. Close follow-up study is required to document involution of the fluid collection, as well as correlation with clinical signs of infection, such as fever and leukocytosis, to exclude the possibility of abscess



Fig. 19 Postoperative abscess following surgery for gallbladder cancer. 67-year-old male's status postsurgery for gallbladder cancer with fever and leukocytosis. Axial contrast-enhanced MSCT image reveals a fluid collection with a thick rim of peripheral enhancement and internal gas bubbles in the surgical bed (*arrows*), compatible with abscess

approaches 0 % when there are intrahepatic metastases. Surgery offers the best outcome [24]. The risk factors for ICC are shared with other cholangiocarcinomas [25].

3.2 Clinical Presentation

The clinical symptoms of ICC are nonspecific. Patients can present with general malaise and discomfort, weight loss, abdominal pain, or nausea [26]. In contrast to ECC, jaundice due to biliary obstruction is not a common finding, and ICC typically presents with signs and symptoms associated with a large mass in the liver. On physical examination, an enlarged liver may be detected. In contrast to patients with HCC, patients do not present with ascites, cirrhosis, or portal hypertension. Some cases present with an incidental liver mass, and imaging studies or endoscopic evaluation are performed searching for a possible primary GI cancer.

Laboratory abnormalities include elevation of CA 19-9 and carcinoembryonic antigen (CEA); this is also seen in ECC [26]. The bilirubin level is usually normal. α -Fetoprotein (AFP), frequently elevated in patients with HCC, is usually normal in ICC. In combined tumors of HCC-ICC pathology, there will be elevated α -fetoprotein and modestly elevated CA19-9 versus elevation of AFP alone for HCC and CA19-9 for ICC [22].

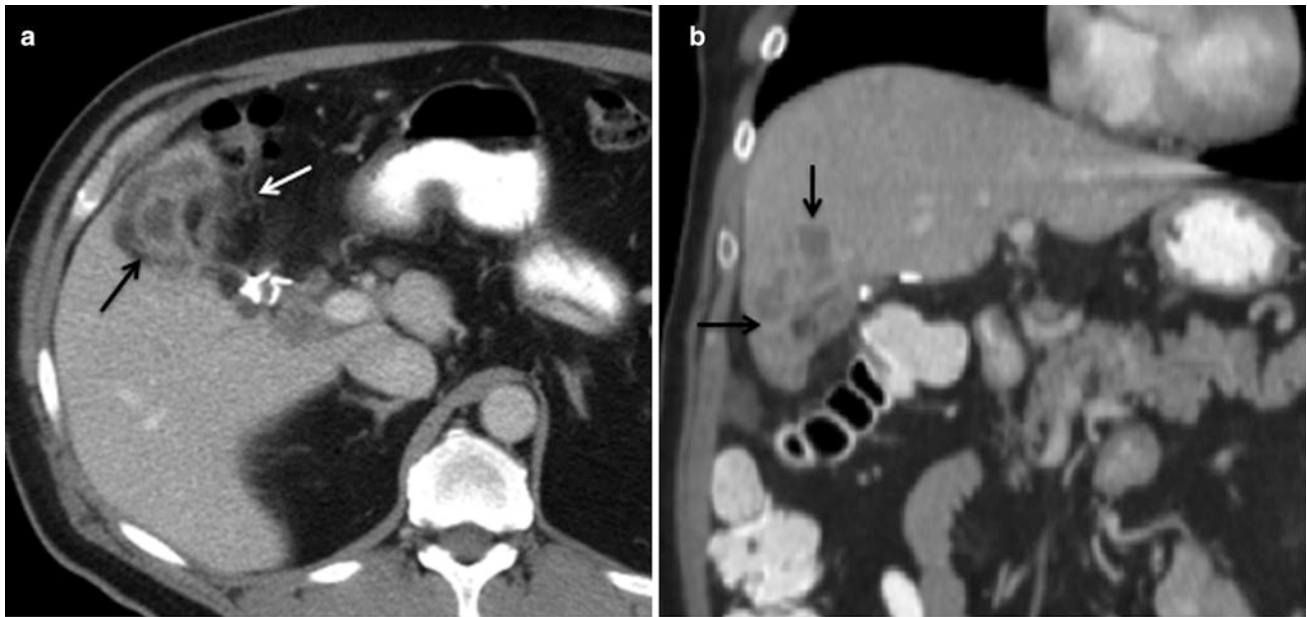


Fig. 20 Recurrent disease after surgery for gallbladder cancer. **a** Axial contrast-enhanced MSCT image shows heterogeneous enhancement in the surgical bed (*black arrow*), with associated fat stranding and nodular thickening of the adjacent peritoneal fat (*white*

arrow), consistent with tumor recurrence. **b** Coronal contrast-enhanced MSCT image reveals invasion of segment V at the resection margin of the liver (*black arrows*)



Fig. 21 Recurrent gallbladder cancer; 68-year-old male with surgery for gallbladder cancer 1 year prior to the study. Axial contrast-enhanced MSCT image demonstrates subtle nodularity and increased fat attenuation near the gallbladder fossa and in the gastropiploic region (*white arrow*), worrisome for peritoneal neoplastic infiltration

4 Imaging

4.1 Imaging Protocol and Technique

Computed tomography plays a key role in the clinical evaluation of patients with ICC. The availability and short scan duration makes CT the main diagnostic tool in oncologic imaging, including patients with ICC. The CT protocol for a patient with a liver mass is a multiphasic examination. The liver protocol consists of pre-contrast and postcontrast images obtained during the late arterial, portal venous, and excretory phases of contrast administration. 150 ml of intravenous iodinated contrast is injected at a rate of 5 ml/s. SmartPrep® (GE Healthcare, Milwaukee) is used for contrast bolus tracking, monitoring the aorta at the level of the celiac artery until 100 Hounsfield units is obtained; this takes roughly 20 s. Dynamic imaging through the liver in the late arterial phase is then obtained at 13 s post-threshold. Portal venous phase imaging is then obtained after 60 s, and delayed images through the liver are obtained at 90 s. The images are obtained at 5 mm and reconstructed at 2.5 mm for each phase of contrast administration.

Fig. 22 ICC enhancement pattern; 81-year-old male presenting with weight loss. **a** Pre-contrast MSCT image shows hypodense mass involving segments VII and VIII of the right liver lobe. **b** Postcontrast MSCT image in late arterial phase shows hypodense center with only mild peripheral enhancement. **c** Postcontrast MSCT image in portal venous phase shows continuous centripetal contrast enhancement. A satellite smaller peripheral enhancing lesion (*arrow*) is also appreciated posteriorly. **d** Postcontrast MSCT image in delayed phase shows almost complete opacification with contrast of the tumor. Incidental right pleural effusion also visualized



4.2 Imaging Findings

There are three macroscopic presentations of ICC described by the Liver Cancer Study Group: mass-forming (MF), periductal-infiltrating (PD), and intraductal (ID) types [27]. A fourth group has been created to combine tumors that exhibit mixed characteristics, for example, MF + PD. The MF and the combination types are the most common (70 % of cases).

4.3 Mass-Forming Type

On the pre-contrast images, ICC presents as a large, hypodense mass with lobular or irregular margins

(Fig. 22a). During the late arterial phase of contrast administration, commonly there is minimal to no peripheral enhancement of the tumor (Fig. 22b). On the portal venous phase, the enhancement will continue in a centripetal fashion and continue to progress on the delayed and excretory phases of contrast (Fig. 22c). The delayed enhancement in ICC is due to the presence of a dense fibrous stroma, which retains contrast over time [28] (Fig. 22d). This enhancement pattern is distinct from other common liver malignancies such as, HCC or hypervascular metastases (e.g., neuroendocrine carcinoma). However, ICC can also present with arterial enhancement similar to other hypervascular tumors of the liver (Fig. 23). Associated findings include peripheral ill-defined calcification due to

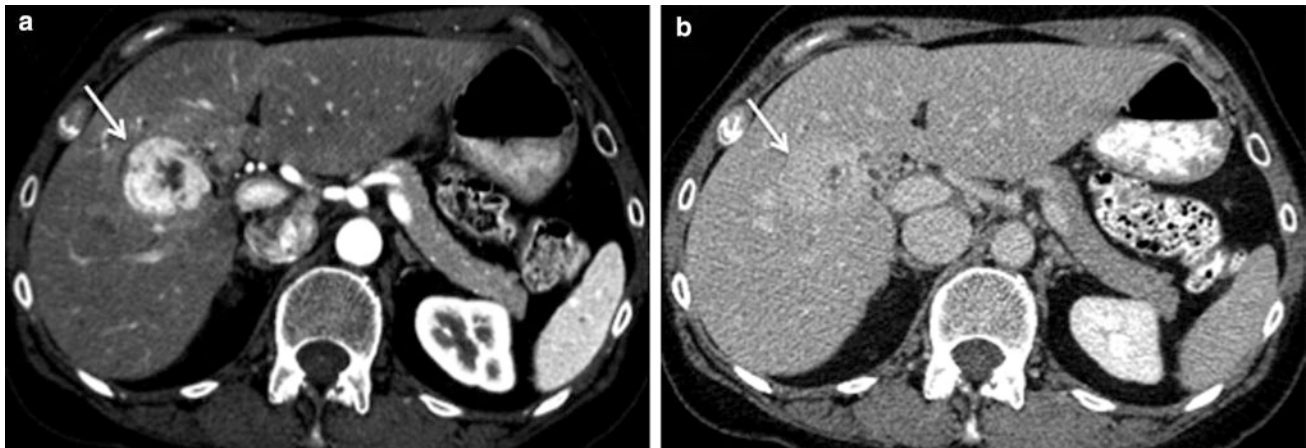


Fig. 23 Enhancement pattern; 62-year-old female with ICC. **a** Post-contrast MSCT image shows mass in segment V of the right liver lobe (*arrow*) with avid contrast enhancement in late arterial phase. **b** Postcontrast MSCT image in delayed phase shows progressive

contrast enhancement with almost complete filling (*arrow*) of the tumor. ICC may present with arterial enhancement similar to other hypervascular liver tumors

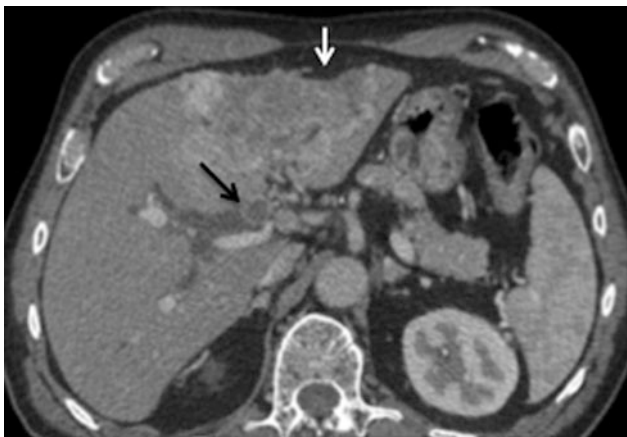


Fig. 24 Capsular retraction; 72-year-old male presenting with weakness and anorexia. Axial portal venous phase MSCT image shows large, heterogeneous enhancing space-occupying lesion in segment II and III of the left liver lobe with associated capsular retraction (*white arrow*), due to desmoplastic reaction. Heterogeneous soft tissue in the proximal right hepatic duct (*black arrow*) with associated proximal ductal dilation represents ductal invasion of tumor

mucin production in 18 % and capsular retraction due to desmoplastic response (Fig. 24) [29, 30]. The differential diagnosis includes metastasis from the GI tract. Features that will suggest the diagnosis of ICC include a large single mass, the absence of a primary GI tumor, and the absence of multiple nodules.

4.4 Periductal-Infiltrating

The PD type accounts for approximately 15–20 % of cases [27, 31]. This tumor pattern presents as a proximal ductal dilation without a discrete mass or as periductal soft tissue

in the noncontrast CT. On the arterial phase, minimal ductal wall or periductal soft tissue enhancement is observed [32]. In the portal venous phase, more intense enhancement is seen in the ductal wall and periductal soft tissues [27]. These tumors have a higher incidence of satellite nodules and also of nodal metastases. The differential diagnosis of this type includes benign strictures. The presence of portal vein obliteration and lymph node involvement is more suggestive of a malignant etiology.

4.5 Intraductal Type

The ID type of ICC accounts for approximately 5 % of cases [27, 31]. This pattern of ICC is considered similar to the intraductal papillary mucinous neoplasm (IPMN) of the pancreas and has the best prognosis [33]. On noncontrast CT, a dilated duct with or without a mass >1 cm can be seen. On the arterial and portal venous phases, a hypoattenuating mass or a hyperattenuating duct may be seen [34]. The differential diagnosis of a high-attenuation ID mass includes stone, tumor such as HCC or metastatic disease, or stricture with debris. ID HCC will usually have the typical pattern of HCC enhancement, being slightly less hypoattenuating on the noncontrast images and showing marked enhancement on arterial phase and washout on the portal venous images [35].

4.6 Combined HCC-ICC

Mixed HCC and ICC tumors (cHCC-ICC) can present as large, solitary tumors with irregular margins [22]. The contrast enhancement pattern is dependent on the

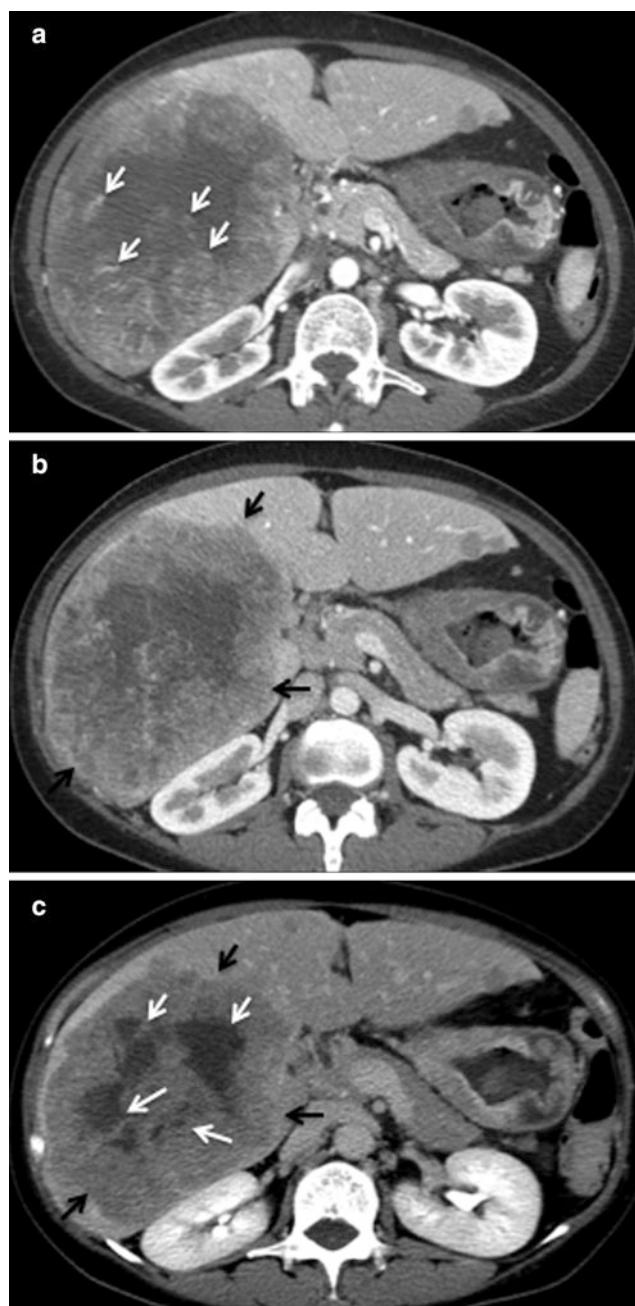


Fig. 25 Enhancement pattern; 65-year-old male with chHCC-ICC. **a** Postcontrast MSCT image in late arterial phase shows few central focal areas of increased vascularity (*white arrows*) and minimal peripheral enhancement. **b** Postcontrast MSCT image in portal venous phase shows increased peripheral contrast enhancement (*black arrows*). **c** Postcontrast MSCT image in delayed phase shows washout of the previously enhancing central foci (*white arrows*) and peripheral contrast enhancement of the tumor (*black arrow*)

percentage of each tumor. An HCC pattern will present with intense arterial enhancement. It may be very difficult to predict prospectively whether the mass represents HCC or ICC. On noncontrast CT there is a large hypoattenuating

tumor. During the arterial phase, the HCC portion will enhance avidly, while the ICC portion may stay hypodense. At the portal venous phase, the enhancing portion representing HCC will wash out and the portion representing ICC continues to enhance. On the delayed phase, the HCC component is hypoattenuating, and the ICC portion is homogeneously enhancing (Fig. 25).

5 Staging

5.1 Primary Tumor

Mass-forming ICC and chHCC-ICC are staged according to the AJCC hepatocellular carcinoma system [21, 36]. The discriminating factors for T-staging are tumor size, satellite nodules, vascular invasion, and extra-capsular extension. Any solitary tumor without vascular invasion, regardless of size, is classified as T1. A T2 tumor is defined as solitary tumors with vascular invasion or multiple tumors none larger than 5 cm. T3 tumors are classified as multiple masses larger than 5 cm or with major vascular invasion of a major branch of the portal or hepatic veins. A T4 lesion is defined as a tumor that demonstrates direct invasion to adjacent organs other than the GB or in the setting of perforation to the visceral peritoneum [37].

Similar to HCC, ICC has a propensity to encase and even invade branches of portal veins and hepatic arteries through direct extension [34] (Figs. 26, 27). There can be wedge-shaped enhancement of the liver surrounding the tumor due to arterial supply as a result of portal vein encasement [35] (Fig. 28). Biliary ductal dilation and gallbladder involvement due to direct extension of the tumor into the gallbladder fossa can also be seen (Figs. 29, 30).

5.2 Nodal Disease

Lymph node status is an important prognostic indicator. The radiologic evaluation of lymph nodes is based on size, morphology, and location. Helpful clues for metastatic lymph nodes include size >1 cm, low-density center due to necrosis, or delayed enhancement. Nodes located near the primary tumor are likely to be malignant, even if they measure <1 cm in minimum diameter. A round node is also more likely to be malignant than an oval node. CT has a high negative predictive value but a low positive predictive value for lymph node involvement [38].

Nodes along the hepatic hilum are a common site of central tumor spread. Central ICC typically spreads into the hepatoduodenal ligament first, and then into the para-aortic nodes, retropancreatic nodes, and common hepatic artery

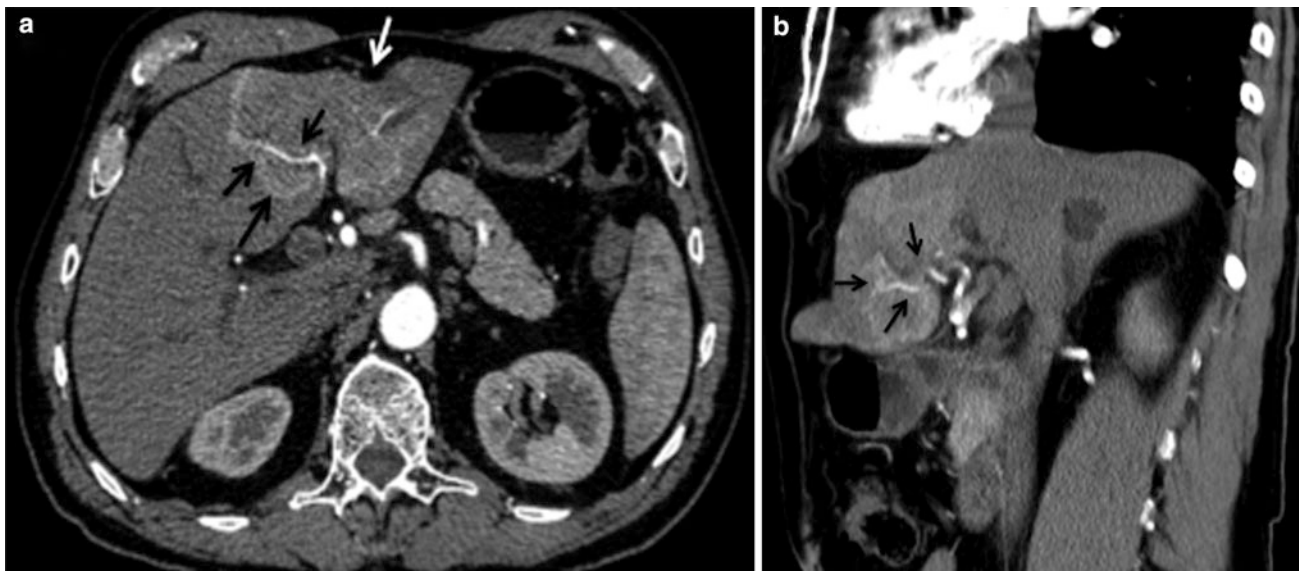


Fig. 26 Hepatic artery involvement; 72-year-old male with weakness and anorexia. **a** Axial and **b** coronal MSCT images in late arterial phase show heterogeneous enhancing mass in the left liver lobe with

encasement (*black arrow*) of the left hepatic artery. Capsular retraction (*white arrow*) is appreciated as well

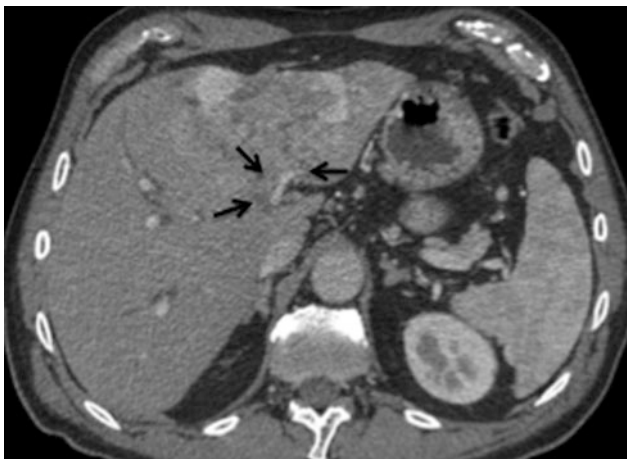


Fig. 27 Vascular involvement; 72-year-old male with ICC. Axial MSCT image in portal venous phase shows heterogeneous mass with encasement of the left portal vein (*arrows*)

nodes, in that order. Diaphragmatic nodes in the anterior, posterior, or middle diaphragmatic nodal stations, as well as, para-cardiac and lesser curvature nodes may be considered as N1 nodes for ICC that are located near the dome and in the left lobe of the liver [39] (Fig. 31). The nodal disease defines N1 and N2 nodes as regional or distant nodal spread of disease, respectively. Examples of N1 are perihepatic, periportal, portocaval, and periceliac nodes (Figs. 32, 33). Common distant lymph node involvement includes mediastinum and retroperitoneal para-aortic nodes (Fig. 33).



Fig. 28 Transient asymmetric hepatic enhancement secondary to portal vein invasion; 79-year-old female presenting with chronic right upper quadrant pain. Late arterial phase axial MSCT image demonstrates large mass with enhancement of the surrounding liver parenchyma of the left liver lobe (*arrows*), representing preferential arterial flow secondary to portal vein invasion

5.3 Metastatic Disease: Radial Tumor Growth and Organ Invasion

The pattern of tumor spread for ICC may be extrahepatic or intrahepatic. The most common site of metastatic spread is to the hepatic parenchyma via the portal venous system [40]. Intrahepatic spread may include extension of tumor



Fig. 29 Adjacent organ involvement; 62-year-old female with ICC. Axial MSCT image in late arterial phase shows heterogeneous mass in segment V of the left liver lobe (arrows) invading the gallbladder by direct extension

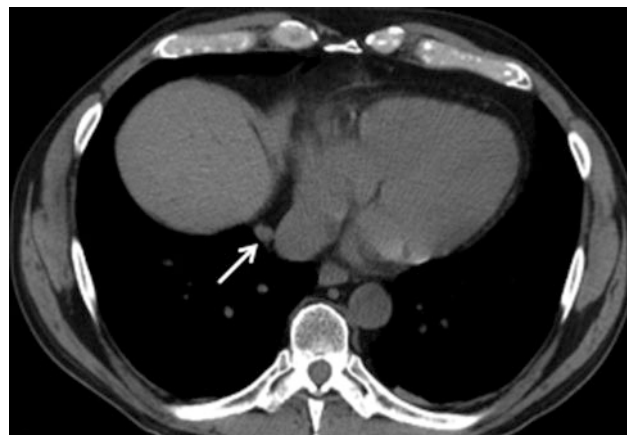


Fig. 31 Lymphatic spread of ICC. 61-year-old male with ICC in the left hepatic lobe. Noncontrast MSCT axial image shows a mildly enlarged lymph node in the middle diaphragmatic nodal station (arrow), consistent with N1 node. The node is enlarged when compared to prior exam, favoring neoplastic involvement

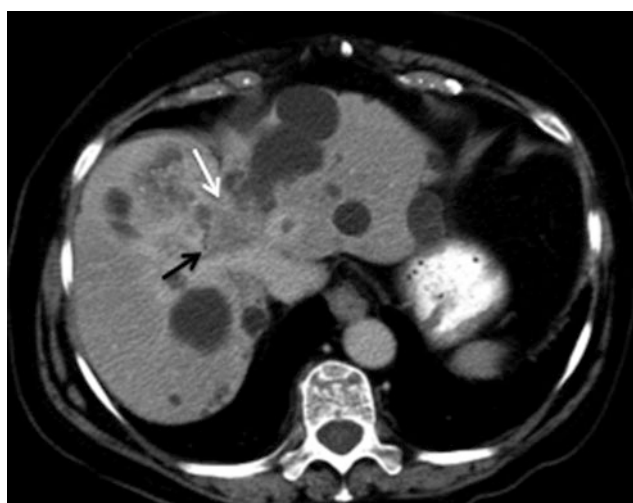


Fig. 30 Invasive ICC; 83-year-old female with polycystic kidney disease and ICC. Axial MSCT image in delayed phase shows heterogeneous mass in segments VIII and IVA of the liver with encasement of the mid-hepatic vein (white arrow). Bile duct invasion is also appreciated with mild biliary ductal dilation (black arrow)

into the portal vein, which will demonstrate postcontrast enhancement features similar to those of ICC [41]. The presence of hepatic satellite nodules is a poor prognostic factor [26, 42]. In the portal venous phase images, enhancing nodules with peripheral enhancement are seen in the noncontiguous lobe (Figs. 22, 34).

Computed tomography has high sensitivity for assessing distal metastatic liver disease but underestimates the extent of peritoneal disease, spread along Glisson's capsule, and metastatic liver disease [43]. Invasion through the liver capsule and extension to adjacent sites is more common in ICC than in HCC (Fig. 34). ICC can also present with distant metastases, and common sites are the lungs, bone,

adrenals, peritoneum, and brain. Lung metastasis can be present as single or multiple nodules. Osseous involvement is most commonly seen as lytic lesions. Adrenal metastases present as large, irregular masses. Spread to the peritoneum results in peritoneal carcinomatosis and thickening of the omentum (Figs. 34, 35). Extrahepatic spread has been reported as 50–67 % based on autopsy series [44].

5.4 Treatment Response and Recurrence

Surgery provides the best outcome and possibility of cure for patients with ICC [20, 37]. Factors that preclude surgical resection are bilobar involvement, main portal vein involvement, involvement of two or more hepatic veins, and metastatic spread. Other factors that can impact surgery are degree of cirrhosis, degree of steatosis, and comorbid factors. The surgery for ICCs is usually resection with right or extended left hepatectomy, similar to the management of hepatocellular carcinoma [45].

Computed tomography volumetric analysis of the liver is usually performed to assess residual liver volume, which provides key information for procedure planning. The liver is marked into individual segments by the radiologist, and then, 3D volumetric reconstructions are performed using 1.25-mm collimation images (Fig. 36). Calculations of the future liver remnant (FLV/TELV ratio) are made. If this ratio is <20 % in noncirrhotic liver or <40 % in cirrhotic livers, then portal vein embolization is utilized to increase liver volume and avoid post-operative complications, such as liver failure [46].

While orthotopic liver transplantation (OLT) is a recommended surgical option for the management of HCC, OLT indications have recently been modified to include

Fig. 32 Lymphatic spread of ICC; 60-year-old female with left ICC. **a** Contrast-enhanced MSCT axial image shows an enlarged hypodense lymph node in the gastrohepatic nodal station (*arrow*), consistent with N1 node. **b** Contrast-enhanced MSCT axial image shows few enlarged hypodense lymph nodes in the hepatic artery nodal station (*black arrow*), consistent with N1 nodes. Left hepatic lobe segment III biliary dilation seen (*white arrow*). **c** Contrast-enhanced MSCT coronal image shows enlarged necrotic lymph nodes in the gastrohepatic and periportal nodal stations (*arrows*), consistent with N1 nodes. **d** Contrast-enhanced MSCT axial image shows enlarged lymph nodes in the celiac nodal station (*white arrows*), consistent with N1 nodes. Small lymph node in the retroperitoneal para-aortic nodal station (*black arrow*), consistent with N2 node

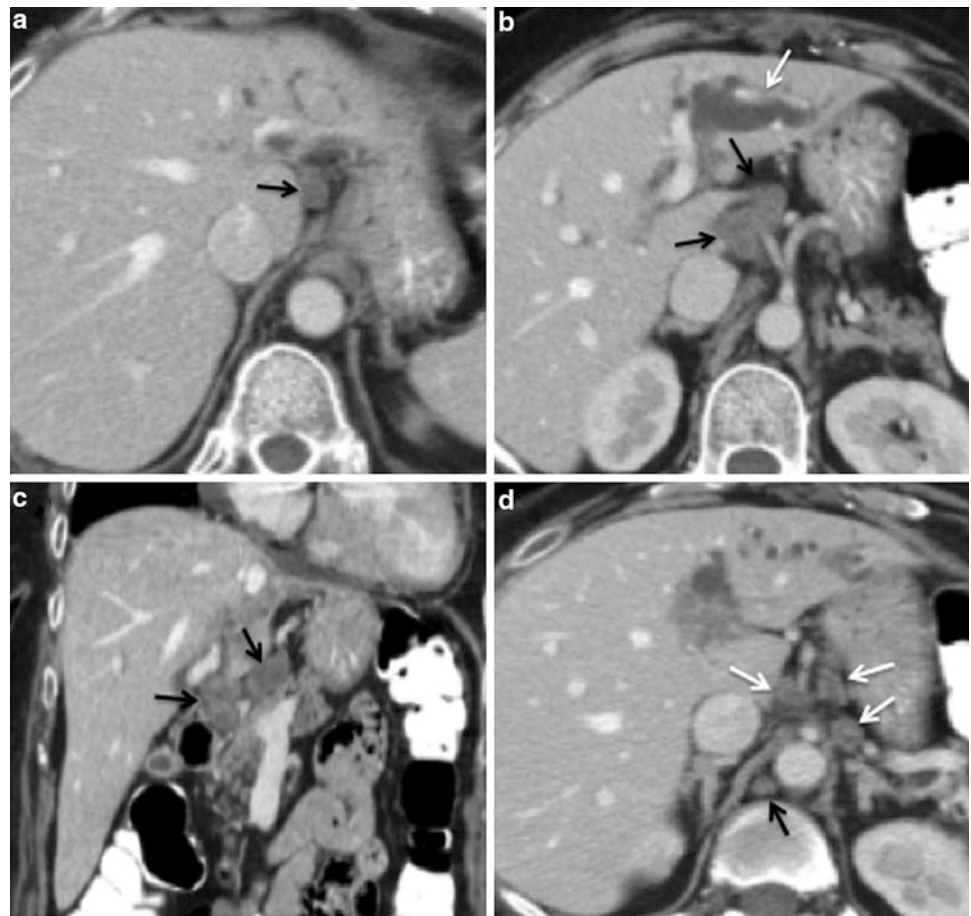


Fig. 33 Lymphatic spread of ICC. Contrast-enhanced MSCT axial image shows two enlarged lymph nodes in the periaortic and pre-caval retroperitoneal nodal stations (*arrows*), consistent with N2 nodes. Few metastatic lesions identified in the right liver lobe as well

patients with unresectable ICC. The initial results were not very promising due to poor survival outcomes. The addition of neoadjuvant therapy has been suggested to improve the survival following OLT for ICC [47].

Postoperative changes include stranding of the peritoneal fat and seroma, which appears as low-density fluid collection at the surgical site and resolves within 3–6 months (Fig. 37). The most common site of recurrence is local. On MSCT, you can see a nodular mass underneath the hemidiaphragm or contiguous to the surgical site. Recurrence can also present with liver mass (Fig. 38).

Chemotherapy can be used alone or combined with radiotherapy for preoperative debulking of the tumor or for palliation. Imaging findings after chemotherapy include decrease in the size of the mass, decrease in enhancement of solid components, and decrease in the adjacent perfusion abnormalities (Fig. 39). Findings after radiotherapy include low attenuation in the liver parenchyma adjacent to the radiation port (Fig. 40). This is due to peritumoral edema, which appears as a low-density area compared to adjacent liver [48].

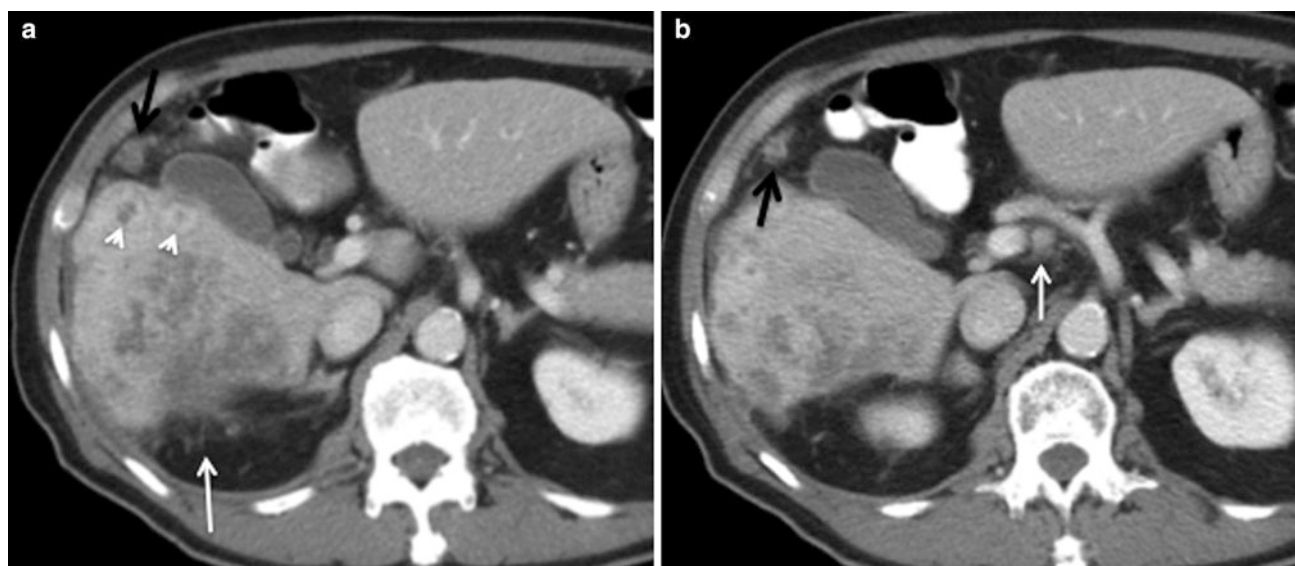


Fig. 34 Advanced ICC. 73-year-old male patient with ICC. **a** Axial MSCT contrast-enhanced image shows a large heterogeneous mass in the posterior right liver lobe with invasion and extension through the liver capsule (*white arrow*). Few satellite hypoattenuating peripheral enhancing nodules (*arrowheads*) anterior to the primary mass. Nodular

soft tissue density in the peritoneal fat anteroinferior to the liver (*black arrow*) represents peritoneal metastatic deposit. **b** Lymph node in the hepatic artery nodal station (*white arrow*), N1 node. Peritoneal implant again observed (*black arrow*)

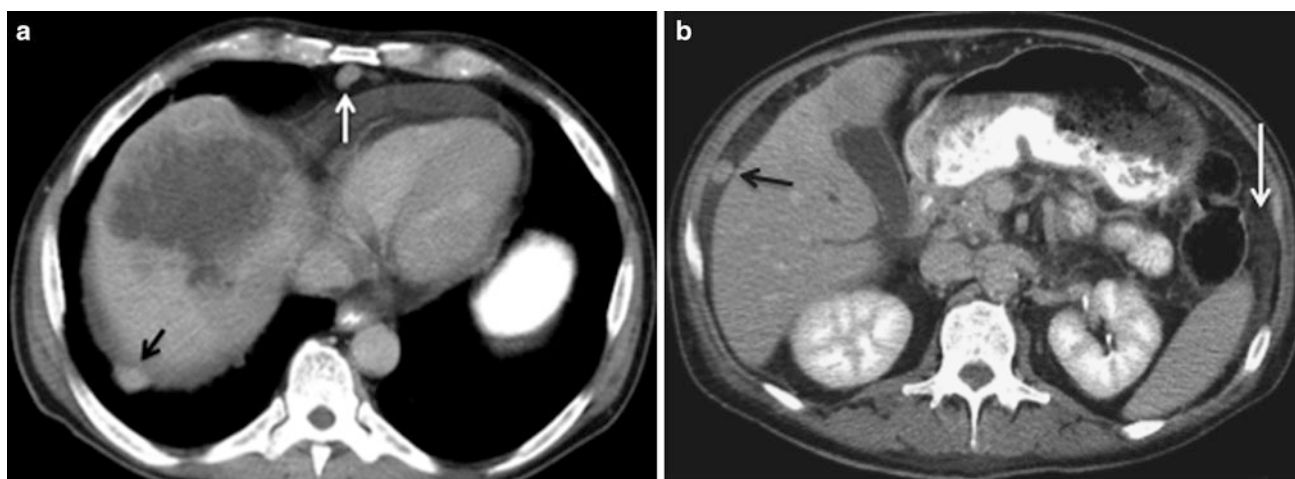


Fig. 35 Peritoneal carcinomatosis from ICC. **a** Axial MSCT contrast-enhanced image shows a large heterogeneous mass with central necrosis in the liver. Perihepatic metastatic implant seen (*black*

arrow). Anterior diaphragmatic node also appreciated (*white arrow*). **b** Nodular perihepatic soft tissue density (*black arrow*) and omental thickening (*white arrows*) represent metastatic peritoneal spread

6 Extrahepatic Cholangiocarcinoma

6.1 Epidemiology

Extrahepatic cholangiocarcinomas (ECC) are adenocarcinomas of the bile ducts that arise proximal in the right or left hepatic ducts, at the confluence of the ducts, or in the common hepatic or common bile ducts. They are subdivided into proximal (hilar) or distal cholangiocarcinomas if they

arise at the confluence of the ducts or in the distal bile duct near the ampulla, respectively [49]. Staging, management, and survival are different for proximal and distal tumors [50]. ECC is usually seen in patients over 65 years of age. The annual incidence of bile duct cancer in the United States is 1 per 100,000. The prognosis is poor, and, if untreated, it usually leads to death in approximately 12 months.

Hilar cholangiocarcinomas are the most prevalent type of ECC [2]. Tumors of the distal bile duct are less common

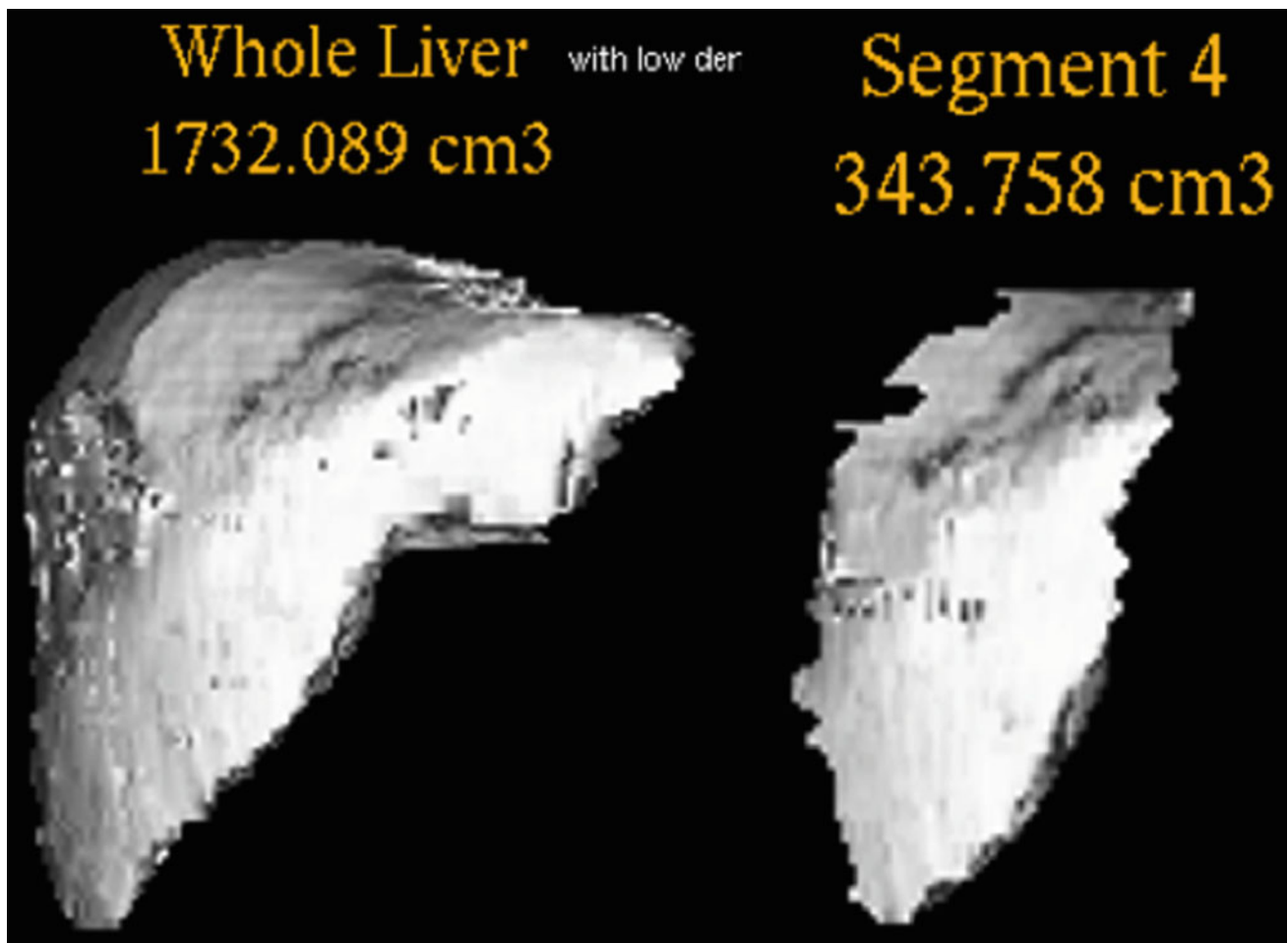


Fig. 36 Liver volume calculation; 45-year-old male patient with cholangiocarcinoma. Multiple volume-rendered images of the whole liver and residual liver segment; whole liver volume of approximately 1,732 cc and segment IV volume of approximately 344 cc



Fig. 37 Postoperative seroma following extended right hepatectomy for ICC; Axial contrast-enhanced MSCT image reveals a small fluid collection without peripheral enhancement in the surgical bed (arrows), compatible with seroma

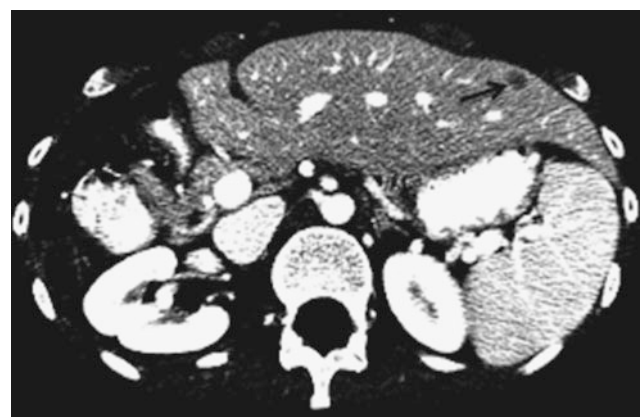


Fig. 38 Recurrent tumor; 29-year-old female with ICC status post-right hepatectomy. MSCT contrast-enhanced image shows a small hypoattenuating rim-enhancing lesion in segment III of left liver lobe (arrow), in keeping with recurrent disease



Fig. 39 Postchemotherapy changes; 66-year-old male with ICC after 1 year of chemoradiation. Axial contrast-enhanced MSCT image shows a sharply demarcated low-attenuation region in segment VI of right liver lobe (*black arrows*) without significant enhancement, correlating with chemoradiation changes. Nodular soft tissue densities posterior to the liver (*white arrow*), representing peritoneal carcinomatosis

and a very small number of ECC are diffuse tumors. Worldwide, there is a higher incidence in Israel and Japan and among American Indians. The risk factors for ECC are the same as for other cholangiocarcinomas. PSC (Primary Sclerosing Cholangitis) appears to be the most widely recognized risk factor. These patients are at risk for multifocal disease. An increased incidence has also been reported in patients with choledochal cysts and Caroli's disease. Oriental cholangiohepatitis has shown an association with a higher incidence of cholangiocarcinoma, particularly in Japan and parts of Southeast Asia, where the disease is more prevalent. Other risk factors are ulcerative colitis (UC), drugs (oral contraceptives, methyldopa, and isoniazid), chemical exposure (thorotrast, radionuclides, asbestos, arsenic, and dioxin), and primary biliary cirrhosis. The risk of ECC in patients with UC is approximately 0.5 %. The risk also increases with biliary cirrhosis, cholelithiasis, alcoholic liver disease, diabetes, and chronic pancreatitis [25]. Infectious pathogens associated with ECC include liver flukes (*Clonorchis sinensis*).

6.2 Clinical Presentation

Due to the mechanical obstruction of the bile ducts, most patients with ECC present with obstructive jaundice. Obstruction may be seen in both distal and proximal tumors. The degree of jaundice is a function of the extent of the mechanical obstruction of the biliary tree. In the setting of bilobar or common hepatic duct (CHD) involvement, the clinical sign of obstructive jaundice may appear early in the disease. Jaundice may not occur if biliary obstruction is not

complete. Abdominal pain, pruritus, weight loss, diarrhea, fever, and anorexia may be present. In the setting of chronic obstruction, tumor may be complicated by an inflammatory or infectious process of the bile ducts.

Laboratories such as alkaline phosphatase (ALP) and aspartate aminotransferase (AST) levels are usually elevated, especially in the case of late disease. Tumor markers such as CEA and CA 19-9 are elevated after the mass has become very large. In patients with PSC and a value of CA19-9 >100 U/mL, there is a sensitivity and specificity of 89 and 86 % for ECC, respectively [51]. The CA19-9 is not specific because a portion of the population cannot synthesize the protein or may be elevated secondary to inflammation rather than tumor [51].

6.3 Imaging

The imaging approach and radiologic interpretation for ECC should focus on providing a comprehensive noninvasive resectability evaluation in order to stratify patients preoperatively into operable and nonoperable categories [49, 52, 53]. The CT imaging protocol tailored to provide accurate information on the following factors: biliary anatomy, location of the biliary obstruction, cause of obstruction, location of the primary tumor within the biliary tree, extension to adjacent organs, vascular anatomy and involvement by the tumor, nodal disease, and the presence of metastases.

6.4 Imaging Protocol and Technique

For proximal and distal tumors, CT is the most commonly used modality for the evaluation and workup of patients with suspected biliary cancer. Technological developments in MSCT have made possible high-speed and high-resolution imaging of the entire abdomen. These capabilities decrease volume-averaging artifacts and significantly lower the chance of motion artifacts that may hinder the detection of small tumors and their true extent along the biliary tree.

The CT evaluation of patients with suspected ECC is based on the pancreas protocol. This protocol consists of a pre-contrast evaluation and two phases after the administration of contrast. The timing of the first phase (pancreas parenchymal phase) is at 20 s after reaching the threshold of 100 HU in the aorta, at the level of the celiac artery. The second phase is 15 s after the first phase. In order to increase tumor conspicuity, rapid intravenous injection (5 cc/s) of 120–150 cc of iodinated contrast is required. Our dual-phase protocol for imaging on a 64-detector row scanner results in a 5 s image acquisition for the entire

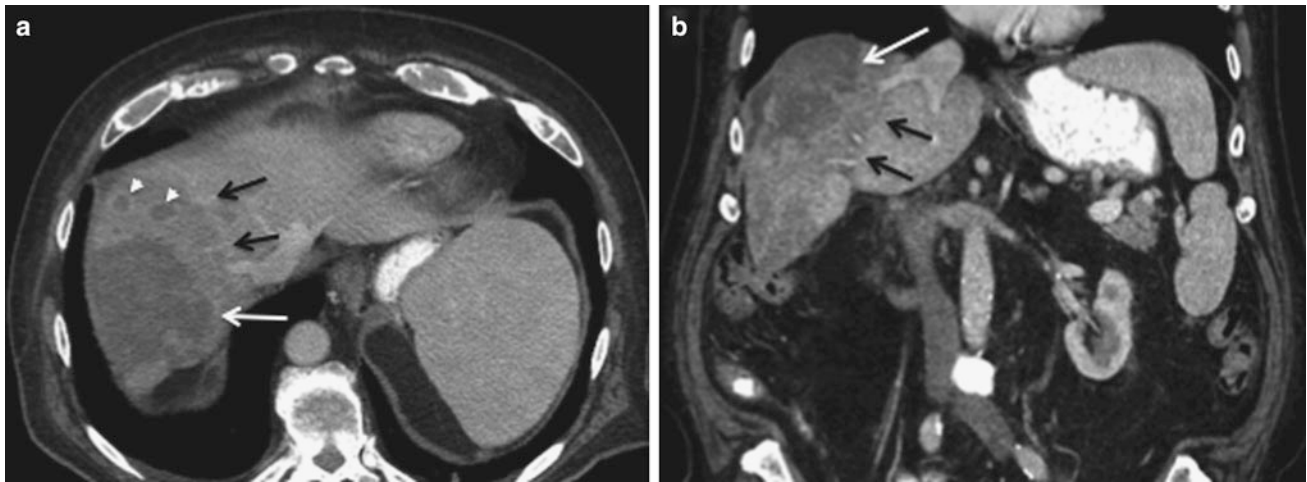


Fig. 40 Radiotherapy changes; 77-year-old male with right liver lobe ICC with satellite nodules after 8 months of radiation. **a** Axial and **b** coronal contrast-enhanced MSCT images show a sharply demarcated low-attenuation area in the right liver lobe (*black arrows*), correlating

with edema secondary to radiation. Large hypodense mass (*white arrow*) and nodular rim-enhancing hypodense lesions (*arrowheads*) in segments VII and VIII of the right liver, representing ICC

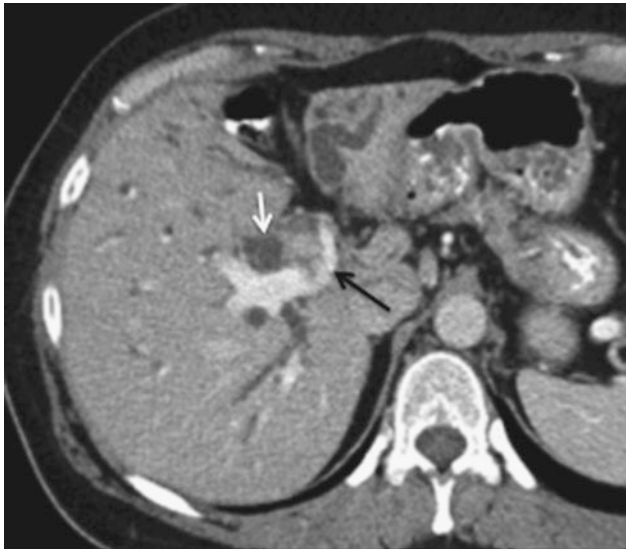


Fig. 41 T4 hilar cholangiocarcinoma; vascular and biliary involvement. Axial MSCT image in portal venous phase shows heterogeneous mass in the hepatic hilum with encasement and invasion of the portal vein (*black arrow*). Bile duct invasion is also appreciated with associated biliary ductal dilation (*white arrow*)

abdomen. Images are acquired with a slice thickness 2.5 mm and then reconstructed for both phases to 0.63 mm for problem-solving and multiplanar 3D reconstructions. Biphasic scanning technique of the entire abdomen during the pancreas parenchymal phase and during the peak of portal venous enhancement (delayed phase) optimizes detection of the primary tumor, visualization of arterial and venous structures for staging, and identification of hepatic metastases.



Fig. 42 Hilar ECC Type I. Axial MSCT image in late arterial phase shows heterogeneous mass in the common hepatic duct within 1 cm of the bifurcation (*white arrow*). There is associated biliary ductal dilation; bile duct to segments VII and VIII, coursing posterior to the portal vein (*black arrow*), anatomical variant

6.5 Normal Anatomy

A thorough understanding of the normal CT anatomy of the hilar fissure of the liver is essential for the correct interpretation [54]. The intrahepatic bile ducts follow portal vein anatomy; they have three main branches: right, left, and

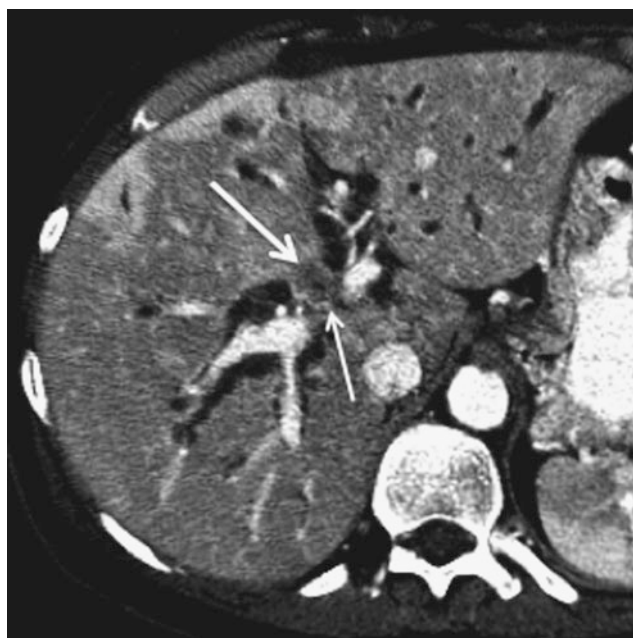


Fig. 43 Hilar ECC Type IV. Axial MSCT image in late arterial phase shows heterogeneous mass involving both the right and left hepatic ducts (arrows). There is associated bilateral intrahepatic biliary ductal dilation

caudate. In the hilar fissure, the left hepatic duct (which drains segments II, III, and IV) joins the right hepatic duct (which drain segments V, VIII, VII, and VI) to form the CHD. Variants of portal vein and bile ducts may be seen. The confluence of the hepatic ducts is commonly located anterior to the origin of the left portal vein. The intrahepatic biliary ducts are not visible on the contrast-enhanced CT unless dilated. The normal right and left hepatic ducts and the CHD may be seen as thin tubular structures of water attenuation and imperceptible walls (<3 mm) anteriorly to the portal vein. The left hepatic duct is usually longer than the right duct, measuring approximately 2–5 cm, while the right duct measures approximately 1 cm.

The CBD (common bile duct) is divided into thirds—the upper third extends from the confluence of the intrahepatic bile ducts to the level of the cystic duct, the middle third is from the level of the cystic duct to the duodenum, and the distal third is to the level of the duodenal ampulla. Proximal and distal ECC are located above and below the cystic duct, respectively. The hepatic ducts and the upper and middle CBDs receive arterial supply from the cystic artery. The middle portion of the duct receives blood supply from the right hepatic and posterosuperior pancreaticoduodenal arcade. The posterior superior pancreaticoduodenal arcade also supplies the distal CBD. The portal vein drains the lower CBD, and the upper portion drains through the liver. This knowledge is essential to evaluate the regional pattern of spread of the tumor.

The portal triad is formed by the combination of hepatic artery, bile duct, and portal vein. These structures are very closely related, which can lead to poor prognosis even in early disease. To provide the best evaluation of radiologic imaging, familiarity with the variant arterial supply to the liver is of great significance. Michel's classification of the hepatic artery describes variants of arterial supply to the liver [55]. The most common variants include a replaced/accessory right hepatic artery from the superior mesenteric artery (SMA) and a replaced/accessory left hepatic artery from the left gastric artery.

7 Staging

7.1 Imaging Findings

The staging for the bile duct tumors is based on the local extension of tumor (T), nodal disease (N), and distant metastases. The seventh edition of the AJCC TNM staging criteria for EHCC divides the tumors into proximal tumors and distal tumors [36].

For the proximal bile duct tumors, the T-staging is based on the thickness of the tumor. In the early stage, the tumor is confined to the bile duct (T1). In T2-staging, the tumor extends to the adipose tissue beyond the wall (T2a) or invades the liver (T2b). Unilateral extension into the portal vein or hepatic artery classifies the tumor as T3. T4 tumors are defined by the extension into the main portal vein and branches, common hepatic artery, contralateral vascular extension, and degree of second biliary radicle involvement (Fig. 41).

The Bismuth and Corlette Classification System is used to stage patients with hilar cholangiocarcinoma, also known as Klatzkin tumor. This system takes into account the location of the primary tumor in relation to the confluence of the right and left ducts and the extent of ductal involvement [56]. According to this system, hilar cholangiocarcinomas are classified into five types. Type I lesions involve only the CHD within 1 cm of the bifurcation (Fig. 42). Type II lesions involve the confluence of the right and left hepatic ducts, without extension into the secondary biliary radicles. Type IIIa lesions involve the right intrahepatic bile ducts, and type IIIb, the left intrahepatic bile ducts. Type IV lesions involve both left and right intrahepatic bile ducts (Fig. 43).

For the distal bile duct tumors, the T-staging is based on tumors confined to the bile duct histologically (T1) or invade beyond the bile duct wall (T2). Tumors that invade adjacent organs without arterial vascular invasion are T3. T4 tumors have involvement of the celiac and superior mesentery arteries. This classification is similar to that for pancreatic tumors.

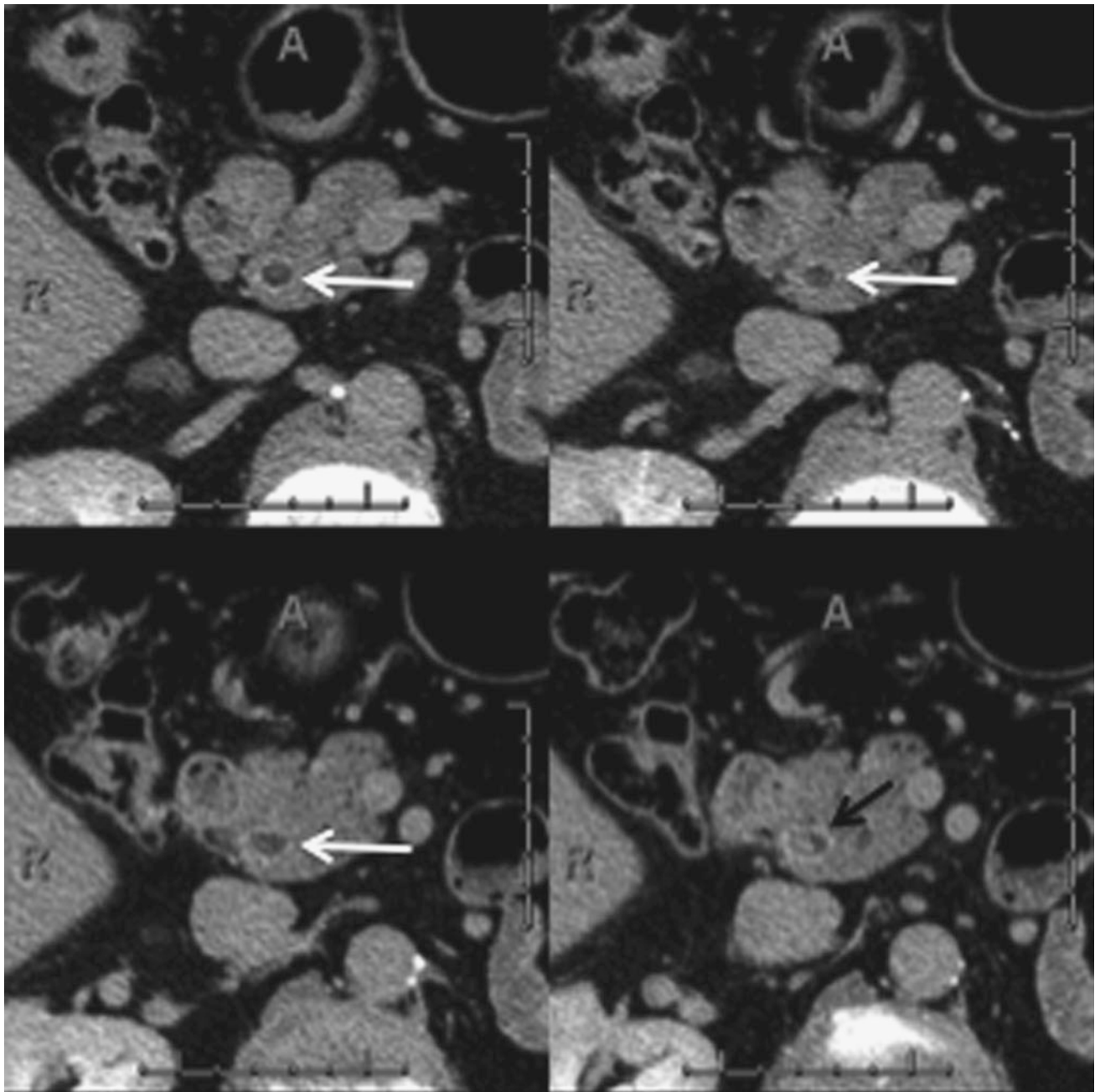


Fig. 44 Periductal-infiltrating ECC. Sequential contrast-enhanced axial MSCT images in delayed phase of a 76-year-old male show circumferential thickening and enhancement of the distal common bile duct (*white arrow*). This is always an abnormal finding and should

raise concerns for tumor involvement in the absence of recent instrumentation or infection. There is soft tissue component protruding into the duct distally (*black arrow*)

7.2 Primary Tumor

The Liver Cancer Study Group described three macroscopic presentations of ECC: periductal-infiltrating (PD) (sclerosing), mass-forming (MF) (nodular), and intraductal (ID) (papillary) types [27]. The PD type causes annular thickening of the bile duct and accounts for 70 % of hilar cholangiocarcinomas (Fig. 44). The MF tumors are solid nodules that project into the lumen (Fig. 45). The ID tumors

have an intraluminal growth pattern (Fig. 46). The most common type is the PD type, with the ID the least common type. The PD type is most commonly seen in the hilar region, whereas the ID type is more commonly seen in the distal bile duct. The ID type has a better outcome owing to its association with low-grade histology.

The PD-type tumors present on contrast-enhanced CT as areas of irregular circumferential thickening and luminal narrowing of the bile duct associated with proximal biliary

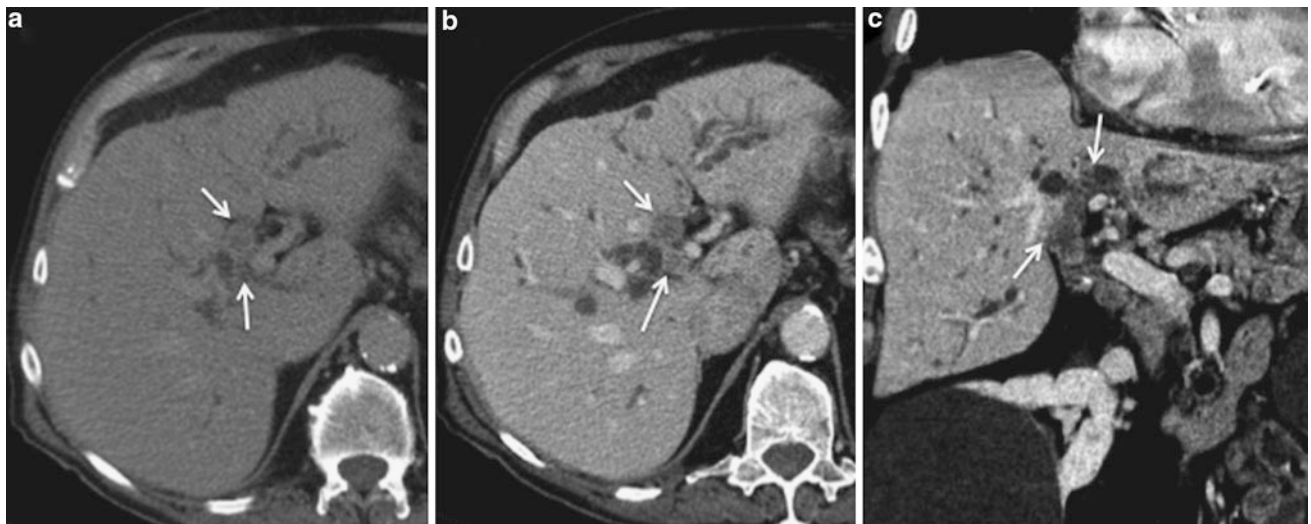
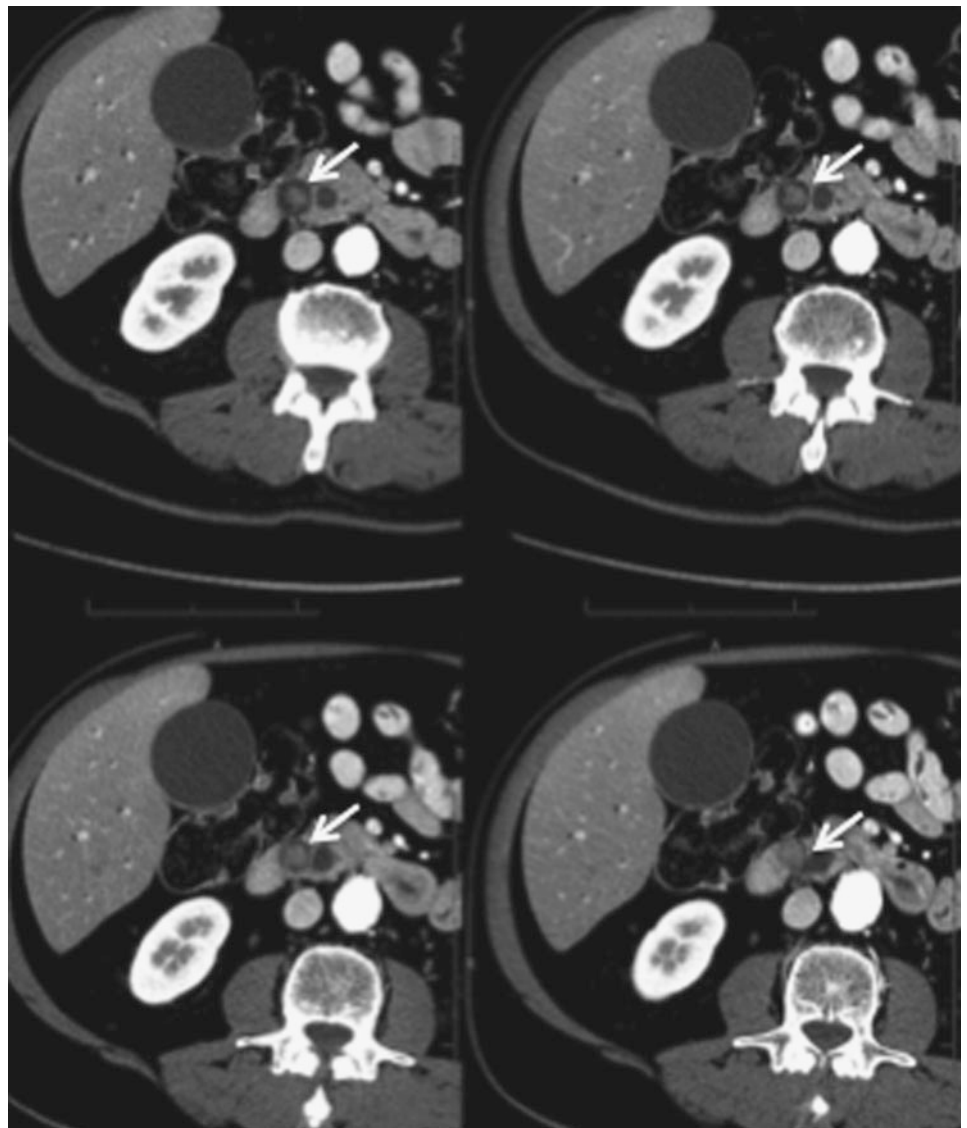


Fig. 45 Mass-forming hilar cholangiocarcinoma. 80-year-old male with jaundice and anorexia. **a** Axial noncontrast, and contrast-enhanced **b** axial and **c** coronal MSCT images show a soft tissue

mass involving both the right and left hepatic ducts (*arrows*), compatible with type IV hilar cholangiocarcinoma. There is associated bilateral intrahepatic biliary ductal dilation

Fig. 46 Intraductal ECC. Sequential contrast-enhanced axial MSCT images show intraluminal enhancing soft tissue density partially filling the lumen of the CBD (*arrows*)



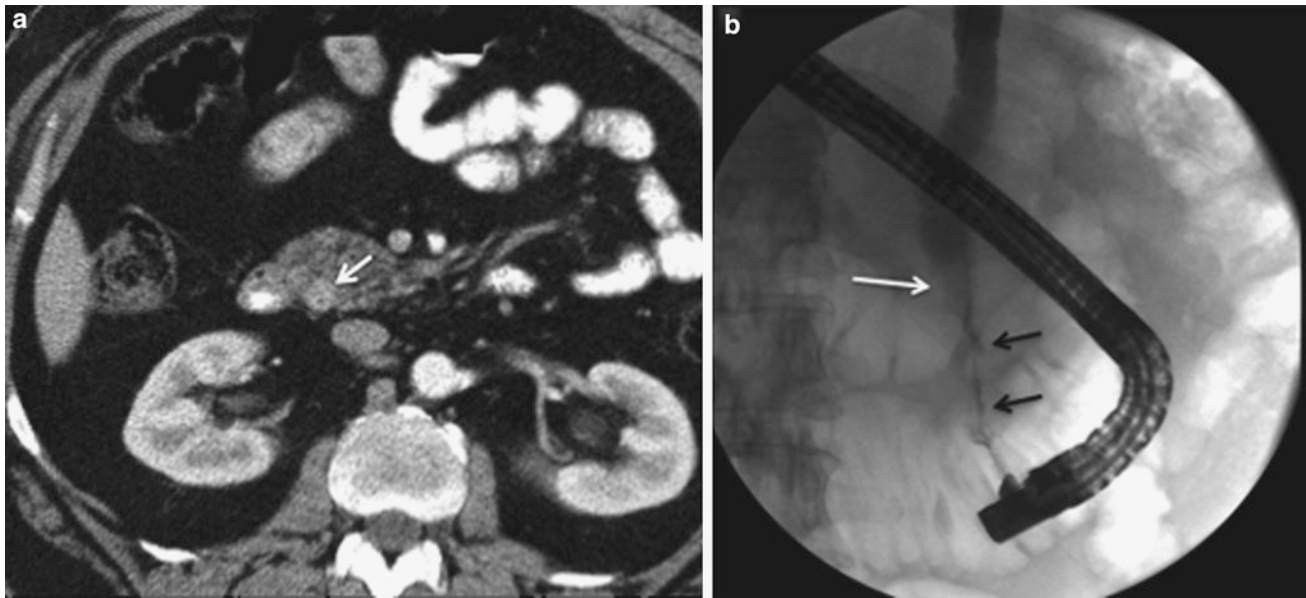


Fig. 47 Periductal-infiltrating ECC. 55-year-old male with jaundice. **a** Contrast-enhanced axial MSCT image shows annular thickening and enhancement (arrow) of the distal CBD, consistent with cholangiocarcinoma. **b** Spot fluoroscopic image of an ERCP shows bile duct

dilation with abrupt change in caliber (white arrow) proximal to a segment with irregular thickening and luminal narrowing (black arrows) in the distal common bile duct. This is commonly seen in periductal-infiltrating cholangiocarcinomas

dilatation (Fig. 47). Bile duct wall enhancement or thickening is always an abnormal finding and is an indication of tumor involvement in the absence of inflammatory disease or recent instrumentation. MF and ID-growing types of ECC present as an enhancing soft tissue mass filling and expanding the lumen of the bile duct (Fig. 45) [57]. The PD cholangiocarcinoma shows a tendency to disseminate submucosally, which underestimates the extent of disease on imaging studies.

The CT imaging features we use to determine tumor location and extension are enhancement of the bile duct wall (Fig. 44), changes of attenuation from water to soft tissue within the bile ducts (Fig. 46), abrupt caliber changes (Fig. 48), and the presence of a mass within the bile duct with proximal dilatation of the biliary tree (Fig. 49).

7.3 Radial Growth and Adjacent Organ Invasion

After the anatomic site of the primary tumor has been determined by imaging, attention needs to be paid to the extent of radial growth and adjacent organ invasion. The PD type of cholangiocarcinoma commonly invades the surrounding periductal fat and has a propensity to spread along the peribiliary nerve plexus and arteries. Soft tissue attenuation with obliteration of the fat surrounding the vessels in the hepatic hilum is a feature of periarterial and perineural spread of cholangiocarcinoma (Fig. 50). It is important to carefully evaluate the ducts and vessels in the hilar fissure for signs of invasion. For example, portal vein involvement

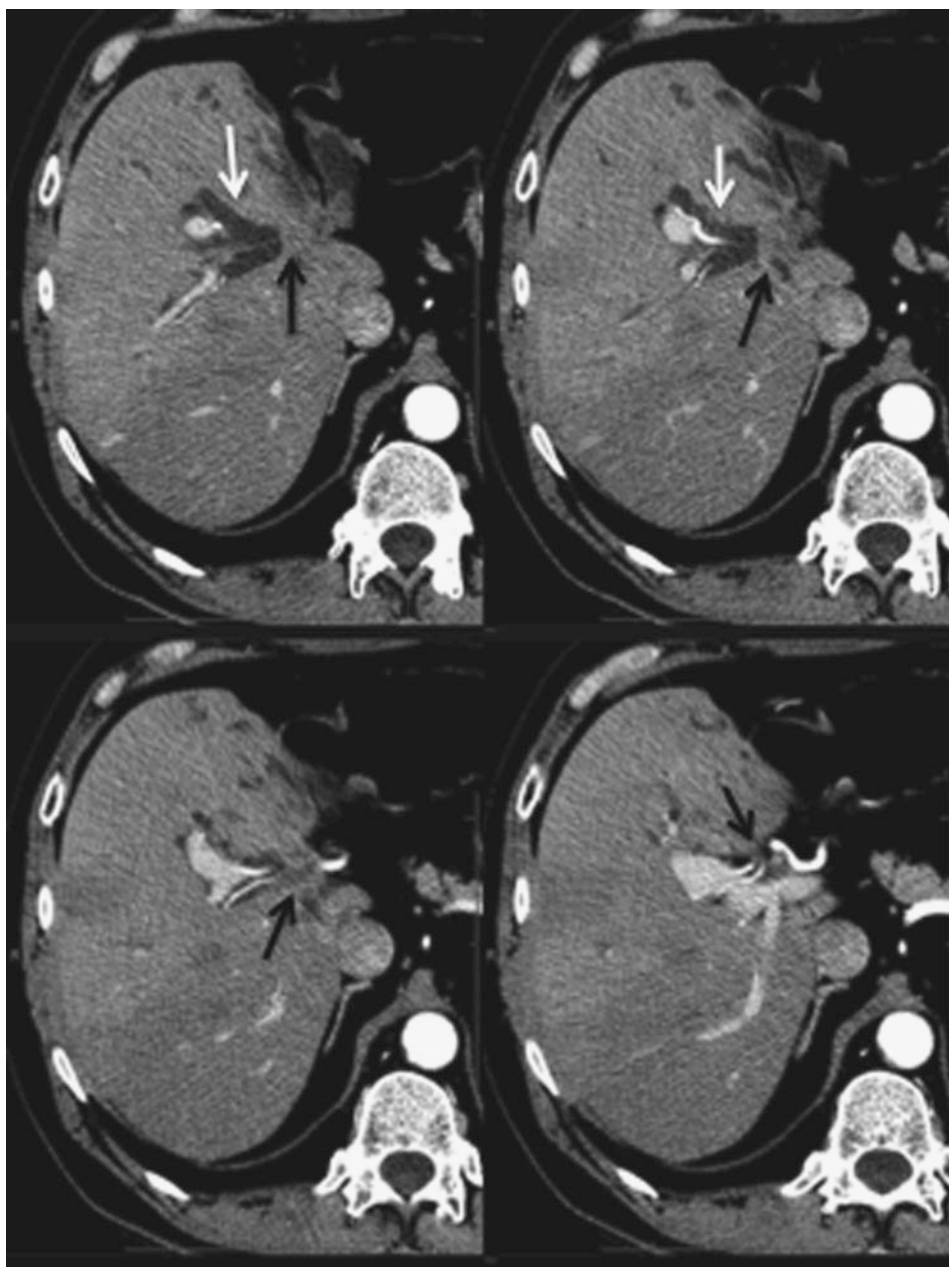
will result in lobar or segmental atrophy [58]. Similar to GB CA, ECC can invade adjacent structures, which is usually readily apparent on CT as direct tumor extension into the hepatic parenchyma, gallbladder, bowel, or pancreas.

7.4 Nodal Disease

Lymphatic spread represents essential information with direct impact in patient management and prognosis [59]. The N1 nodes for the perihilar tumors are nodes in the hilar, hepatoduodenal ligament, cystic, CBD, hepatic artery, and portal vein nodal stations (Fig. 51). N2 nodes are periaortic, pericaval, superior mesenteric, and celiac nodal stations (Fig. 51). The higher the T-staging, the higher the prevalence of nodal spread of disease. The N1 nodes are usually resected with the primary tumor and do not represent a contraindication to surgery. N2 nodes usually represent a contraindication to surgical resection [36, 59] (Fig. 57).

There are only two nodal classifications for the distal tumors, N1 and N0. N1 refers to the regional nodes that include hepatic artery, CBD, celiac, posterior and anterior pancreaticoduodenal, and superior mesenteric nodal stations. Enlarged nodes distant to the primary such as retroperitoneal, para-aortic, and mesenteric nodes should be biopsied prior to surgical planning to exclude the possibility of lymphoproliferative disorders or reactive lymphadenopathy. Similar to the management of cancer of the pancreas head, 12-nodal sampling is required as a minimum during pancreaticoduodenectomy.

Fig. 48 Water and soft tissue attenuation in bile duct with cholangiocarcinoma. Sequential contrast-enhanced axial MSCT of a 63-year-old male shows dilation of bile ducts (*white arrows*). Note abrupt transition from fluid to soft tissue attenuation in the right hilar fissure extending to the main confluence of the ducts (*black arrow*), consistent with hilar cholangiocarcinoma



Imaging features that indicate metastatic involvement of lymph nodes are nodal enlargement (short axis >1 cm) and its internal attenuation. Nodes with lower attenuation are generally necrotic, and necrotic nodes are more likely to harbor metastatic disease, even if not enlarged by CT criteria (other section). MSCT has been proven accurate in predicting nodal metastasis in patients with hilar cholangiocarcinoma with a positive predictive value of 80 %, negative predictive value of 84.4 %, and sensitivity and specificity of 53.3 and 95 %, respectively, yielding an overall accuracy of 83.6 % [59].

7.5 Metastatic Disease

The metastases for the perihilar type are most commonly found in the liver. The peritoneum, lung, brain, and bone are less common sites. The metastases from the distal tumors may be local to the pancreas, duodenum, stomach, colon, or omentum but may also be seen in the liver, lungs, and peritoneum.

Small hepatic and peritoneal metastasis are a well-recognized cause of nonresectability. Benign lesions, such as cysts or hemangiomas, can generally be confidently

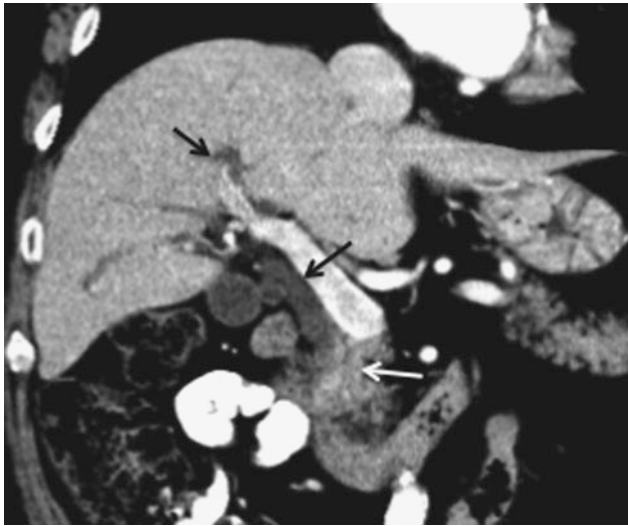


Fig. 49 Periductal-infiltrating cholangiocarcinoma. Coronal contrast-enhanced MSCT image in portal venous phase shows luminal narrowing, circumferential thickening and enhancement of the distal common bile duct (*white arrow*). There is associated proximal biliary ductal dilation (*black arrow*). This type of tumor infiltrates the periductal soft tissues and causes a desmoplastic response that results in significant enhancement

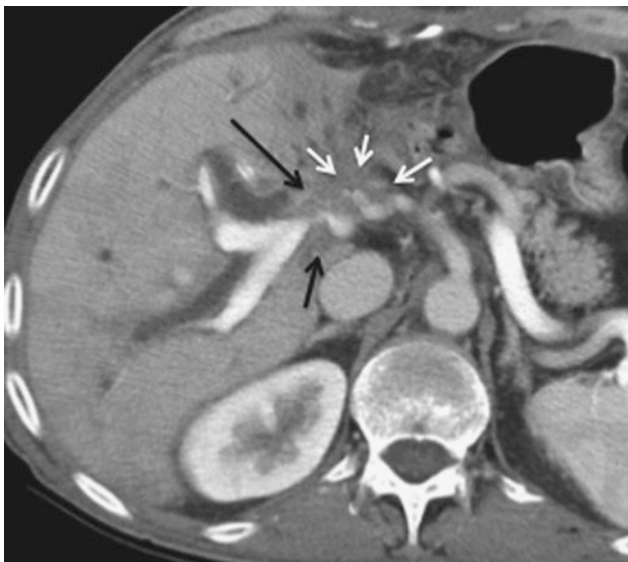


Fig. 50 Periaxial perineural spread in cholangiocarcinoma. Axial MSCT image in portal venous phase shows infiltrating soft tissue encasing the hepatic artery (*white arrows*) in the hilar fissure. Note atrophy of the left liver lobe. There is also encasement and invasion of the main portal vein (*black arrow*). Periaxial perineural spread of tumor may cause angulation, narrowing or occlusion depending on the degree of invasion into the adventitia

differentiated from metastatic lesions larger than 1 cm on thin slice MSCT. Liver metastases generally present as hypoattenuating lesions in relation to the contrast-enhanced hepatic parenchyma and are best appreciated during the portal venous phase of contrast enhancement. Subcentimeter

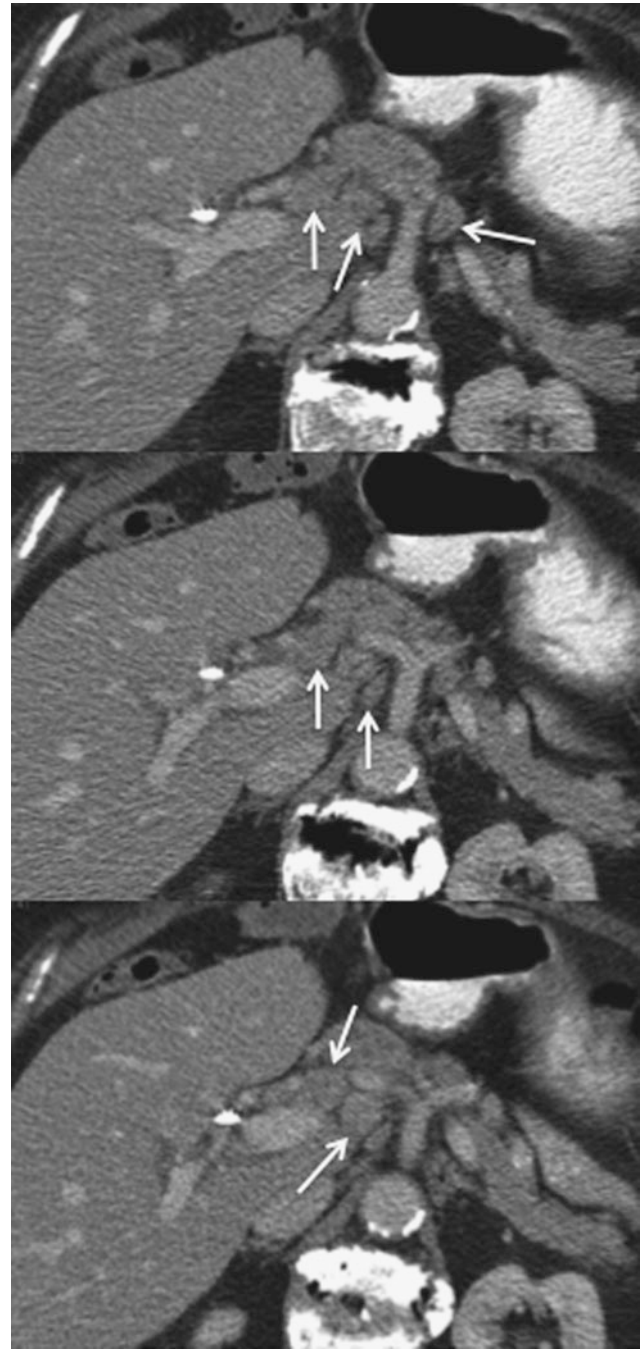


Fig. 51 Lymphangitic spread in ECC. Sequential axial contrast-enhanced images of a patient with cholangiocarcinoma show multiple enlarged lymph nodes along the common hepatic, periportal (*black arrows*), and celiac nodal stations (*white arrows*), consistent with N1 and N2 nodes, respectively

liver metastases remain a diagnostic dilemma since even state-of-the-art MSCT may not be able to accurately characterize these lesions. Peritoneal implants usually present as soft tissue infiltration or discrete nodularity against the normal low-attenuation intraperitoneal fat (Fig. 52). Subtle peritoneal disease is a common source of error in the



Fig. 52 Peritoneal carcinomatosis; 50-year-old male with metastatic cholangiocarcinoma. Axial contrast-enhanced image shows large enhancing soft tissue nodule in the omentum in the right side (*arrow*). There is mild stranding and infiltration of the surrounding fat

imaging analysis of resectability and may result in understaging (Fig. 52). The evaluation of retroperitoneal nodes is essential because this may make the patient a nonsurgical candidate.

7.6 Differential Diagnosis

Several nonneoplastic and neoplastic conditions may mimic ECC on imaging studies. In our opinion, inflammatory conditions of the biliary tree can pose a significant diagnostic challenge. Mirizzi's syndrome is defined as compression of the CHD due to inflammation associated with an impacted stone in the cystic duct or neck of the gallbladder (Fig. 53). In the setting of inflammation, thickening and enhancement of the bile duct wall is commonly seen and can be virtually indistinguishable from biliary cancer. The clinical history and presentation, the presence of gallstones, and the surrounding inflammatory changes may help the radiologist make the appropriate distinction.

Autoimmune pancreatitis is another disease in the differential diagnosis of distal tumors. It is a chronic inflammatory process that affects the pancreas, as well as, the bile ducts. It usually causes segmental, irregular narrowing of the main pancreatic duct, usually accompanied by an extrinsic-appearing stricture of the distal common bile duct. As compared to autoimmune pancreatitis, ECC usually present with shorter strictures and/or a mass, and are more frequently associated with biliary obstruction [60].

Likewise, neoplastic processes, such as lymphoma and plasmacytoma, may occasionally involve the biliary tree and need to be included in the differential diagnosis in the appropriate clinical setting. Lymphoproliferative disorders are also in the differential and may show diffuse

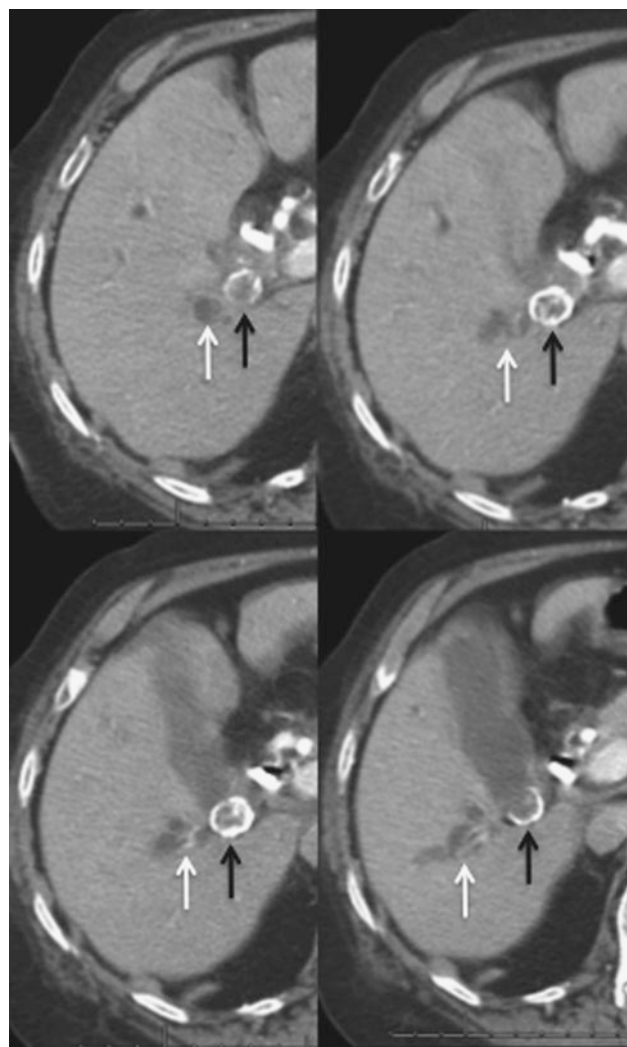


Fig. 53 Mirizzi's syndrome; Sequential contrast-enhanced axial MSCT images show a large rim-calcified stone in the gallbladder neck (*black arrows*) with associated intrahepatic biliary ductal dilation (*white arrows*), representing Mirizzi's syndrome

homogeneous soft tissue thickening and infiltration in the biliary tree.

7.7 Treatment Response and Recurrence

Similar to gallbladder cancer, CT is the imaging modality of choice to monitor patients with ECC after curative surgical resection. Familiarity of the normal postoperative CT appearance following partial hepatectomy with bilioenteric anastomosis is essential for adequate interpretation of the images. A nonenhancing fluid collection at the hepatic resection margin is a normal finding following surgery. This collection should progressively decrease in size on serial follow-up examinations. No soft tissue nodule or solid components should be present. Soft tissue infiltration and

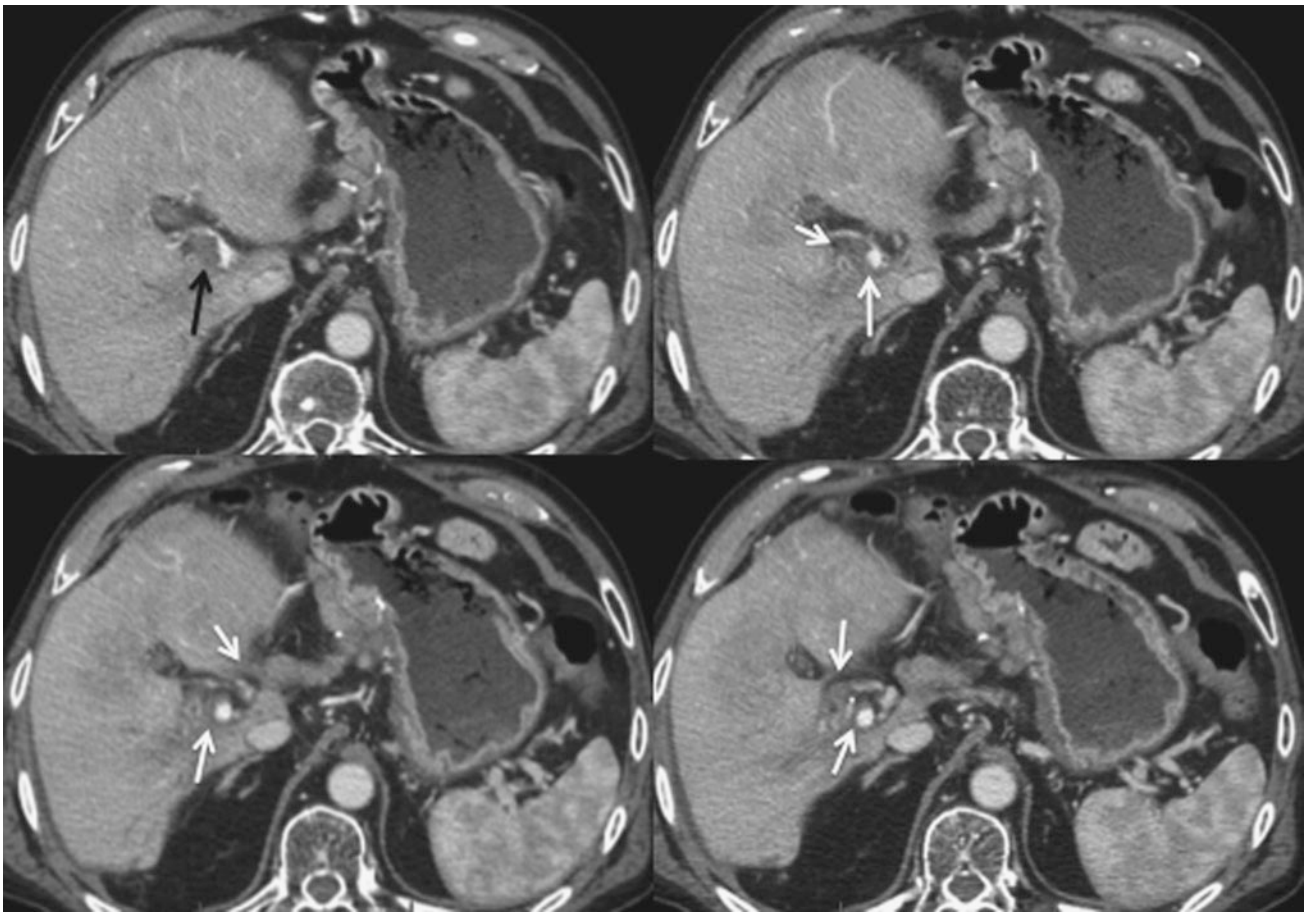


Fig. 54 Recurrent hilar cholangiocarcinoma. Sequential contrast-enhanced axial images of a patient with history of cholangiocarcinoma show soft tissue infiltration surrounding the portal vein and hepatic

artery (white arrows) along the hilar fissure, compatible with recurrent disease. Note also nonenhancing filling defect in the main portal vein (black arrow), compatible with bland thrombus

blurring is commonly noted at the site of choledochojejunostomy or hepaticojejunostomy and does not necessarily indicate recurrent disease. It usually becomes less prominent on serial examinations. In our experience, postradiation fibrosis can be very difficult to differentiate from recurrent disease. The importance of serial imaging with similar technique (collimation, reconstruction interval, field of view, etc.) cannot be overemphasized, since post-treatment changes such as scarring and postradiation fibrosis can masquerade underlying disease and decrease sensitivity of CT for recurrence (Fig. 54, 55). Despite state-of-the-art CT technique, early diagnosis of recurrence in the setting of prominent radiation fibrosis remains a diagnostic dilemma. Findings that indicate recurrent disease on imaging are progressive enhancement and irregular thickening of the remaining bile duct wall, development of a soft tissue mass within the residual ducts, and obstruction of the choledochojejunostomy or hepaticojejunostomy with development of biliary obstruction (Figs. 54, 55).

7.8 Vascular Information

Recognition and adequate description regarding variant vascular anatomy, particularly involvement of the portal vein and hepatic artery, is extremely important for proper staging and surgical planning of hepatobiliary cancers [61]. The presence of vascular anatomy variations or vascular invasion plays major role in determining resectability. The most common vascular variants include replaced right hepatic artery from the SMA (Fig. 56), accessory right hepatic artery (Figs. 57, 58) and common hepatic artery replaced from the SMA, and replaced left hepatic artery from the left gastric artery (Fig. 59). The overall incidence of hepatic branches from the SMA is approximately 20 %. It is critical to make careful evaluation of the hepatic artery. The presence of an accessory or replaced right hepatic artery may change management of a patient with unresectable biliary malignancy due to encasement of the common hepatic artery by tumor. Variation of the portal venous

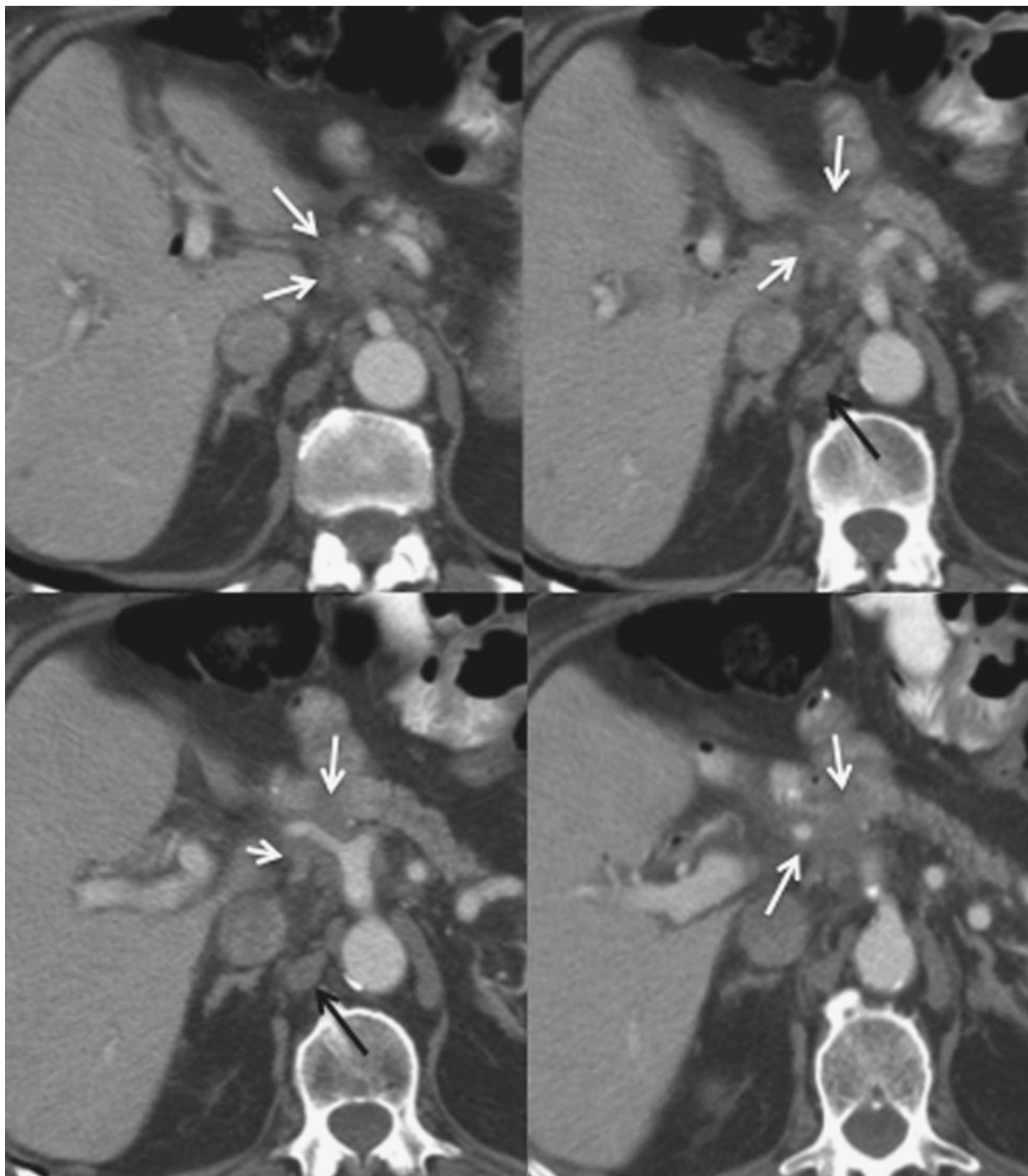


Fig. 55 Recurrent distal cholangiocarcinoma; 78-year-old male patient with history of distal cholangiocarcinoma. Sequential contrast-enhanced axial images show soft tissue infiltration surrounding

the celiac artery and common hepatic artery (*white arrows*), representing recurrent disease. Note lymph node in the retroperitoneal right para-aortic nodal station (*black arrow*)

anatomy is also equally important (Fig. 60). In patients with trifurcation of the main portal vein, the right anterior portal vein arises from the left portal vein (Fig. 60). Resection of the left portal vein proximal to the origin of the right anterior portal vein in such cases would compromise

perfusion to the right liver. MSCT is accurate for the pre-operative vascular evaluation of patients with hepatobiliary neoplasms.

The presence of vascular invasion has a major impact on surgical planning. Encasement or occlusion of the main

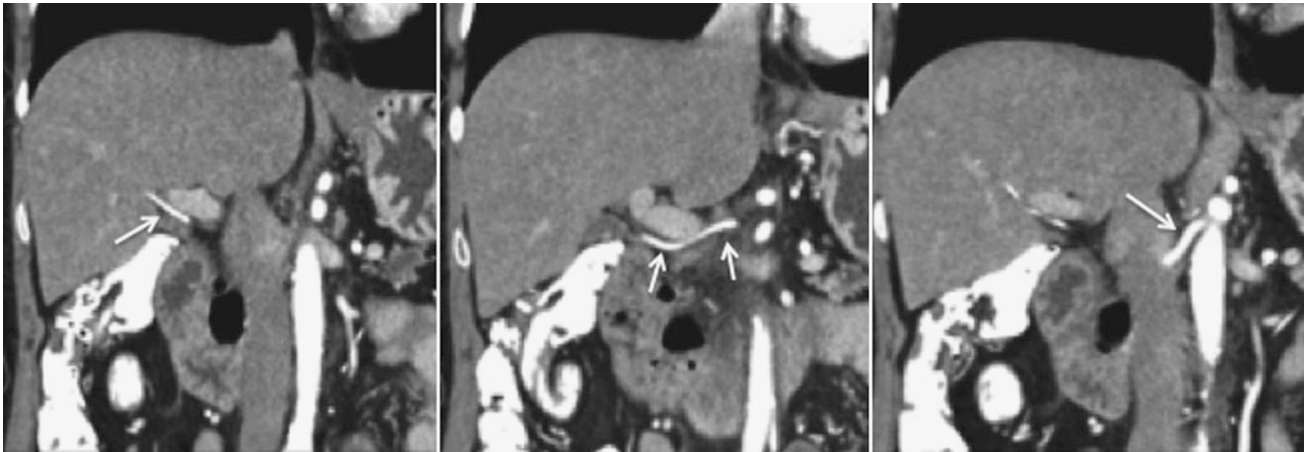


Fig. 56 Variant vascular anatomy; replaced right hepatic artery (RHA). Coronal MSCT contrast-enhanced images of a 70-year-old female show a RHA (arrows) arising from the SMA

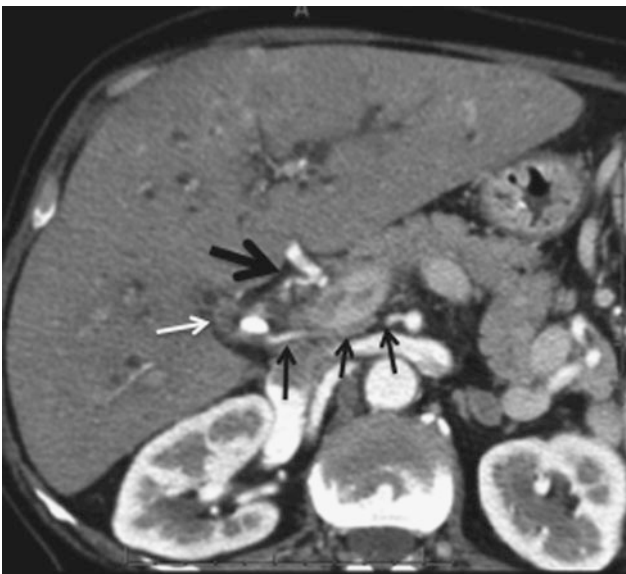


Fig. 57 Variant vascular anatomy; accessory right hepatic artery (RHA) in a patient with hilar cholangiocarcinoma (white arrow). Axial contrast-enhanced MSCT image show a RHA (thin black arrows) arising from the SMA. The main right and left hepatic arteries are also observed (thick black arrow)

portal vein or hepatic artery, or involvement of the portal vein contralateral to the primary tumor, constitutes criteria for irresectability in most institutions. A cholangiocarcinoma in the left duct may extend inferiorly along the hilar fissure and occlude the main or the contralateral portal vein. The imaging criteria commonly used in CT to determine vascular invasion includes occlusion, irregular luminal narrowing, and loss of the fat plane between the tumor and

the vessel wall with tumor encasing more than 180° of vessel circumference.

8 Conclusion

Gallbladder and biliary tract cancers are highly lethal diseases. The prognosis is dismal, and surgical resection remains the only chance for cure. Cross-sectional imaging with MSCT is a valuable technique in the preoperative evaluation of gallbladder and intra- and extrahepatic cholangiocarcinoma. MSCT is capable of providing, in a single study, information on tumor location and depth of hepatic invasion, extent into the biliary tree, adjacent organ invasion, regional lymphadenopathy, peritoneal extension and distant metastases. Angiographic images of the vascular structures in multiple planes can be generated for detection of variant anatomy and vascular invasion. This information constitutes the basis for proper staging and effectively predicting resectability preoperatively.

In our institution, state-of-the-art MSCT is the imaging modality of choice for the staging workup and in monitoring treatment response in patients with gallbladder and biliary tract cancers. Careful attention to the detection of soft tissue nodules at the surgical bed, wound site, and peritoneum is essential for the detection of residual disease. The response to treatment is evaluated with the RECIST and WHO criteria. Nonsurgical approaches utilizing systemic and directed therapies are now available. In addition, newer therapies are being developed for treatment of nonsurgical candidates. Improvements in these and other therapeutic approaches

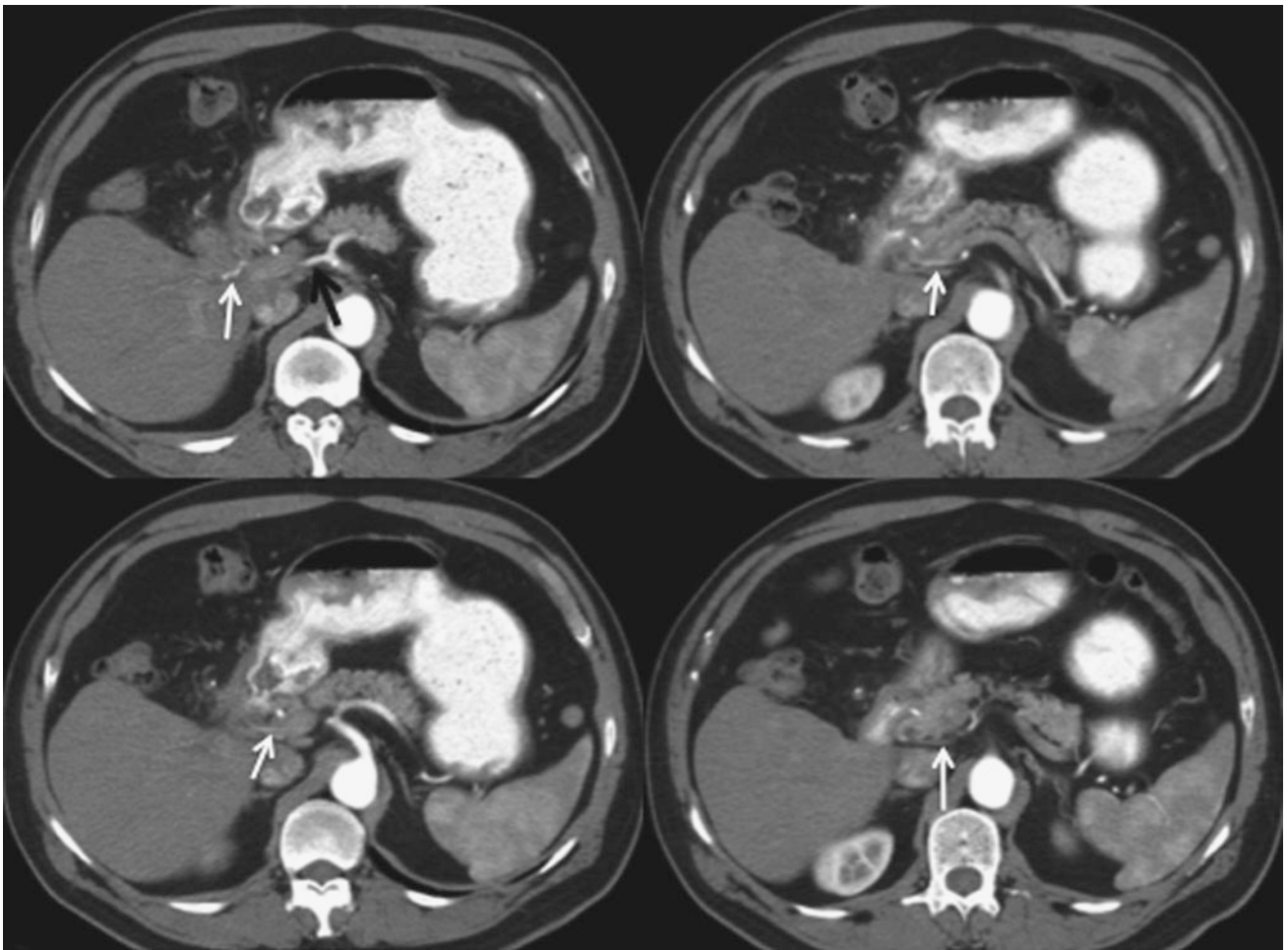


Fig. 58 Variant vascular anatomy. Sequential axial contrast-enhanced MSCT images show an accessory right hepatic artery (*white arrows*) from the common hepatic artery (*black arrow*)

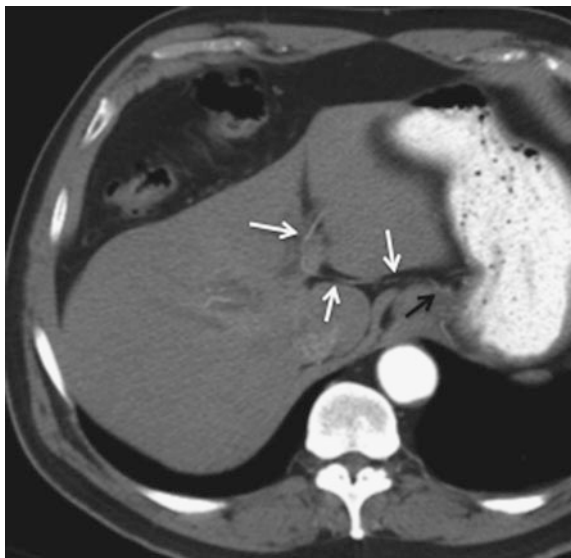


Fig. 59 Variant vascular anatomy; replaced left hepatic artery (HA). Axial contrast-enhanced MSCT image shows the left HA (*arrows*) arising from left gastric artery (*black arrow*) within the gastrohepatic ligament

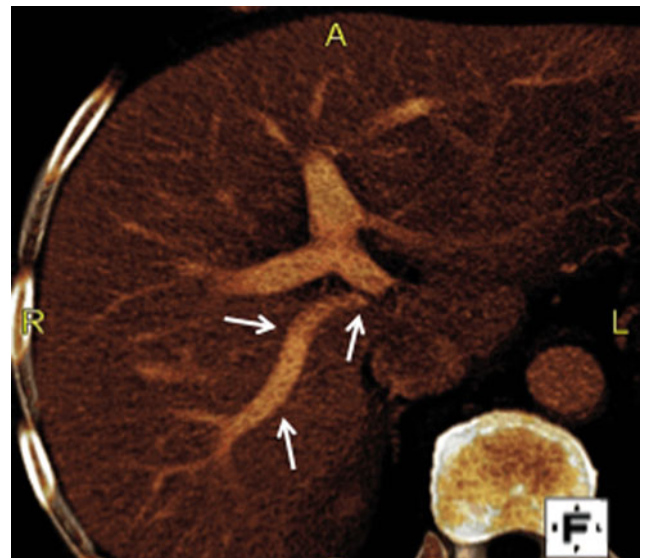


Fig. 60 Variant vascular anatomy. Axial MSCT volume-rendered image shows the portal vein to segments VI and VII of the right liver lobe (*arrows*) arising from the main portal vein

with the goal of downstaging the tumor may permit longer survival or allow patients to receive surgical options that would not have been otherwise indicated.

References

- Greenlee RT, Murray T, Bolden S, Wingo PA (2000) Cancer statistics. *CA Cancer J Clin* 50(1):7–33
- Albores-Saavedra J, Henson DE, Armed Forces Institute of Pathology (U.S.) (1986) Tumors of the gallbladder and extrahepatic bile ducts. Atlas of tumor pathology, vol 2nd ser, fasc 22. Armed Forces Institute of Pathology: Supt. of Docs., U.S. G.P.O. For sale by the Armed Forces Institute of Pathology, Washington, DC
- Rustagi T, Dasanu CA (2012) Risk factors for gallbladder cancer and cholangiocarcinoma: similarities, differences and updates. *J Gastrointest Cancer* 43(2):137–147. doi:10.1007/s12029-011-9284-y
- Goldin RD, Roa JC (2009) Gallbladder cancer: a morphological and molecular update. *Histopathology* 55(2):218–229. doi:10.1111/j.1365-2559.2008.03192.x (HIS3192 [pii])
- Liang JL, Chen MC, Huang HY, Ng SH, Sheen-Chen SM, Liu PP, Kung CT, Ko SF (2009) Gallbladder carcinoma manifesting as acute cholecystitis: clinical and computed tomographic features. *Surgery* 146(5):861–868. doi:10.1016/j.surg.2009.04.037 (S0039-6060(09)00347-X [pii])
- Shukla VK, Gurubachan, Sharma D, Dixit VK, Usha (2006) Diagnostic value of serum CA242, CA 19-9, CA 15-3 and CA 125 in patients with carcinoma of the gallbladder. *Trop Gastroenterol* 27(4):160–165
- Fong Y, Jarnagin W, Blumgart LH (2000) Gallbladder cancer: comparison of patients presenting initially for definitive operation with those presenting after prior noncurative intervention. *Ann Surg* 232(4):557–569
- Lim KS, Peters CC, Kow A, Tan CH (2012) The varying faces of gall bladder carcinoma: pictorial essay. *Acta Radiol* 53(5):494–500. doi:10.1258/ar.2012.110684 (ar.2012.110684 [pii])
- Tongdee R, Marongrope P, Suthikeree W (2011) The value of MDCT scans in differentiation between benign and malignant gallbladder wall thickening. *J Med Assoc Thai* 94(5):592–600
- Kim SJ, Lee JM, Lee JY, Choi JY, Kim SH, Han JK, Choi BI (2008) Accuracy of preoperative T-staging of gallbladder carcinoma using MDCT. *AJR Am J Roentgenol* 190(1):74–80. doi:10.2214/AJR.07.2348 (190/1/74 [pii])
- Coakley FV, Choi PH, Gougoutas CA, Pothuri B, Venkatraman E, Chi D, Bergman A, Hricak H (2002) Peritoneal metastases: detection with spiral CT in patients with ovarian cancer. *Radiology* 223(2):495–499
- Deshmukh SD, Johnson PT, Sheth S, Hruban R, Fishman EK (2012) CT of gallbladder cancer and its mimics: a pattern-based approach. *Abdom Imaging*. doi:10.1007/s00261-012-9907-1
- Bartlett DL, Fong Y, Fortner JG, Brennan MF, Blumgart LH (1996) Long-term results after resection for gallbladder cancer. Implications for staging and management. *Ann Surg* 224(5):639–646
- Noji T, Kondo S, Hirano S, Tanaka E, Suzuki O, Shichinohe T (2008) Computed tomography evaluation of regional lymph node metastases in patients with biliary cancer. *Br J Surg* 95(1):92–96. doi:10.1002/bjs.5920
- Ohtani T, Shirai Y, Tsukada K, Muto T, Hatakeyama K (1996) Spread of gallbladder carcinoma: CT evaluation with pathologic correlation. *Abdom Imaging* 21(3):195–201
- Levin B (1999) Gallbladder carcinoma. *Ann Oncol* 10(Suppl 4):129–130
- Kalra N, Suri S, Gupta R, Natarajan SK, Khandelwal N, Wig JD, Joshi K (2006) MDCT in the staging of gallbladder carcinoma. *AJR Am J Roentgenol* 186(3):758–762. doi:10.2214/AJR.04.1342 (186/3/758 [pii])
- Levy AD, Murakata LA, Abbott RM, Rohrmann CA Jr (2002) From the archives of the AFIP. Benign tumors and tumorlike lesions of the gallbladder and extrahepatic bile ducts: radiologic-pathologic correlation. *Armed Forces Institute of Pathology. Radiographics* 22(2):387–413
- Owen CC, Bilhartz LE (2003) Gallbladder polyps, cholesterosis, adenomyomatosis, and acute acalculous cholecystitis. *Semin Gastrointest Dis* 14(4):178–188
- DeOliveira ML, Cunningham SC, Cameron JL, Kamangar F, Winter JM, Lillemoe KD, Choti MA, Yeo CJ, Schulick RD (2007) Cholangiocarcinoma: thirty-one-year experience with 564 patients at a single institution. *Ann Surg* 245(5):755–762. doi:10.1097/01.sla.0000251366.62632.d3 (00000658-200705000-00013 [pii])
- Razumilava N, Gores GJ (2013) Classification, diagnosis, and management of cholangiocarcinoma. *Clin Gastroenterol Hepatol* 11(1):13–21 e11. doi:10.1016/j.cgh.2012.09.009 (S1542-3565(12)01050-6 [pii])
- Yin X, Zhang BH, Qiu SJ, Ren ZG, Zhou J, Chen XH, Zhou Y, Fan J (2012) Combined hepatocellular carcinoma and cholangiocarcinoma: clinical features, treatment modalities, and prognosis. *Ann Surg Oncol* 19(9):2869–2876. doi:10.1245/s10434-012-2328-0
- Patel T (2001) Increasing incidence and mortality of primary intrahepatic cholangiocarcinoma in the United States. *Hepatology* 33(6):1353–1357. doi:10.1053/jhep.2001.25087 (S0270-9139(01)42325-1 [pii])
- Nakagohri T, Kinoshita T, Konishi M, Takahashi S, Gotohda N (2008) Surgical outcome and prognostic factors in intrahepatic cholangiocarcinoma. *World J Surg* 32(12):2675–2680. doi:10.1007/s00268-008-9778-3
- Welzel TM, Graubard BI, El-Serag HB, Shaib YH, Hsing AW, Davila JA, McGlynn KA (2007) Risk factors for intrahepatic and extrahepatic cholangiocarcinoma in the United States: a population-based case-control study. *Clin Gastroenterol Hepatol* 5(10):1221–1228. doi:10.1016/j.cgh.2007.05.020 (S1542-3565(07)00542-3 [pii])
- Singal AG, Rakoski MO, Salgia R, Pelletier S, Welling TH, Fontana RJ, Lok AS, Marrero JA (2010) The clinical presentation and prognostic factors for intrahepatic and extrahepatic cholangiocarcinoma in a tertiary care centre. *Aliment Pharmacol Ther* 31(6):625–633. doi:10.1111/j.1365-2036.2009.04218.x (APT4218 [pii])
- Yamasaki S (2003) Intrahepatic cholangiocarcinoma: macroscopic type and stage classification. *J Hepatobiliary Pancreat Surg* 10(4):288–291. doi:10.1007/s00534-002-0732-8
- Asayama Y, Yoshimitsu K, Irie H, Tajima T, Nishie A, Hirakawa M, Nakayama T, Kakihara D, Taketomi A, Aishima S, Honda H (2006) Delayed-phase dynamic CT enhancement as a prognostic factor for mass-forming intrahepatic cholangiocarcinoma. *Radiology* 238(1):150–155. doi:10.1148/radiol.2381041765 (2381041765 [pii])
- Blachar A, Federle MP, Brancatelli G (2002) Hepatic capsular retraction: spectrum of benign and malignant etiologies. *Abdom Imaging* 27(6):690–699. doi:10.1007/s00261-001-0094-8
- Chung YE, Kim MJ, Park YN, Choi JY, Pyo JY, Kim YC, Cho HJ, Kim KA, Choi SY (2009) Varying appearances of cholangiocarcinoma: radiologic-pathologic correlation. *Radiographics* 29(3):683–700. doi:10.1148/rg.293085729 (29/3/683 [pii])
- Nanashima A, Abo T, Murakami G, Matsumoto A, Tou K, Takeshita H, Kunizaki M, Hidaka S, Sakamoto I, Hayashi H, Fukuda T, Kudo T, Nagayasu T (2012) Intrahepatic cholangiocarcinoma: relationship between tumor imaging

- enhancement by measuring attenuation and clinicopathologic characteristics. *Abdom Imaging*. doi:[10.1007/s00261-012-9974-3](https://doi.org/10.1007/s00261-012-9974-3)
32. Park HS, Lee JM, Kim SH, Jeong JY, Kim YJ, Lee KH, Choi SH, Han JK, Choi BI (2006) CT Differentiation of cholangiocarcinoma from periductal fibrosis in patients with hepatolithiasis. *AJR Am J Roentgenol* 187(2):445–453. doi:[10.2214/AJR.05.0247](https://doi.org/10.2214/AJR.05.0247) (187/2/445 [pii])
 33. Gulluoglu MG, Ozden I, Poyanli A, Cevikbas U, Ariogul O (2007) Intraductal growth-type mucin-producing peripheral cholangiocarcinoma associated with biliary papillomatosis. *Ann Diagn Pathol* 11(1):34–38. doi:[10.1016/j.anndiagpath.2006.09.003](https://doi.org/10.1016/j.anndiagpath.2006.09.003) (S1092-9134(06)00127-4 [pii])
 34. Lee JW, Han JK, Kim TK, Kim YH, Choi BI, Han MC, Suh KS, Kim SW (2000) CT features of intraductal intrahepatic cholangiocarcinoma. *AJR Am J Roentgenol* 175(3):721–725
 35. Jung AY, Lee JM, Choi SH, Kim SH, Lee JY, Kim SW, Han JK, Choi BI (2006) CT features of an intraductal polypoid mass: differentiation between hepatocellular carcinoma with bile duct tumor invasion and intraductal papillary cholangiocarcinoma. *J Comput Assist Tomogr* 30(2):173–181 (00004728-200603000-00002 [pii])
 36. Edge SB, American Joint Committee on Cancer (2010) *AJCC cancer staging manual*, 7th edn. Springer, New York
 37. Lim JH (2003) Cholangiocarcinoma: morphologic classification according to growth pattern and imaging findings. *AJR Am J Roentgenol* 181(3):819–827
 38. Grobmyer SR, Wang L, Gonen M, Fong Y, Klimstra D, D'Angelica M, DeMatteo RP, Schwartz L, Blumgart LH, Jarnagin WR (2006) Perihilar lymph node assessment in patients undergoing partial hepatectomy for malignancy. *Ann Surg* 244(2):260–264. doi:[10.1097/01.sla.0000217606.59625.9d](https://doi.org/10.1097/01.sla.0000217606.59625.9d) (00000658-200608000-00013 [pii])
 39. Suji T, Hiraoka T, Kanemitsu K, Takamori H, Tanabe D, Tashiro S (2001) Lymphatic spreading pattern of intrahepatic cholangiocarcinoma. *Surgery* 129(4):401–407. doi:[10.1067/msy.2001.111873](https://doi.org/10.1067/msy.2001.111873) (S0039-6060(01)49159-8 [pii])
 40. Shimoda M, Kubota K (2007) Multi-disciplinary treatment for cholangiocellular carcinoma. *World J Gastroenterol* 13(10):1500–1504
 41. Uenishi T, Hirohashi K, Haba T, Wakasa K, Kubo S, Shuto T, Tanaka H, Yamamoto T, Tanaka S, Kinoshita H (2003) Portal thrombosis due to intrahepatic cholangiocarcinoma following successful treatment for hepatocellular carcinoma. *Hepatogastroenterology* 50(52):1140–1142
 42. Suzuki S, Sakaguchi T, Yokoi Y, Okamoto K, Kurachi K, Tsuchiya Y, Okumura T, Konno H, Baba S, Nakamura S (2002) Clinicopathological prognostic factors and impact of surgical treatment of mass-forming intrahepatic cholangiocarcinoma. *World J Surg* 26(6):687–693. doi:[10.1007/s00268-001-0291-1](https://doi.org/10.1007/s00268-001-0291-1)
 43. Ohtsuka M, Ito H, Kimura F, Shimizu H, Togawa A, Yoshidome H, Miyazaki M (2002) Results of surgical treatment for intrahepatic cholangiocarcinoma and clinicopathological factors influencing survival. *Br J Surg* 89(12):1525–1531. doi:[10.1046/j.1365-2168.2002.02268.x](https://doi.org/10.1046/j.1365-2168.2002.02268.x) (2268 [pii])
 44. Choi BI, Lee JM, Han JK (2004) Imaging of intrahepatic and hilar cholangiocarcinoma. *Abdom Imaging* 29(5):548–557. doi:[10.1007/s00261-004-0188-1](https://doi.org/10.1007/s00261-004-0188-1)
 45. Ali SM, Clark CJ, Zaydfudim VM, Que FG, Nagorney DM (2012) Role of major vascular resection in patients with intrahepatic cholangiocarcinoma. *Ann Surg Oncol*. doi:[10.1245/s10434-012-2808-2](https://doi.org/10.1245/s10434-012-2808-2)
 46. Ebata T, Yokoyama Y, Igami T, Sugawara G, Takahashi Y, Nagino M (2012) Portal vein embolization before extended hepatectomy for biliary cancer: current technique and review of 494 consecutive embolizations. *Dig Surg* 29(1):23–29. doi:[10.1159/000335718](https://doi.org/10.1159/000335718) (000335718 [pii])
 47. Rana A, Hong JC (2012) Orthotopic liver transplantation in combination with neoadjuvant therapy: a new paradigm in the treatment of unresectable intrahepatic cholangiocarcinoma. *Curr Opin Gastroenterol* 28(3):258–265. doi:[10.1097/MOG.0b013e32835168db](https://doi.org/10.1097/MOG.0b013e32835168db)
 48. Apisarnthanarak P, Kanchanarat K, Muangsomboon K, Chaiyasoot W, Ieumwananonthachai N, Pattaranutaporn P (2012) CT appearances of post-radiation livers in patients with unresectable cholangiocarcinoma. *J Med Assoc Thai* 95(2):221–228
 49. Ruys AT, van Beem BE, Engelbrecht MR, Bipat S, Stoker J, Van Gulik TM (2012) Radiological staging in patients with hilar cholangiocarcinoma: a systematic review and meta-analysis. *Br J Radiol* 85(1017):1255–1262. doi:[10.1259/bjr/88405305](https://doi.org/10.1259/bjr/88405305) (85/1017/1255 [pii])
 50. Ganesan D, Moron FE, Szklaruk J (2012) Extrahepatic biliary cancer: new staging classification. *World J Radiol* 4(8):345–352. doi:[10.4329/wjr.v4.i8.345](https://doi.org/10.4329/wjr.v4.i8.345)
 51. Patel AH, Harnois DM, Klee GG, LaRusso NF, Gores GJ (2000) The utility of CA 19-9 in the diagnoses of cholangiocarcinoma in patients without primary sclerosing cholangitis. *Am J Gastroenterol* 95(1):204–207. doi:[10.1111/j.1572-0241.2000.01685.x](https://doi.org/10.1111/j.1572-0241.2000.01685.x) (S0002-9270(99)00744-3 [pii])
 52. Izbicki JR, Tsui TY, Bohn BA, Bockhorn M (2012) Surgical strategies in patients with advanced hilar cholangiocarcinoma (Klatskin tumor). *J Gastrointest Surg*. doi:[10.1007/s11605-012-2109-x](https://doi.org/10.1007/s11605-012-2109-x)
 53. Kim HJ, Lee DH, Lim JW, Ko YT (2009) Multidetector computed tomography in the preoperative workup of hilar cholangiocarcinoma. *Acta Radiol* 50(8):845–853. doi:[10.1080/02841850903092366](https://doi.org/10.1080/02841850903092366) (913478021 [pii])
 54. Friesen BR, Gibson RN, Speer T, Vincent JM, Stella D, Collier NA (2011) Lobar and segmental liver atrophy associated with hilar cholangiocarcinoma and the impact of hilar biliary anatomical variants: a pictorial essay. *Insights Imaging* 2(5):525–531. doi:[10.1007/s13244-011-0100-9](https://doi.org/10.1007/s13244-011-0100-9) (100 [pii])
 55. Michels NA (1966) Newer anatomy of the liver and its variant blood supply and collateral circulation. *Am J Surg* 112(3):337–347
 56. Cha JH, Han JK, Kim TK, Kim AY, Park SJ, Choi BI, Suh KS, Kim SW, Han MC (2000) Preoperative evaluation of Klatskin tumor: accuracy of spiral CT in determining vascular invasion as a sign of unresectability. *Abdom Imaging* 25(5):500–507. doi:[10.1107/s002610000081](https://doi.org/10.1107/s002610000081)
 57. Sahani D, Saini S, Pena C, Nichols S, Prasad SR, Hahn PF, Halpern EF, Tanabe KK, Mueller PR (2002) Using multidetector CT for preoperative vascular evaluation of liver neoplasms: technique and results. *AJR Am J Roentgenol* 179(1):53–59
 58. Sugiura T, Nishio H, Nagino M, Senda Y, Ebata T, Yokoyama Y, Igami T, Oda K, Nimura Y (2008) Value of multidetector-row computed tomography in diagnosis of portal vein invasion by perihilar cholangiocarcinoma. *World J Surg* 32(7):1478–1484. doi:[10.1007/s00268-008-9547-3](https://doi.org/10.1007/s00268-008-9547-3)
 59. Noji T, Miyamoto M, Kubota KC, Shinohara T, Ambo Y, Matsuno Y, Kashimura N, Hirano S (2012) Evaluation of extra capsular lymph node involvement in patients with extra-hepatic bile duct cancer. *World J Surg Oncol* 10:106. doi:[10.1186/1477-7819-10-106](https://doi.org/10.1186/1477-7819-10-106) (1477-7819-10-106 [pii])
 60. Maeda E, Akahane M, Yoshioka N, Takao H, Matsuda I, Kamiya K, Hirano K, Tada M, Ohtsu H, Fukushima N, Ohtomo K (2012) Comparison of CT findings of biliary tract changes with autoimmune pancreatitis and extrahepatic bile duct cholangiocarcinoma. *Jpn J Radiol* 30(3):227–234. doi:[10.1007/s11604-011-0035-6](https://doi.org/10.1007/s11604-011-0035-6)
 61. Fukami Y, Ebata T, Yokoyama Y, Igami T, Sugawara G, Takahashi Y, Suzuki K, Nagino M (2012) Diagnostic ability of MDCT to assess right hepatic artery invasion by perihilar cholangiocarcinoma with left-sided predominance. *J Hepatobiliary Pancreat Sci* 19(2):179–186. doi:[10.1007/s00534-011-0413-6](https://doi.org/10.1007/s00534-011-0413-6)

Interventional Radiology Management of Unresectable Intrahepatic Cholangiocarcinoma

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Abstract

Inoperable intrahepatic cholangiocarcinoma (ICC) carries a dismal prognosis. Transarterial therapies have been shown by numerous small- and medium-sized series to prolong survival in these patients well beyond 1 year. Studies of drug-eluting bead transarterial chemoembolization (DEB-TACE) and yttrium-90 transarterial radioembolization (TARE) suggest longer survival may be achieved with these newer transarterial modalities. Research to date suggests that patient factors associated with prolonged survival after transarterial therapy include the absence of cirrhosis or the presence of at most Child A cirrhosis, normal or near normal performance status (Eastern Cooperative Oncology Group, ECOG, 0–1), peripheral tumor morphology, tumor hypervascularity, small tumor size, low tumor grade, low tumor burden, and the absence of portal thrombus. The presence of extrahepatic disease has not been found significantly to impact survival, confirming the high mortality from the primary disease. Several studies have directly compared different transarterial therapies. Several have found that transarterial chemoembolic (TACE) therapy is more effective than transarterial chemoinfusion (TACI); however, no study has been conducted to evaluate whether this difference between TACE and TACI persists in the subpopulation of hypovascular tumors. There is evidence that dual-agent conventional TACE with gemcitabine and cisplatin may be more effective than single-agent TACE. In addition to progress being made with transarterial therapies, early results of percutaneous thermal ablation for selected patients with small-to-moderate-sized unresectable ICC are promising. Three recent studies of patients receiving thermal ablation each reported median overall survival of over 30 months post-treatment. Prospective studies of transarterial and percutaneous ablative therapies are needed.

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1 Introduction

The prognosis for patients diagnosed with intrahepatic cholangiocarcinoma (ICC) remains poor. Surgical resection is the only established curative option for patients with ICC, and only 30 % of those diagnosed with this disease are eligible for resection at the time of diagnosis [1]. With surgery, 5-year survival has been reported at rates ranging from 14 to 40 % [2]. While there has been incremental progress through the years, traditional nonsurgical options of systemic chemotherapy and external beam radiation have yet to significantly alter the course of disease. Over the last decade, transarterial and percutaneous ablative therapies have become the standard of care for unresectable hepatocellular carcinoma (HCC). This has in recent years led to the incorporation of these interventional treatment modalities in the case of those suffering from unresectable ICC. This chapter describes the indications for and general techniques of transarterial therapies for ICC, followed by a summary of the current scientific literature supporting effectiveness and associated complications. Finally, recent studies of ICC successfully treated with percutaneous thermal ablation will be reviewed.

2 Indications for and General Technique of Transarterial Therapy

2.1 Indications and Contraindications

Transarterial embolization (TAE), chemoembolization (TACE), chemoinfusion (TACI), and most recently radioembolization (TARE) are indicated for patients with unresectable ICC that is either isolated to the liver or predominantly localized within the liver and likely to be the patient's principal source of morbidity and mortality.

Contraindications to transarterial therapies can be organized according to the different elements of the procedure being considered: angiography, chemotherapy, embolization, and/or radiation therapy. Absolute contraindications to angiography are few and include, principally, severe anaphylactoid reaction to radiographic contrast media and uncorrectable coagulopathy. Contraindications to the administration of chemotherapy generally include thrombocytopenia ($<50,000$ platelets) or leukopenia (white blood cell count <1000), renal insufficiency (creatinine >2 mg/dL), and severe cardiac or pulmonary disease (e.g., NYHA III or IV congestive heart failure). These chemotherapy contraindications may be considered relative since transarterial therapies largely bypass the systemic circulation.

Most of what is known regarding contraindications for transarterial embolic therapies in ICC is derived from that

which has been established in the treatment for HCC. There are few absolute contraindications to transarterial embolic therapies as a whole, but there are several relative contraindications. Decompensate liver disease (Child-Pugh class C cirrhosis) is generally considered a contraindication to transarterial embolization, since any further deterioration liver function or worsening of portal hypertension brought on by even partial or temporary occlusion of arterial supply can provoke liver failure or a life-threatening complication such as esophageal variceal hemorrhage. The Child-Pugh scoring system has been shown to be a better predictor of survival in HCC patients treated with TACE than the Model for End-stage Liver Disease (MELD) score; however, a MELD score greater than 10 has also been negatively associated with survival after transarterial therapy for HCC [3]. Poor performance status is, likewise, a relative contraindication to embolization. While no exact cutoff has been described, generally an Eastern Cooperative Oncology Group (ECOG) score >2 or Karnofsky index <70 % signals a patient without sufficient hepatic functional or systemic reserve to allow for safe treatment.

There is no individual laboratory value that represents an absolute contraindication to transarterial embolic therapy. Serum total bilirubin >3.0 mg/dL has been described as a contraindication to lobar treatment; however, the degree of hepatic arterial occlusion is largely subject to control by the treating interventional radiologist based on the type, quantity and location of embolic infusion. Many would argue this limit need not apply to segmental or subsegmental embolic treatment, as very little hepatic arterial supply maybe sacrificed in this setting. The constellation of >50 % liver volume replacement by tumor, serum bilirubin >2.0 mg/dL, lactate dehydrogenase >425 mg/dL, and aspartate aminotransferase (AST) >100 IU/L has a strong anecdotal association with post-treatment mortality; however, individual elevations of these laboratory values are of uncertain significance.

The absence of an intact sphincter of Oddi is a relative contraindication that raises significantly the risk of abscess complicating any transarterial embolic intervention. Society of Interventional Radiology (SIR) and Cardiovascular and Interventional Radiology Society of Europe (CIRSE) guidelines recommend that tumor burden generally be less than 50 % of liver volume. Society guidelines also advise that there be antegrade flow in the main portal vein or well-established collaterals; however, some exceptions exist in the form of less embolic therapies, such as may be achieved with certain drug-eluting bead formulations [4, 5].

Portal vein thrombus is also less of a concern with TARE, since the radiomicrospheres, which range from 20 to 60 microns in diameter and are rarely infused in greater than 1 mL volumes, serve as carriers of yttrium-90 radioisotope rather than primarily as agents of arterial occlusion. From

data gathered in treatment for liver tumors with external beam therapy, a 50 Gy whole-liver limit has been established beyond which radiation-induced liver disease (RILD) has been known to occur. For this reason and because some radiomicrospheres will inevitably pass through the hepatic sinusoids and tumor microcirculation into the hepatic veins and the lungs, TARE is always preceded by mapping angiography and test administration of ^{99m}Tc -labeled macroaggregated albumin (MAA) radiotracer and calculation of the fraction of radiopharmaceutical shunted to the lungs. A maximum of 30 Gy administered to the lungs in a single treatment or 50 Gy cumulatively has been established in order to prevent radiation-induced pulmonary fibrosis. In practice, doses to the lungs are routinely well below these thresholds. When large or diffuse liver tumors warrant administration of more radioactivity, doses can usually be reduced as necessary to balance safety to the lungs with need for therapeutic activity in the liver.

Extreme care must be taken during mapping angiography to identify any artery arising from the hepatic circulation providing supply the other organs of the foregut. When these are found, they should be treated with coil embolization. Failure to do so has been associated with severe toxicity in the form of pain and gastrointestinal ulceration which may be refractory to treatment. In the rare case of hepaticocentric collateral arterial anatomy that cannot be corrected or avoided, TARE is absolutely contraindicated.

2.2 Transarterial Technique and Periprocedural Care

2.2.1 Preprocedure Preparation

The plan for transarterial treatment should optimally be established during discussion at an institutional tumor board or other interdisciplinary meeting during which imaging is reviewed and unresectability of the tumor is established. The nature, purpose, risks and alternatives of the planned treatment are explained to the patient by the interventional radiologist during a separate office visit prior to the day of treatment. In addition to setting appropriate patient expectations for treatment, any additional bloodwork, imaging, medical clearance, or anesthesiology assistance can be arranged as necessary at this time. Relevant laboratories include a complete blood count, prothrombin time, basic metabolic panel, liver function tests, and CA 19-9 tumor marker. Dedicated triphasic CT or MRI should generally be acquired within 30 days of the planned intervention to inform the interventionalist of proximal visceral arterial anatomy and ensure appropriate preprocedure staging.

For patients undergoing TARE, mapping angiography with coil occlusion of hepaticocentric collateral arteries prior to the day of treatment is essential. This is generally

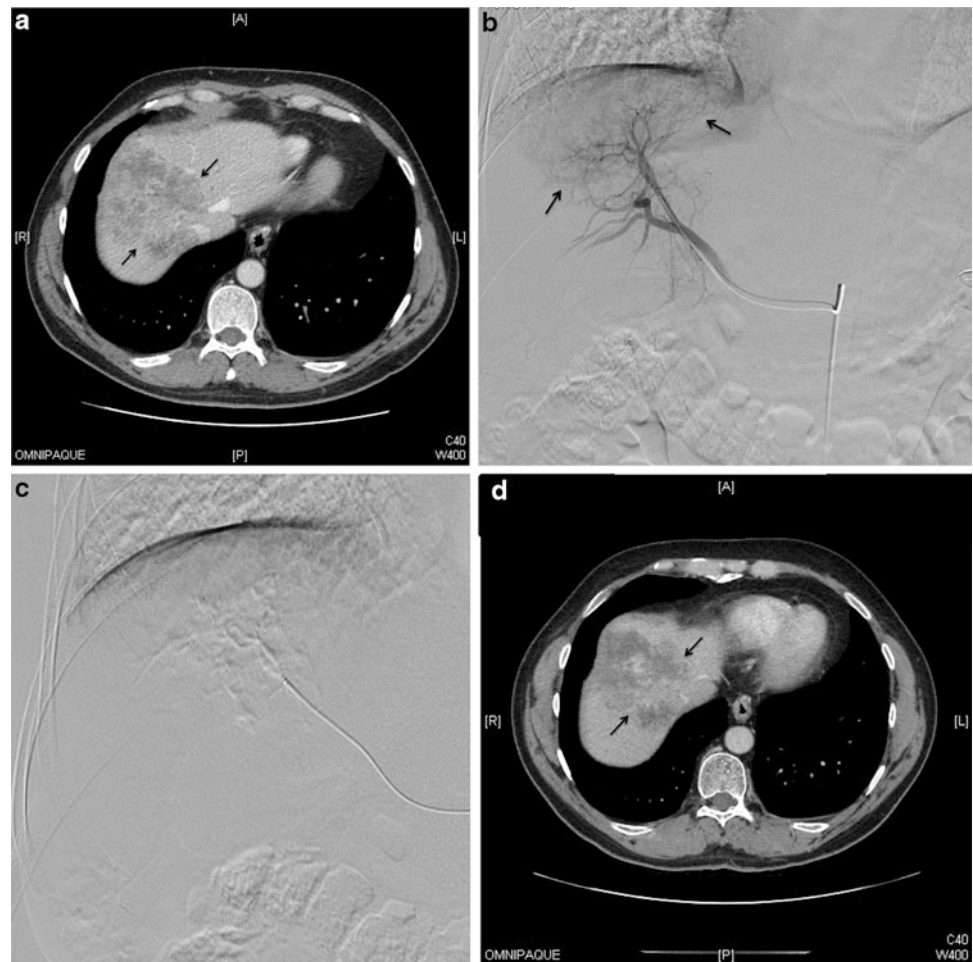
performed as an outpatient procedure during which detailed interrogation of arterial supply to the tumor is identified with conventional and rotational angiography. Multiplanar CT reconstructions from rotational angiograms (also known as C-arm or cone-beam CT) are routinely obtained from rotational angiograms. Review of these images can be tremendously helpful in identifying vessels supplying target tumors or extrahepatic tissues that would be jeopardized by nontarget radioembolization. Parenchymal- and venous-phase images obtained from prolonging conventional arterial angiograms are also helpful in identifying these vessels. Techniques for identifying additional normal and variant hepaticocentric collateral arteries are beyond the scope of this publication, but meticulous technique is required. In patients with native foregut anatomy, coil embolization is routinely performed in the gastroduodenal and right gastric arteries without adverse sequelae. Recently, a microcatheter with a deployable cone tip designed to prevent reflux of microspheres proximal to the site of infusion has been developed (Surefire infusion system; Surefire Medical, Inc., Westminster, CO) and described in the literature with the goal of reducing or avoiding altogether the need for coil embolization during mapping angiography [6]. After skeletonization of the hepatic arterial supply, ^{99m}Tc -MAA radiotracer is injected in the expected site of future TARE, and the patient is routinely evaluated by planar and SPECT-CT for lung shunt calculation and the presence of any extrahepatic deposition of radiopharmaceutical.

Antiplatelet agents, anticoagulation, and insulin are generally held prior to the day of any transarterial procedure. For transarterial therapies, 81 mg aspirin is generally not withheld, and 325 mg aspirin is held or continued at the interventional radiologist's discretion, taking into consideration the patient's cardiovascular risk. Patients are instructed to be NPO except their other routine medications with sips of water for 8 hours prior to the time of the planned procedure. Peripheral venous access is obtained, and intravenous hydration with 150–300 mL/h normal saline is routinely administered unless cardiac or renal function requires fluid restriction, in which case lower rates may be used. Although high-level evidence is lacking, antibiotic prophylaxis to cover skin and enteric flora are generally administered within an hour of procedure commencement. For patients without an intact sphincter of Oddi, bowel preparation beginning the night before the procedure and additional antibiotic prophylaxis for 1–2 weeks may also be beneficial at reducing abscess formation. Anti-emetics, steroids, and proton pump inhibitors may also frequently be administered.

2.2.2 Procedure

Transarterial therapies are performed under moderate sedation with independent radiology nursing supervision for

Fig. 1 49-year-old man with unresectable, liver-dominant intrahepatic ICC. **a** Axial portal-phase post-contrast CT image demonstrating a heterogeneously hypoenhancing mass centered in segments 7 and 8, measuring up to 9.8 cm. **b** Segmental right hepatic artery angiography demonstrating tumor blush and discrete neovascularity despite relative hypovascularity by CT. **c** Follow-up angiography immediately after embolization to segmental vessel stasis with 100 micron Embozene microspheres permanent embolic (CeloNova, Ulm, Germany) demonstrates subtraction artifact from casts of the embolized tumor vessels due to static contrast trapped between microspheres. **d** Follow-up axial portal-phase post-contrast CT image 2 months after embolization demonstrating decreased enhancement and slight decrease in size from 9.8 to 8.4 cm maximally



most patients, including pulse oximetry, cardiac, and blood pressure monitoring. When warranted by a patient's comorbidities, procedures may be performed with light sedation or under deep sedation with anesthesiology assistance. Recently, the wide availability of advanced cross-sectional imaging has allowed the interventionalist to forego aortic and superior mesenteric artery angiography unless arterial pathology or variant anatomy require, thus reducing X-ray exposure and contrast dose at the time of intervention. Focused sonographic examination in the IR suite may also often allow confirmation of portal patency and hepatopetal flow.

Although transradial access for TACE has been described [7], the majority of IR physicians continue to use femoral artery access. After sterile preparation, the common femoral artery is accessed using bony landmarks with or without direct sonographic guidance and a vascular sheath is placed. A reverse-curve 4 or 5 French base catheter is then advanced into the celiac artery, and lobar or segmental hepatic arterial access is obtained with a 3 French or smaller coaxial microcatheter system.

Liver function and hepatic vascular anatomy as well as tumor size, vascularity, and distribution affect the interventional radiologist's decision about where and with what to embolize. Generally, embolic treatments to more than one lobe are staged to decrease risk of liver failure and portal hypertensive complications [8]. More highly embolic treatments tend to be administered on a segmental or sub-segmental level, whereas less embolic treatments may be preferred for lobar administrations in cases of more widely distributed tumor burden. Transarterial lidocaine may be administered immediately prior to embolization and has been shown to decrease pain [9].

A variety of embolic and chemotherapeutic agents are currently in use in transarterial therapy of primary hepatic malignancy. There is no adequately powered prospective trial that demonstrates improved survival for ICC or HCC by adding transarterial chemotherapy to embolization (TACE) versus transarterial embolization (TAE) alone [5]. One of two randomized, controlled trials to demonstrate superiority of TACE over best supportive care (BSC) contained a subgroup treated with transarterial embolization

without chemotherapy (also referred to as bland embolization) (Fig. 1) that had survival similar to those treated with TACE. The trial was stopped when superiority of TACE to BSC was shown, and prior to demonstration of statistical significance in the smaller bland embolization subgroup [10]. As a result of this study and another RCT that validated these findings [11], TACE has become the standard of care for unresectable HCC; however, a meta-analysis of TACE for HCC failed to show superiority of TACE over TAE [12].

There is some evidence that TACE is superior to transarterial chemoinfusion without embolization (TACI) for HCC [13]. More recently, similar data have emerged for the superior efficacy of TACE over TACI in the treatment for ICC as well [14–16]. Results of specific transarterial chemotherapeutic and embolic agents used by different practitioners will be discussed separately in conjunction with individual trials and their results; however, the endpoint of TAE and TACE is generally stasis within the distal small arteries supplying the target tumor(s).

Methodologically, TACE can broadly be divided in two categories: conventional TACE (cTACE) and TACE using drug-eluting bead (DEB-TACE). cTACE uses sterile iodinated poppy seed oil (Lipiodol Ultra-Fluid [previously Ethiodol] Guerbet, Villepinte, France) to create a viscous, radiopaque emulsion with a chemotherapeutic agent or agents that is infused into the tumoral arterial supply. This is usually followed by infusion of temporary or permanent embolic agent, though some practitioners mix the embolic agent with the chemotherapy and Lipiodol and infuse the entire suspension at once. Drug-eDEB-TACE is a modification of TACE in which a single chemotherapeutic agent is bound to the surface of a permanent embolic bead. The beads are mixed suspended in saline and contrast and usually infused without the need for any additional embolic. Once deposited in the tumoral arteries, the chemotherapeutic agent elutes off the beads over a period of several days. Two products are available. LC Beads ([marketed as DC Beads outside the USA], Biocompatibles, BTG, West Conshohocken, USA) are polyvinyl alcohol (PVA) hydrogel microspheres. QuadraSpheres ([HepaSpheres outside the USA], Merit Medical, South Jordan, USA) are hydrophilic microspheres consisting of sodium acrylate alcohol polymer that functions in similar fashion to LC Beads.

TARE, also known as selective internal radiotherapy (SIRT) or radiomicrobrachytherapy (RMB), makes use of neoplastic arterial supply to deposit small glass or starch-resin microspheres within the target tumor(s) that emit beta radiation from directly within the malignancy. Two devices are marketed in North America with which to perform TARE. TheraSphere (^{90}Y microspheres; MDS Nordion, Ottawa, ON, Canada) is composed of nonbiodegradable glass microspheres with ^{90}Y as an integral constituent.

TheraSphere range from 20 to 30 microns diameter and have a specific gravity of 3.6 g/dL and a specific activity of 2500 Bq/sphere. A 3-GBq vial contains 1.2×10^6 microspheres (TheraSphere package insert, MDS Nordion, Kanata, Canada). They were FDA approved in 1999 with a humanitarian device exemption (HDE) for treatment for unresectable HCC. Approval and oversight by an institutional review board is required to administer TheraSphere. Specific doses may be infused by ordering a predetermined dose-vial and coordinating the day and time of administration with published decay curves. More recently, custom dose vials have allowed greater flexibility in timing of treatment.

SIR-Spheres (Sirtex Medical, Lane Cove, Australia) are resin microspheres onto which ^{90}Y is bound. They range from 20 to 60 microns diameter and have a specific gravity of 1.6 g/dL and a specific activity of 50 Bq/sphere. A 3-GBq vial contains $40\text{--}80 \times 10^6$ microspheres (SIR-Spheres package insert). SIR-Spheres received premarket FDA approval for treatment for hepatic metastases from colorectal cancer. Their use does not require IRB oversight, but use in any other capacity is off-label. SIR-Spheres arrive in a standard dose vial on the day of treatment. The receiving institution's radiopharmacist decants an appropriate volume of spheres to achieve the prescribed dose for treatment.

Characteristics of ^{90}Y that facilitates its use in TARE are common to both devices. ^{90}Y is a pure beta emitter that decays to stable ^{90}Zr with a half-life of 64.1 days. The average energy of beta emission is 0.9367 MeV, with a mean and maximum soft tissue penetration of 2.5 and 10 mm, respectively. One GBq (27 mCi) of ^{90}Y per kg of tissue provides a dose of 50 Gy. Doses over 80 Gy are generally considered tumoricidal. TARE takes advantage of the fact that even tumors which appear hypovascular to liver by contrast CT or MRI typically recruit additional arterial supply. There is, therefore, shunting of hepatic arterial flow toward tumors and preferential deposition of radiomicrospheres within the tumors and away from benign liver tissue. Figure 2 depicts treatment of and follow-up imaging for a patient with a partially cystic mixed hepatocellular–intrahepatic cholangiocarcinoma.

2.2.3 Post-procedure Care

After transarterial therapy, intravenous hydration, pain control, and anti-emetics are continued as needed. Some authors recommend continuing antibiotic coverage for gram-negative enteric organism in a 3–7 day course, although data for this practice are lacking [17]. A notable exception is patients lacking an intact Sphincter of Oddi. For these patients, continuation of antibiotic prophylaxis for 7–14 days post-embolization has been advocated [18]. Depending on the degree and distribution of embolization, symptoms typical of post-embolization syndrome (i.e., pain, nausea, and fatigue)

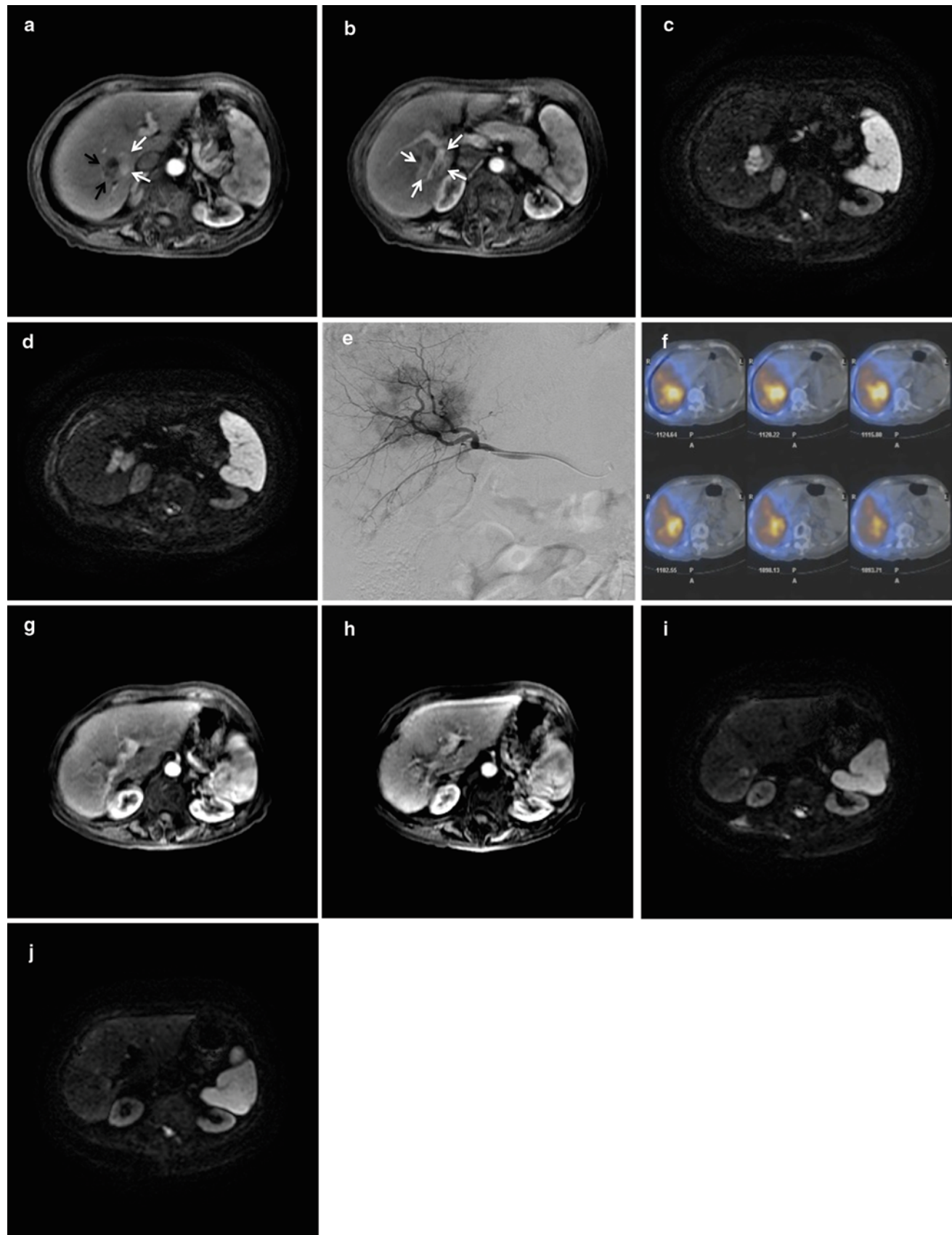


Fig. 2 79-year-old woman with chronic hepatitis B infection and unresectable, biopsy-proven mixed hepatocellular-cholangiocarcinoma (HCC-ICC) with progression of disease on systemic chemotherapy. **a** Axial arterial-phase post-contrast MR image demonstrates a mixed cystic (*black arrows*) and solid (*white arrows*) lesion on the margin of segments 6 and 7 corresponding to biopsy-proven lesion. **b** More inferiorly, lesion is more completely solid (*white arrows*) and surrounds the posterior right portal vein. **c** pre-TARE diffusion weighted image (DWI) demonstrates markedly restricted diffusion in cystic component of mixed HCC-ICC and moderate to markedly restricted diffusion in the more medial, solid portion of the lesion. **d** pre-TARE DWI just inferior to prior image shows restricted diffusion corresponding to the HCC-ICC lesion on either side of the posterior right portal vein branch. **e** Microcatheter angiography performed via the right hepatic artery demonstrates heterogeneous tumor blush corresponding to the known partly cystic segment 6/7 tumor. **f** Axial fused SPECT-CT images from Bremsstrahlung scan

immediately after transarterial radioembolization with a delivered activity of 18.6 mCi (0.69 GBq) of ^{90}Y -resin microspheres infused via the right hepatic artery: white and yellow represent areas of greatest deposition of microspheres, essentially all within the target HCC-ICC, gray is least deposition of microspheres, and light blue is blooming artifact from activity within the right liver. **g** and **h** arterial-phase axial images through the superior and inferior aspects of lesion 1 year after TARE demonstrate near complete resolution of enhancement (EASL/mRECIST complete response). **i** and **j** superior and inferior DWI 1 year after TARE demonstrates a small focus of restricted diffusion corresponding to residual cystic component of lesion. No restricted diffusion is demonstrated corresponding to any residual solid tumor. Findings in figures **g–j** are compatible with RECIST partial response and mRECIST/EASL complete response. The patient was alive and asymptomatic at the time of this publication, 12 months after TARE treatment

may require hospitalization for one or more days. In cases of highly selective embolization, patients may often be discharged on the day of treatment after ambulation criteria related to arterial puncture have been met.

2.3 Follow-up

Patients are typically followed with bloodwork and office visits 2 weeks after each treatment, although some practitioners defer office follow-up for 1 month. As described in SIR and CIRSE guidelines, triphasic pre- and post-contrast CT or pre- and dynamic post-contrast MRI should be obtained between 4 and 6 weeks after transarterial therapy and then at 3-month intervals thereafter [4, 5]. Transarterial therapies are typically repeated as warranted by imaging assessment and as long as tolerated by the patient's clinical and laboratory evidence of functional status.

3 Results

3.1 Conventional TACE and Transarterial Chemoinfusion

Table 1 summarizes results of the TACI and cTACE investigations described here, including also demographic data. Originally described in 1980 for the treatment for HCC, TACE takes advantage of the dual blood supply to benign hepatocytes (from both hepatic artery and portal vein) and the preferential recruitment of arterial neovascularity by HCC tumors to provide more effective treatment for liver tumors with fewer side effects than would be expected from systemic chemotherapy. Shown by two separate randomized, controlled trials in 2002 to improve survival over best supportive care in patients with unresectable HCC, TACE is now considered standard of care for that patient population [10, 11].

Transarterial therapy for ICC was initially reported in 2002 by Tanaka et al. using an implanted subcutaneous port attached to a microcatheter infusing in the common or proper hepatic artery, as had previously been done for HCC. With TACI, no embolic material was administered. Fluorouracil was infused periodically via the port-catheter system with or without doxorubicin, epirubicin, or mitomycin C. Tumor response by follow-up imaging was made using modified WHO criteria: complete response (CR) was disappearance of tumors, partial response (PR) was 50 % or greater reduction in maximum tumor diameter, minor response (MR) was 25–50 % diameter reduction, stable disease (SD) was less than a 25 % change in tumor size, and progressive disease (PD) was 25 % or greater increase in tumor size. Five of 11 patients (45 %) experienced PR, 2/11 (18 %) had MR, 2/11 (18 %) had SD, and 2/11 (18 %) had PD. There has been criticism that the authors did not censor one patient downstaged to resection and reported mean survival after treatment initiation of 26.0 months instead of median survival, which would arguably be lower due to the downstaged patient. Nevertheless, the paper by Tanaka et al. proved the principle of transarterial therapy for ICC [19].

The first case series of TACE therapy for unresectable ICC was described by Burger et al. in 2005 in a retrospective report of 17 patients treated with one or more sessions of triple-agent chemotherapy (100 mg cisplatin, 50 mg doxorubicin hydrochloride, and 10 mg mitomycin C) emulsified with Ethiodol and followed by 300–500 micron-diameter tris-acryl gelatin microsphere embolization (Embospheres, Biosphere Medical, Rockland, MA). In keeping with modern TACE technique, the microcatheter used to administer treatment was removed at the end of each treatment session. Three patients could not be followed with MRI. Eight of 14 (57 %) patients who did receive contrast-enhanced MR exams showed >75 % tumor necrosis, and 3/14 (21 %) patients showed 25–50 % necrosis. The authors did not comment on baseline degree of tumor vascularity, and

Table 1 Results of the TACI and cTACE investigations

Primary author	Year	Study type	Type of treatment	Number of patients	Mean or median # of Rx sessions	Noncirrhotic or CP A	ECOG PS 0	ECOG PS 1	ECOG PS 2+	Prior chemotherapy (%)	Prior external beam radiotherapy (%)	Prior thermal ablation (%)
Tanaka	2002	CS	TACI: fluorouracil ± doxorubicin, epirubicin or mitomycin C	11								
Burger	2005	CS	cTACE: cisplatin, doxorubicin and mitomycin C	17	1.8	88	12	71	88	35	12	
Vogl	2006	CS	TACI: gencitabine	12	6		25	75	0	100	8	
			cTACE: gencitabine + starch microspheres	12	9.2		17	83	0	100	8	
Herber	2007	CS	cTACE: mitomycin C + Lipiodol	15	3.9	93			93	27		7, 7 ^{\$}
Gusani	2008	CS	cTACE: gencitabine, cisplatin, oxaliplatin or gem-cis combo; all w/TAG microspheres	42	3.5							
Kim	2008	CS	cisplatin TACI (n = 13) or cisplatin, Lipiodol and gelfoam cTACE (n = 36)	49	3	82				4 ^{\$\$}	33 ^{\$\$}	
Park	2011	CC	cTACE: cisplatin, Lipiodol and gelfoam	72	2.5		65	32	3	0	0	0
			best supportive care	83								
Kiefer	2011	CS	cTACE: cisplatin, doxorubicin and mitomycin C with PVA spheres	62	2.7		54	41	5	0	0	0
Shen	2011	CC	fluorouracil or carboplatin, epirubicin and hydroxycamptothecin TACI ± Lipiodol cTACE	53		60	89	10	1	29	3	5
			no transarterial therapy	72		61						
Vogl	2012	CS	cTACE: mito C, gem, mito-gem, or mito-gem-cis, + Lipiodol and starch spheres	115	7.1	46						

(continued)

Table 1 (continued)

Primary author	Prior surgical resection (%)	Mean or median tumor size (cm)	Single tumor (%)	Peripheral morphology (%)	Unilobar disease (%)	Extrahepatic (%)	Portal vein thrombus (%)	Hypervascular tumor (%)	Treatment response imaging criteria	RECIST or WHO imaging assessment of response to treatment at 3 months or earliest post-treatment interval (% CR/PR/(MR)/SD/PD)	Median OS (months)
Tanaka	9 ^{\$}	6.7	55			36			mWHO	0/45/18(18MR)/18	26* **
Burger	12 ^{\$}	7.4	53		36	24			% tumor necrosis	57 % > 75 % necrosis, 21 % > 25 % necrosis	23***
Vogl	67				49	67			WHO	0/0/75/25	13.5* *** #
	50								WHO	0/0/92/8	20.2* *** #
Herber	7	10.8	53		40		27	87	RECIST	0/7/60/27	16.3
Gusani		9.8		12		45			RECIST	0/0/57/43	9.1
Kim		8.9	29	90		51		73	RECIST	0/20/35(tumor necrosis)/3/1/14	10
Park	0	8.1	43		49	54		18 ^{##}	RECIST	0/23/67/11	12.2
	0	7.8	53		51	60		12 ^{##}			3.3
Kiefer	11								RECIST	0/11/64/24	15 ^{###}
Shen	100, 4 ^{\$}		63	92		6					12
	100, 4 ^{\$}		68	83		10					5
Vogl			30		23	0	0	54	RECIST	0/9/57/33	13

CP Child-Pugh classification of severity of cirrhosis A–C, ECOG PS Eastern Cooperative Oncology Group performance status 0–4, RECIST Response Evaluation Criteria in Solid Tumors (National Cancer Institute), WHO World Health Organization, CR complete response, PR partial response, MR minor response (WHO criteria only), SD stable disease, PD progression of disease, OS overall survival, TACI transarterial chemoinfusion, cTACE conventional transarterial chemoembolization (chemotherapy in K Lipiodol, ± gelfoam or permanent embolic), TARE transarterial radioembolization, DEB-TACE drug-eluting bead TACE, RFA radiofrequency ablation, MWA microwave ablation; *gem-cis* gemcitabine + cisplatin, CS case series, CC case/control, RCS retrospective analysis of prospectively gathered case series data, TAG tris-acryl gelatin microspheres, PVA polyvinyl alcohol microspheres

* Mean OS reported, not median

** Resections after transarterial therapy not censored from survival data

*** OS reported from time of diagnosis, not first treatment

\$ Subsequent to transarterial therapy

\$\$ Concurrent with transarterial therapy

Study combined patients with ICC and hepatic metastases from pancreatic carcinoma

Authors defined hypervascularity by diagnostic CT or MR, not by angiography as is more typical in this literature

Study combined patients with ICC and intrahepatic adenocarcinoma of unknown primary

European Association for the Study of the Liver (EASL) and modified Response Evaluation Criteria in Solid Tumors (mRECIST) systems were not explicitly used; however, these results would appear to correlate roughly with between 57 and 78 % complete response rates by these modern criteria. Median survival was 23 months from diagnosis. Two patients were downstaged to resection and were censored from the survival data. The authors did not separately report median survival from time of first TACE treatment [20].

Vogl et al. recently published a series of 24 patients with either ICC or hepatic metastases of pancreatic adenocarcinoma treated with a dose-escalation protocol using gemcitabine as either TACI or TACE with a mean of 9 treatment sessions. TACE was performed with EmboCept degradable starch microspheres (PharmaCept GmbH, Berlin, Germany). The transarterial catheters were removed after each treatment session. Nine of 12 (75 %) TACI patients and 8/12 (67 %) TACE patients had ICC. In their study, WHO criteria were used to gauge imaging response to treatment. As is often the case after transarterial therapy, there were no complete or partial responses (disappearance of lesions or significant decrease in product of orthogonal tumor diameters, respectively) in either the TACE or TACI group. Nine of 12 (75 %) patients receiving TACI had SD, while 3/12 (25 %) had progression. In the TACE group, there were 11/12 (92 %) with SD and 1/12 (8 %) with progression. The mean time to progression was 4.2 months and 6.8 months in the TACI and TACE groups, respectively ($p < 0.01$). Mean survival from time of diagnosis was 13.5 months and 20.2 months in the TACI and TACE groups, respectively ($p < 0.01$) [14].

Herber et al. presented a series of 15 patients with unresectable ICC treated with a mean of 3.9 TACE treatments using mitomycin C in Lipiodol without particle embolization. RECIST criteria were assessed after three treatments: 1 patient had partial response, 9 patients had stable disease, and 5 progressed. Mean and median survival were 21.1 months and 16.3 months after first treatment, respectively. The authors noted mean survival in patients very large or miliary tumors was poorest, 3.4 months, whereas mean survival in patients with focal lesions in a single lobe was 27.5 months [21].

Gusani et al. published a retrospective review of 42 patients with ICC receiving transarterial gemcitabine, cisplatin, oxaliplatin, or gemcitabine with cisplatin, each followed by particle embolization with Embospheres (Biosphere Medical, Inc., Rockland, MA, USA). These investigators showed significantly improved median survival from time of first TACE with gemcitabine–cisplatin dual-agent therapy than with gemcitabine alone (13.8 vs. 6.3 months, $p = 0.0005$). A median of 3.5 TACE sessions per patient were administered. Twenty of 42 (48 %) patients showing SD by RECIST criteria after 3 treatments were

found to have a median survival of 13.1 months, whereas 15/42 (36 %) with PD had a median survival of 6.9 months ($p = 0.017$). The investigators additionally noted that patients with peripheral tumors treated with TACE had median survival of 18.7 months, while those with central tumors survived a median of 8.2 months after TACE (0 = 0.012). There was no difference seen between patients with and without extrahepatic spread of disease at baseline [22].

Kim et al. published a series of 49 patients receiving either TACE, TACI, or both. Forty of 49 patients (82 %) were Child A, with the remainder being Child B. There were a median of 3 TACE or TACI treatments per patient. Twenty patients received TACE alone, 13 patients received TACI alone, and 16 patients received both TACE and TACI treatments. TACE was performed using cisplatin in Ethiodol followed by 1-mm-diameter gelfoam microsphere embolization of the vessel supplying the tumor. If no tumor hypervascularity was noted at angiography, chemoinfusion was performed without Ethiodol or gelfoam embolic. Median survival from time of first treatment was 10 months. Imaging assessment was performed a month after treatment. RECIST criteria were used with an additional category, tumor necrosis, added by the investigators characterized by lack of tumor enhancement. Ten of 49 patients (20 %) had RECIST PR, 17/49 (35 %) had tumor necrosis, 15/49 (31 %) had SD, and 7/49 (14 %) had PD. The authors defined clinical success as achievement of either RECIST PR or tumor necrosis on imaging follow-up, findings present in a total of 27/49 (55 %) patients. Student's *t* and Fischer exact tests were used with uni- and multivariate logistic regression analysis to compare rates of clinical success associated with the following factors: age; sex; child class; tumor size, type (peripheral or periductal-infiltrating), multiplicity and vascularity; prior radiation therapy; treatment group (TACE vs. TACI); and treatment frequency. Two of these variables, treatment modality and tumor vascularity, were found to be significant by univariate regression analysis: 15/20 patients (75 %) receiving TACE had clinical success versus 1/13 (8 %) receiving TACI ($p < 0.001$), and 26/36 patients (72 %) with hypervascular tumors had clinical success versus 1/13 (8 %) of those with hypovascular tumors ($p < 0.001$). With multivariate regression analysis, only tumor vascularity was found significantly related to clinical success (OR 31.2, $p = 0.002$).

Similar analysis was performed assessing these factors' impact on likelihood of dying during the study period. Tumor hypovascularity (OR = 10.6, $p < 0.001$), Child-Pugh class B (OR = 4.1, $p = 0.006$), and treatment with TACI (OR = 4.7, $p = 0.002$) were associated with decreased survival by univariate analysis. Tumor size of 8 cm or larger approached but did not reach significance (OR = 2.1, $p = 0.116$). By multivariate analysis,

hypovascularity (OR = 13.5, $p < 0.001$), and Child class B were again associated with decreased likelihood of survival (OR = 3.6, $p = 0.014$), but treatment group was not found to be significant. Large tumor size, though not found significantly related to survival by univariate analysis, did result in significantly decreased odds of survival by multivariate regression (OR = 2.6, $p = 0.048$) [15].

Park et al. retrospectively reviewed 155 patients with unresectable ICC, 72 of whom received a mean of 2.5 cTACE treatments and 83 of whom received best supportive care (BSC). TACE was performed as 2 mg/kg cisplatin via the lobar or proper hepatic artery over 15 min, followed by selective embolization of 3–10 mL of 1:1 cisplatin in Ethiodol, followed by embolization to stasis with 1-mm-diameter gelfoam sponge spheres. Overall survival was measured from time of diagnosis in both groups. Log-rank test was used with Student's *t* test or the Fischer exact test to identify demographic differences between the cTACE and BSC groups, to detect differential survival between these groups and to perform subgroup analyses of those with liver-only disease, extrahepatic disease, and those showing radiological response to treatment by RECIST criteria versus nonresponders. There were no significant differences between BSC and cTACE groups regarding age; sex; cancer stage; ECOG performance status (PS); tumor location, size, vascularity or multiplicity; or baseline bloodwork including white blood cell count, hemoglobin level, platelet count, serum albumin, total bilirubin, AST, ALT, or ALP (alkaline phosphatase). Median survival from diagnosis was 12.2 months with cTACE versus 3.3 months with BSC ($p < 0.001$). This difference was upheld in subanalysis of patients with disease confined to the liver (13.3 months with cTACE vs. 4 months with BSC, $p < 0.001$) and those with extrahepatic spread at baseline (11.3 months with cTACE vs. 3.2 months with BSC, $p < 0.001$). In those receiving cTACE, survival was longer among those demonstrating objective response (defined by the authors as RECIST PR or CR) than those who displayed none (22 months vs. 10.9 months, $p = 0.001$). Tumor response to treatment by RECIST criteria from CT scans obtained in 66/72 patients 1–3 months post-cTACE was 15/66 (23 %) PR, 44/66 (67 %) SD, and 7/66 (11 %) PD. ECOG PS; tumor stage, focality, lobar distribution and vascularity; and liver function or other serological characteristics were not found associated with differential survival, possibly, the authors suggested, due to power limitations from small sample size [23].

Kiefer et al. treated 62 patients with biopsy-proven ICC or adenocarcinoma of unknown primary compatible with pancreatobiliary origin thought to represent cholangiocarcinoma at 2 institutions with a mean of 2.7 cTACE sessions using identical TACE technique comprised of 100 mg cisplatin, 10 mg mitomycin C, and 50 mg doxorubicin 1:1 with Ethiodol followed by 0.2 mL of 150–250-micron-

diameter spherical PVA particles (Contour SE, Natick, MA). The angiographic goal was stasis in the tumor vessel(s) with forward flow preserved in the infused segmental or lobar artery. Standard pre- and post-procedure medical care was provided. Survival and time to progression (TTP) were calculated for all patients and analyzed by subgroup for differences between pathologic groups. RECIST 1.0 was determined 1 month after completion of TACE. Forty-five of 62 patients had complete imaging follow-up. Five of 45 (11 %) demonstrated PR, 29/45 (64 %) had SD, and 11/45 (24 %) had PD. Three of 29 (10 %) patients with pathology-proven cholangiocarcinoma had PR, 19/29 (66 %) had SD, and 7 (24 %) had PD. In the adenocarcinoma of unknown primary group, there were 2 (13 %) PR, 10 (63 %) SD and 4 (25 %) PD. Median OS in the entire group was 20 and 15 months from time of diagnosis and first TACE, respectively. There was no difference in median survival from diagnosis or first TACE between patients with ICC or adenocarcinoma of unknown primary (20 and 15 months vs. 19 and 14 months, $p = 0.88$ and 0.51). Prior systemic chemotherapy was associated with prolonged survival (28 vs. 16 months, $p = 0.02$). The absence of extrahepatic disease trended toward prolonged survival but was not statistically significant (18 vs. 13 months, $p = 0.12$). Median TTP in any organ was 8 months regardless of the presence or absence of extrahepatic disease. Eighty-two percent of patients had no change in ECOG PS after treatment. The remainder were evenly split between improvement and worsening PS after treatment. Twenty-one patients had abnormally elevated serum CA 19–9 levels at baseline (> 37 U/mL); 4/21 (20 %) normalized after TACE (CR), 8/21 (40 %) declined by 50 % (PR), 7/21 (35 %) changed < 50 % (SD), and 2/21 (10 %) increased > 50 % (PD). A statistical comparison of CA 19–9 levels and survival was not performed. The parity of survival and imaging response to treatment between those with ICC and adenocarcinoma of unknown primary was cited by the authors to support the hypothesis that the two cohorts represent well-differentiated and poorly differentiated ends of a common spectrum of ICC malignancy [24].

Shen et al. published the first dedicated study of adjuvant transarterial therapy after surgical resection with curative intent. In a retrospective series of 125 patients having undergone hepatectomy for ICC, 53/125 (42 %) received TACI or TACE 1.5–2.0 months after resection at the discretion of the surgeon. TACI with fluorouracil 500 mg or a mix of carboplatin 100 mg, epirubicin 20 mg, and hydroxycamptothecin 10 mg was performed via the proper hepatic artery in all patients. For patients with angiographic evidence of recurrent tumor, 3–5 mL of iodinated oil was added to the chemotherapeutic agents. Demographics, OS, and PFS were compared between groups with the chi-squared test. There were no statistically significant

differences in these baseline characteristics between those patients receiving transarterial therapy and those who did not. There also were no differences between the two groups regarding age, amount of blood transfused during hepatectomy, adequacy of resection margin, TNM staging, or serum CA 19-9. Demographic variables that did differ between treatment groups were sex and the presence of microvascular invasion. Of those receiving TACE or TACI, only 8/38 (21 %) were women, while 29/72 (40 %) were women in the historical control ($p = 0.002$). Twenty-three of 53 (43 %) patients treated with TACE/TACI had microvascular invasion, compared to only 15/72 (21 %) of those who did not receive adjunct therapy ($p = 0.007$). One-, 3-, and 5-year recurrence-free survival periods were not different between the two groups ($p = 0.659$), but the TACE/TACI group did experience slightly better overall survival (69.8, 37.7 and 28.3 % vs. 54.2, 25.0 and 20.8 %, $p = 0.045$). Early recurrence was found in 54/125 (43 %) of patients, 27/53 (51 %) of TACE/TACI patients and 27/72 (38 %) of non-TACE/TACI patients. Subgroup analysis showed that median OS in those with early recurrence was 12 months in the TACE/TACI group versus 5 months in the nonadjuvant cohort ($p < 0.001$). The only demographic variable differing between groups in the subgroups analysis was age: only 10/27 (37 %) of patients in the adjuvant therapy group were 54 years old or older versus 18/27 (67 %) of those not receiving adjuvant treatments. It is possible that this difference confounded improved OS in the TACE/TACI group in the setting of early recurrence. It should also be noted that since TACE was provided if and only if tumor recurrence (i.e., hypervascularity or blush) was seen at angiography, patients in the early recurrence subgroup analysis who received adjuvant transarterial therapy would all have received TACE and not TACI. In this manner, the improved OS in the treatment group may at least in part reflect a response to embolic therapy and/or hypervascular tumor histology and should probably not be construed as a response to transarterial chemoinfusion. These factors may have, to a lesser extent, also accounted for the slightly improved OS with TACE/TACE in the entire study population [16].

Vogl et al. treated 115 patients with a mean 7.1 cTACE treatments at 4-week intervals using 4 chemotherapeutic regimens consisting of mitomycin C, gemcitabine, both mitomycin and gemcitabine, or gemcitabine, mitomycin, and cisplatin. Chemotherapy was administered in Lipiodol and followed by 200-micron degradable starch microspheres. The authors compared several patient factors' effects on survival using the log-rank test: Child-Pugh class; tumor variables of number, localization, and vascularity; TACE regimen; and imaging response to treatment by RECIST using noncontrast MRI every month during the first 3 months of treatment. Tumor hypervascularity was

defined as the presence of demonstrable tumor vessels by angiography and localization of Lipiodol solely within tumor by noncontrast CT performed 4–6 h after each embolization. Tumor hypovascularity was defined as the presence of only faintly demonstrable tumor vessels on angiography and only scant uptake of Lipiodol by the tumor by post-procedure noncontrast CT. Patients were excluded if they had cardiac or pulmonary failure, tumor burden >70 %, Child C cirrhosis, portal vein thrombosis, extrahepatic metastases, serum bilirubin >3.0 mg/dL, albumin <2.0 mg/dL, creatinine >2.0 mg/dL, or Karnofsky PS of 70 % or less. Ten of 115 patients (9 %) had a PR by RECIST criteria, 66/115 (57 %) had SD, and 39/115 (34 %) had PD. Maximal imaging response was typically seen 3 months after first treatment. The median survival from first treatment in the entire group was 13 months. There was no survival difference between the different chemotherapeutic protocols or based on tumor focality or localization. Factors found to favor increased survival included the following: Child class A (21.7 months median OS vs. 11.0 months for child B, $p < 0.001$) and tumor hypervascularity (24.0 vs. 9.0 months median OS, $p < 0.001$). PD by RECIST at initial imaging follow-up was associated with shorter survival (9.0 vs. 17.0 months for SD, $p < 0.001$, and 25.2 months for PR) [25].

Knuppel et al. described retrospective review of 195 patients treated at the gastrointestinal clinic at a single center during a 6-year period with surgical resection, systemic chemotherapy, photodynamic therapy, and/or TACE. These investigators, however, failed to separate patients with ICC from those with extrahepatic cholangiocarcinoma in their analyses, so interpretation of their results is difficult [26].

3.2 Drug-eluting Bead TACE

Table 2 summarizes results of these DEB-TACE and TARE studies. In 2010, Lammer et al. reported results of a randomized, controlled, multicenter trial of doxorubicin-DEB-TACE versus cTACE for HCC. They found that in addition to experiencing fewer chemotherapy-associated side effects, patients with more advanced disease, such as those with ECOG 1 or poorer performance status, Child B cirrhosis or bilobar or recurrent disease, had significantly greater objective response to treatment by EASL criteria at 6 months than those treated with cTACE [27]. Based on these results and other trials suggesting safety and efficacy of DEB-TACE for HCC, investigators have more recently studied the DEB-TACE for ICC.

Aliberti et al. described a cohort study comparing 11 patients receiving doxorubicin-DEB-TACE (DEBDOX) with 9 patients receiving systemic chemotherapy comprised of mainly fluorouracil, cisplatin, or doxorubicin regimens.

Table 2 Results of these DEB-TACE and TARE studies

Primary author	Year	Study type	Type of treatment	Number of patients	Mean or median # of Rx sessions	Noncirrhotic or CP A	ECOG 0	ECOG 1	ECOG 2+	Prior chemotherapy (%)	Prior external beam radiotherapy (%)	Prior thermal ablation (%)
Aliberti	2008	CC	doxorubicin-DEB-TACE	11	2.6					+	+	+
			systemic fluorouracil	9						+	+	+
Poggi	2009	CC	systemic oxaliplatin and gemcitabine	11		91			0			
			oxaliplatin DEB-TACE + systemic gem-ox	9	3.3	100			0			
Kuhlmann	2012	CC	irinotecan DEB-TACE	26	1.6	100	62	35	4	19	4	
			cTACE: mitomycin C + gelfoam	10	1.4	100	70	30	0	20	10	
			systemic oxaliplatin and gemcitabine	31		100	65	32	3	0	3	
Schiffman	2011	RCS	irinotecan and/or doxorubicin-DEB-TACE	24	1.75		38	50	4	58-80, 33 ^{\$\$}		12, 12 ^{\$}
Ibrahim	2008	CS	TARE: glass microspheres	24	2		42	50	8	29		
Saxena	2010	CS	TARE: resin microspheres	25	1		60	28	12	72		8
Hoffman	2012	CS	TARE: resin microspheres	33	1		52	21	27	82	3	6
Rafi	2012	RCS	TARE: resin microspheres	19	1.6		5	74	21	100	0	0

(continued)

Table 2 (continued)

Primary author	Prior surgical resection (%)	Mean or median tumor size (cm)	Single tumor (%)	Peripheral morphology (%)	Unilobar disease (%)	Extrahepatic (%)	Portal vein thrombus (%)	Approx % liver vol replaced by tumor (< 25/ < 50/< 75)	Treatment response imaging criteria	RECIST or WHO imaging assessment of response to treatment at 3 months or earliest post-treatment interval (% CR/ PR/(MR)/SD/PD)	Median OS (months)
Aliberti	+	6.5							RECIST	9/82/0/0	13
	+	6.5									7
Poggi						0			RECIST	0/0/73/27	20
	22 ^{\$}					0			RECIST	0/44/56/0	30 ^{**}
Kuhlmann	4					42			RECIST	0/3/42/50	11.7
	0					40			RECIST	0/12.5/12.5/75	5.7
	23					90			RECIST	0/26/45/29 ⁺⁺	11.0 ⁺⁺
Schiffman	29, 12 ^{\$}	11.5	12			42		38/46/17	RECIST, mRECIST	4/4/83/8, 4/75/12/8	17.5 ^{**} ***
Ibrahim	4 ^{\$}		46	71	33	33	38	83/13/4	WHO, EASL	0/27/68/5, 9/77/7 ⁺⁺⁺ /7 ⁺⁺⁺	14.9 ^{**}
Saxena	40			60	20	48	0	40/60/0	RECIST	0/26/48/22	9.3 ^{**}
Hoffman	36		30	36	36	24	0	76/24/0	RECIST	0/36/52/15	22
Rafi	0		32		58	58			RECIST	0/11/68/21	11.5

CP Child-Pugh classification of severity of cirrhosis A–C, ECOG PS Eastern Cooperative Oncology Group performance status 0–4, RECIST Response Evaluation Criteria in Solid Tumors (National Cancer Institute); WHO World Health Organization, CR complete response, PR partial response, MR minor response (WHO criteria only), SD stable disease, PD progression of disease, OS overall survival, TACI transarterial chemoembolization, cTACE conventional transarterial chemoembolization (chemotherapy in Lipiodol, ± gelfoam or permanent embolic), TARE transarterial radioembolization, DEB-TACE = drug-eluting bead TACE, RFA radiofrequency ablation, MWA microwave ablation, gem-cis gemcitabine + cisplatin, CS case series, CC case/control, RCS retrospective analysis of prospectively gathered case series data, TAG tris-acryl gelatin microspheres, PVA polyvinyl alcohol microspheres, mRECIST modified RECIST criteria for measuring enhancing tumor, EASL European Association for the Study of the Liver criteria for measuring enhancing tumor

* Mean OS reported, not median

** Resections after transarterial therapy not censored from survival data

*** OS reported from time of diagnosis, not first treatment

\$ Subsequent to transarterial therapy

\$\$ Concurrent with transarterial therapy

Study combined patients with ICC and hepatic metastases from pancreatic carcinoma

All patients by this finding deemed to have recurrence post-resection

Study combined patients with ICC and intrahepatic adenocarcinoma of unknown primary

+ All patients had prior chemotherapy and/or surgery, specific quantities not specified

++ 55 % of systemic therapy patients had extrahepatic cholangiocarcinoma or gallbladder carcinoma

+++ SD and PD not reported

Patients receiving doxorubicin-DEBDOX had a median survival of 13 months versus a median survival of 7 months in the systemic chemotherapy group. Imaging response to treatment was assessed by RECIST criteria from CT datasets at 3 months after initial treatment. In the treatment group, there was 1 CR (9 %), and 9 PRs (82 %), with a mean 45 % reduction in tumor volume demonstrated by 3D CT. The authors also assessed quality of life using the Edmonton Symptom Assessment System (ESAS) for patient receiving DEBDOX. Ten of 11 DEBDOX patients reported improved quality of life by ESAS scores. ESAS scores and imaging assessment were not performed for the group of patients receiving systemic chemotherapy and/or palliative care alone. There was no demographic comparison of the two historical groups [28].

Poggi et al. reported a study in which 9 patients with unresectable ICC received a mean of 3.3 rounds of TACE with oxaliplatin-eluting microspheres (OEM-TACE) using HepaSpheres/QuadraSpheres followed upon completion of the final TACE session by standard systemic chemotherapy. This experimental group was then compared with a historical control cohort of 11 patients receiving only systemic oxaliplatin and gemcitabine chemotherapy. In the experimental group, 50–100-micron-diameter Hepaspheres were mixed with 50 mg oxaliplatin and diluted in isosmolar contrast to a total volume of 30 mL. TACE was performed to stasis as selectively within the right or left hepatic artery branch as possible. Eight of 11 patients (73 %) receiving only systemic chemotherapy were found to have PD by RECIST criteria after 3 and 6 cycles of chemotherapy, and 3/11 (27 %) had SD. Response 3 months after the first TACE session in the experimental group was PR in 4/9 (44 %) and SD in 5/9 (56 %). Three patients who had a PR were able to undergo curative resection. The fourth patient showing PR was not eligible for resection but had an FDG-PET scan showing the absence of metabolic activity in the treated lesion. Progression-free survival (PFS) and OS were compared between groups using the log-rank test. The OEM-TACE group had median PFS and OS from first treatment of 8.4 and 30 months, respectively, versus 2.9 and 12.7 months in the systemic chemotherapy cohort ($p < 0.004$) [29].

Kuhlmann et al. retrospectively compared 26 patients who received a mean of 1.6 irinotecan DEB-TACE treatments (iDEB-TACE or DEBIRI) with 10 patients receiving mitomycin and gelfoam cTACE and 31 patients receiving systemic chemotherapy comprised of oxaliplatin and gemcitabine. Treatments did not overlap. Of note, 23/26 (88 %) iDEB-TACE patients and 9/10 (90 %) cTACE patients had ICC; and 3/26 (12 %) and 1/10 (10 %), respectively, had carcinoma of the gallbladder while only 14/31 (45 %) systemic chemotherapy patients had ICC, 10/31 (32 %) had gallbladder cancer, and 7/31 (23 %) had extrahepatic cholangiocarcinoma. Patients treated with iDEB-TACE had

a median age of 67 versus 62 and 63 years for cTACE and systemic chemotherapy groups, respectively. Patients receiving TACE therapies had approximately even amounts of extrahepatic disease and liver-only disease, while those receiving systemic chemotherapy mostly had extrahepatic spread (28/31, 90 %). Rates of prior surgery and endoscopic stenting were slightly higher in the chemotherapy group, perhaps reflecting the variety of tumors treated in this group. Imaging assessment of the iDEB-TACE group with RECIST criteria 8 weeks after the first treatment revealed 1/26 (4 %) PR, 11/26 (42 %) SD, and 13/26 (50 %) PD (1 patient was lost to follow-up). Response in the cTACE group was 1/10 (10 %) PR, 1/10 (10 %) SD, and 6/10 PD (2 patients died before re-staging) while response in the systemic chemotherapy group was 8/31 (26 %) PR, 14/31 (45 %) SD, and 9/31 (29 %) PD. Median OS was 11.7 months for the iDEB-TACE group, 5.7 months for the cTACE group, and 11.0 months for those receiving systemic chemotherapy. Statistical analysis between groups was not performed [30].

Schiffman et al. described a retrospective review of prospectively gathered data on 24 patients with unresectable ICC entered into the International Bead Registry receiving a mean of 1.75 treatments with either irinotecan (35 treatments) or doxorubicin (7 treatments) DEB-TACE with LC Beads (DC Beads as marketed outside the United States). Median irinotecan dose per treatment was 75 mg (40–100 mg range). Doxorubicin dose was always 150 mg per treatment. The size of the beads used was usually 100–300 micron (71 % of cases), with the remainder of cases using either 300–500-micron beads or 100–300 followed by 300–500-micron beads. In only 1 case (2 %) were 500–700-micron beads used. Complete stasis of the infused arteries was reported in 46 % of cases, with near stasis reported in 33 % and partial stasis reported in 21 %. Treatment was lobar in 88 % of cases and segmental or subsegmental in 12 %. Tumor response to treatment at 3 months by RECIST criteria was 1/24 (4 %) CR, 1/24 (4 %) PR, 20/24 (83 %) SD, and 2/24 (8 %) PD. Response by mRECIST was 1/24 (4 %) CR, 18/24 (75 %) PR, 3/24 (12 %) SD, and 2/24 (8 %) PD. Median OS was 17.5 months from time of diagnosis. Three patients were downstaged to resection and radiofrequency ablation (RFA). The authors did not report the time between diagnosis and treatment or factors associated with prolonged survival [31].

3.3 Transarterial Radioembolization

Ibrahim et al. published a prospective single-arm series of 24 patients treated with a mean of 2.0 treatments of glass TARE. The median OS from time of initial TARE for the

entire cohort was 14.9 months. One patient of 24 (4 %) was downstaged to resection. The log-rank test was used to assess differences in survival based on a variety of baseline patient characteristics. Factors associated with improved survival were intact ECOG PS 0 (median OS 31.8 months vs. 6.1 months for ECOG PS 1 and 1.0 months for PS 2, $p < 0.0001$), the absence of portal vein thrombus (median OS 31.8 months vs. 5.7 months with PVT, $p < 0.0003$), peripheral (versus periductal infiltrative) tumor type (median OS 31.8 months for peripheral tumors vs. 5.7 months for infiltrative, $p < 0.0005$) and the absence of prior systemic chemotherapy (4.4 months vs. 31.8 months, $p = 0.0274$). The presence of a solitary intrahepatic tumor (14.9 months vs. 5.7 months, $p = 0.1826$) and the absence of extrahepatic disease (31.8 months vs. 6.1 months, $p = 0.3493$) trended toward improved survival but did not reach statistical significance. Imaging assessment of response to treatment was obtained in 22 of 24 (92 %) patients using both WHO and EASL criteria. Response by WHO criteria was PR 6/22 (27 %), SD 15/22 (68 %), and PD 1/22 (5 %). Response by EASL was CR 2/22 (9 %) and PR 17/22 (77). Nineteen of 22 patients showed an objective response to treatment, defined by the authors as any measurable decrease in tumor size [32].

Saxena et al. reported a retrospective series of 25 patients treated with ^{90}Y resin microspheres with a median survival from time of treatment of 9.3 months. Patients with bilobar disease had both lobes treated in a single session when feasible. Patient characteristics associated with differential survival were assessed, with the log-rank test and categorical variables assessed with chi-squared or Fischer's exact test, as appropriate. With univariate analysis, the authors found peripheral tumor type (median OS 18.3 months vs. infiltrative 4.5 months, $p = 0.004$) and ECOG PS 0 (18.3 months vs. 2.4 months for PS > 0 , $p < 0.001$) as being significantly associated with prolonged survival. The absence of extrahepatic disease trended toward but did not achieve statistical significance (16.3 vs. 4.8 months, $p = 0.140$). Variables that were not shown to affect survival included age, sex, prior chemotherapy, tumor burden (<25 % vs. 25–50 %), time between diagnosis and treatment, and unilobar versus bilobar disease. The authors suggested the lack of significant difference in survival associated with these variables might be due to small sample size. Imaging response by RECIST at 1 and 3 months for the remaining 23 patients was 6/23 (26 %) PR, 11/23 (48 %) SD, and 5/23 (22 %) PD. One patient with PR was downstaged to resection [33].

Hoffman et al. reported a retrospective series in which they administered a mean of 1.0 treatments of resin-based TARE to 33 patients with unresectable ICC. Patients were a mean of 21.2 months from date of diagnosis when they underwent TARE. Median OS from TARE and from time of

diagnosis were 22.0 and 43.7 months, respectively. Differences in survival between patient groups were assessed with the log-rank test. Factors associated with prolonged survival included ECOG PS 0 (29.4 months from treatment vs. 10.0 months for PS 1 and 5.1 months for PS 2, $p < 0.001$), response by RECIST criteria (35.3 months for PR vs. 17.7 SD and 5.7 PD, $p < 0.001$), and tumor burden <25 % (26.7 vs. 6.0 months if 26–50 % burden, $p < 0.001$). Decrease in CA 19–9 levels after treatment trended toward but did not reach statistical significance (29.4 vs. 10.0 months, $p = 0.29$). The presence or absence of prior chemotherapy or surgery did not significantly affect survival. By RECIST at 3 months from treatment, there were 12/33 PR, 17/33 SD, and 5/13 PD [34].

Rafi et al. prospectively collected data including survival and RECIST response in 19 patients receiving a mean of 1.6 treatments with resin microsphere TARE after having progressed on systemic chemotherapy. The investigators reported median survival of 11.5 months from first treatment and 25.1 months from diagnosis. Log-rank test, independent t test and chi-squared test were used to identify significant patient variables affecting survival. The only variable these authors found to prolong survival was prior TACE (22.1 vs. 11.5 months, $p = 0.047$). The authors speculated that since all patients having received prior TACE also had an ECOG PS of 1, whereas 4 of 19 patients in the study had ECOG PS of 2, the apparent difference made by prior TACE might have been confounded by better PS in this group. Other variables were analyzed but did not reach statistical significance. These included ECOG performance status, the presence of extrahepatic disease, multifocality of intrahepatic tumor, tumor size, unilobar versus bilobar tumor distribution, RECIST response, and change in serum bilirubin or AST from baseline. The investigators hypothesized that small sample size may have contributed to their results. They did not publish demographic data on peripheral versus infiltrative tumor histology. RECIST response assessed 3 months post-TARE was PR 2/19 (11 %), SD 13/19 (68 %), and PD 4/19 (21 %) [35].

4 Complications

Since its inception, the rationale for transarterial oncologic intervention has been the promise of equal or greater efficacy with fewer side effects than available systemic alternatives. As such, a brief review of the toxicity profile of state-of-the-art systemic chemotherapy at the time of this publication may provide the best frame of reference from which to interpret the toxicity profiles of the transarterial therapies detailed below. By way of reference, in their recent landmark paper describing dual-agent cisplatin-gemcitabine systemic chemotherapy, Valle et al. reported Common

Terminology Criteria for Adverse Events (CTCAE) 3.0 grade 3 or 4 toxicities in 140/198 patients (71 %) receiving the dual-agent regimen [36]. Unless otherwise specified, complications reviewed below are grade 3, 4, or 5.

4.1 Conventional TACE and TACI

There were no procedure-related complications reported in Tanaka et al.'s original series of 11 patients treated with TACI port placement. One of the 11 patients (9 %) developed hearing loss and weakness. There was 1 case of pancytopenia (9 %) and 2 cases of cholangitis (18 %) [19].

Five of 17 patients (29 %) treated by Burger et al. experienced self-limited post-embolization symptoms: nausea, vomiting, diarrhea, hypertension, tachycardia, and/or right upper quadrant abdominal pain which did not require prolonged hospitalization or significant further treatment. One patient (6 %) with grade 3 esophageal varices and large tumor suffered massive upper gastrointestinal hemorrhage and died 11 days post-treatment. The authors cautioned against treating large tumors in patients with decompensated cirrhosis. One patient (6 %) experienced severe right upper quadrant pain thought to be due to chemical cholecystitis or acute post-embolization syndrome that resolved with patient-controlled analgesia and intravenous fluids. One patient (6 %) developed ascites, mild jaundice, and left rib pain that resolved after 2 weeks with paracentesis of 3 L and cox-2 inhibitors [20].

In their report of 12 patients treated with gemcitabine TACI, Vogl et al. described 1 case (8.3 %) of pulmonary edema requiring intubation. The maximum-tolerated dose (MTD) in their dose-escalation protocol was reached due to WHO grade 3 myelosuppression in 2 of 3 patients at the 1600-mg/m² dose group. In the report of 12 patients treated with gemcitabine-starch microsphere TACE by the same authors, there was no severe adverse event. MTD was reached due to WHO grade 3 myelosuppression in 2 of 3 patients at 2,000 mg/m² [14].

Herber et al. reported post-embolization symptoms including right upper quadrant abdominal pain, nausea, and/or vomiting in 6/15 (40 %) of patients resolving with minimal medical therapy (minor, Class B). There was one case (6.7 %) of nontarget Lipiodol embolization leading to gastric ulceration requiring 7 days of intravenous proton pump inhibitor (PPI) therapy and 1 case (6.7 %) of anaphylaxis from iodinated contrast material requiring ICU care (each major, Class D). Two cases of severe hepatic arterial spasm resolved with catheter withdrawal from the artery and sublingual nitroglycerin (minor, class B) [21].

Gusani et al. reported CTCAE grade 4 toxicity in 2 of 42 patients (4.8 %): acute myocardial infarction resolving with

percutaneous coronary intervention and abscess leading to sepsis and grade 4 thrombocytopenia requiring percutaneous drainage and prolonged hospitalization. Five additional patients (11.9 %) developed grade 3 adverse events (AEs). One patient developed mild respiratory distress from oversedation, 2 developed hyperbilirubinemia and 2 developed thrombocytopenia. Grade 1 and 2 AEs were seen in 7 (16.7 %) and 9 (21.4 %) of patients, respectively. These included elevations in serum bilirubin and creatinine, thrombocytopenia, hyperglycemia, hypertension, pulmonary edema, and pancreatitis. Nearly all patients experienced some degree of post-embolization syndrome [22].

Kim et al. reported nausea, vomiting, and/or fever compatible with post-embolization syndrome in most patients. One patient (2.0 %) with bilioenteric anastomosis and persistent fevers 34 days after treatment required percutaneous drainage, hospitalization, and antibiotic therapy for hepatic abscess [15].

Park et al. reported 9/72 (13 %) grade 3 or higher cases of hematological toxicity to their cisplatin cTACE therapy, 3/72 (4 %) being anemia, 6/72 (8 %) thrombocytopenia, 1/72 (1 %) neutropenia, and 1/72 (1 %) elevation in INR. There was 17/72 (24 %) nonhematological CTCAE grade 3 or higher toxicities: 2/72 (3 %) AST elevation, 1/72 (1 %) ALT elevation, 2/72 (3 %), alk. phos. elevation, 11/72 (15 %) bilirubin elevation, 5/72 (7 %) albumin decrease, 3/72 (4 %) pain, and 1/72 (1 %) nausea. There were no deaths within 30 days of treatment [23].

Kiefer et al. evaluated toxicity associated with their treatments according to CTCAE 3.0 criteria. Median hospital length of stay was one day. Post-embolization syndrome, defined as CTCAE grade 1 or greater pain, nausea, vomiting or fever, was experienced after 65 % of TACE procedures but was generally mild. Major complications occurred in 5 of 165 treatments (3 %). They included pulmonary edema and myocardial infarct on post-procedure day 2 (grade 4), readmission for management of severe post-embolization syndrome, readmission for hyperglycemia, and acute renal failure from dehydration [24].

Shen et al. reported nausea and/or vomiting in 25/53 (47 %) patients, abdominal pain in 19/53 (36 %), and fever in 6/53 (11 %). They did not quantify with WHO or CTCAE criteria but reported no severe complications such as liver or kidney failure or bone marrow suppression [16].

In their report of Lipiodol and starch microsphere multidrug TACE in 115 patients, Vogl et al. reported 15 patients (13.0 %) had post-embolization symptoms of pain, nausea, and vomiting requiring 2–7 days of hospital treatment. No major complications were reported [25].

Knuppel et al. did not report on complications in their retrospective review of patients receiving surgery, systemic chemotherapy, photodynamic therapy, and/or TACE [26].

4.2 Drug-eluting Bead TACE

Aliberti et al. reported hepatic abscess in one of 20 patients (5.0 %) treated with DEBDOX-TACE. Almost all TACE treatments (27/29, 93 %) were associated with WHO grade 2 nausea and vomiting within 12 h of treatment. Right upper quadrant pain resolving after an average of 10 h and neoplastic fever beginning 72 h and lasting an average of 2 days occurred after 29 of 29 treatments (100 %) [28].

Poggi et al. compared frequencies of CTCAE 3.0-graded adverse events in the OEM-TACE-systemic chemotherapy group versus AEs in the systemic chemotherapy-only group. Pain was a common complaint in both groups, with all grades of pain occurring in 42 % of OEM-TACE patients versus 25 % of systemic only patients, although 9 % of TACE patients suffered grade 3 pain versus none of the receiving only systemic chemotherapy ($p = 0.042$). Nausea and vomiting, however, were much more common in patients receiving only systemic chemotherapy (72 % all grades and 16 % grade 3) than in the TACE group (30 % all grades and no grade 3; $p < 0.001$). Mild and severe asthenia, peripheral neuropathy, and leukopenia were all significantly more frequent in the systemic chemotherapy-only group than in the TACE group: grade 1–2 asthenia 25 versus 3 % and grade 3 asthenia 9 versus 0 %, peripheral neuropathy 40 versus 4 % and 16 versus 0 %, and leukopenia 25 versus 4 % and 9 versus 0 %. Cholangitis was more common in the TACE group: 7 and 1 % versus 0 and 0 %. Mild transaminitis was also more frequent in the TACE group: 30 versus 16 %. There was no grade 3 transaminitis in either group [29].

In their comparison of systemic chemotherapy, cTACE and DEB-TACE, Kuhlmann et al. reported 1 death from cholangitis in each of the cTACE and iDEB-TACE groups (10 and 4 %, respectively). In each case, the patient had a disrupted sphincter of Oddi with a biliary stent. The patient in the cTACE group who died of cholangiosepsis also suffered a pulmonary embolism. One patient in the cTACE group died of liver failure associated with bacterial peritonitis 14 days after treatment. Three patients died (10 %) of treatment-related complications in the systemic chemotherapy group, 2 of cholangitis and 1 from tumor rupture. There were no other CTCAE grade 3 or 4 adverse events in the cTACE group. There were 2 liver abscesses and 1 empyema requiring drainage in the iDEB-TACE group. The empyema was thought by the authors to be related to biliary leak. Post-embolization pain was worse in the DEB-TACE group than in the cTACE group, with 7/26 (27 %) of DEB-TACE patients experiencing grade 3 or 4 pain but no 3 or 4 pain in the cTACE group. Overall, there were 11/26 (42 %) grade 3 or higher adverse events in the DEB-TACE group, 3/10 (30 %) grade 3 or higher AEs in the cTACE group, and

23/30 (77 %) grade 3 or greater AEs in the systemic chemotherapy group. Hematological AEs accounted for 17 of 23 AEs in patients receiving systemic chemotherapy, a rate of 57 %. Specific grade 3 or greater AEs in the chemotherapy group were leukocytopenia 5/30 (16 %), febrile neutropenia 2/30 (7 %), thrombocytopenia 7/30 (23 %), anemia 3/30 (10 %), and peripheral neuropathy 6/30 (19 %) [30].

Schiffman et al. reported grade 3 or higher adverse events in 4/24 (17 %) of patients treated with iDEB-TACE. One patient (4 %) with 50–75 % liver volume replacement by tumor died 12 days after treatment from hepatorenal syndrome. One patient (4 %) developed sepsis related to his chemoinfusion port. Two patients (8 %) developed self-limited grade 3 hepatic insufficiency [31].

4.3 Transarterial Radioembolization

Ibrahim et al. reported delivering a median transarterial radiation dose to liver of 105.5 Gy. Despite this tumoricidal dose, there were only 5/24 (21 %) grade 3 laboratory toxicities of liver function: 4/24 (17 %) hypoalbuminemia and 1/24 (4 %) hyperbilirubinemia. There were no treatment-related grade 4 hepatic toxicities and no deaths. One patient developed a gastroduodenal ulcer refractory to medical management requiring surgical resection. Eighteen of 24 patients (75 %) complained of fatigue, 9/24 (38 %) of abdominal pain, 4/24 (17 %) of nausea or vomiting, and 2/24 (8 %) of anorexia. The authors did not report what percentage of these was grade 3 versus lower grades. Median dose to lungs was 4.6 Gy per treatment and 8.4 Gy total, well below the generally accepted limits of 30 Gy/dose and 50 Gy cumulatively [32].

Saxena et al. reported serologic grade 3 liver toxicity in 3/25 (12 %) patients, 2 (8 %) with hypoalbuminemia, and 2 (4 %) with elevated alk. phos. No other chemical toxicities were observed. One patient suffered a duodenal ulcer that responded to medical therapy. Clinical toxicities not reported by grade included fatigue in 16/25 (64 %), self-limited abdominal pain in 10/25 (40 %), nausea or vomiting in 6/24 (25 %), anorexia in 4/25 (16 %), and shortness of breath in 2/25 (8 %) [33].

Hoffman et al. likewise reported no RILD, despite delivering a median activity of 1.54 GBq per TARE session. The authors described no deaths and reported finding no clinically relevant acute or delayed toxicities during follow-up. This seems to suggest that toxicities they reported were minor, although no CTCAE grades were provided. The investigators reported some degree of toxicity as follows: 23/33 (70 %) hyperbilirubinemia, 18/33 (54 %) AST elevation, 11/33 (33 %) ALT elevation, 16/33

Table 3 Results of thermal ablation investigations

	Yu	Kim	Xu	Fu
Year	2011	2011	2012	2012
Study type	case series	case series	case series prospective data	case series
Treatment type	MWA	RFA	RFA or MWA	RFA
# of Patients	15	13	18	17
Mean or median # of treatment sessions	2.5	1.3	1.1	1.1
Noncirrhotic or CP A (%)	93		89	82
Single tumor (%)	67	76	72	76
Mean or median tumor size (cm)	3.2	3.0	2.8	4.4
Extrahepatic disease (%)	0		0	70
Median OS (months from treatment)	10	38.5	30.3, 62.5*	33

RFA radiofrequency ablation, MWA microwave ablation, OS overall survival

* Median OS in patients with primary, rather than recurrent ICC

(48 %) LDH elevation, 28/33 (85 %) abdominal pain, 20/33 (61 %) nausea, and 9/33 (27 %) vomiting [34].

Rafi et al. reported no treatment-related deaths, no grade 4 toxicities, and no cases of GI ulceration. Two of 19 (11 %) patients had grade 3 toxicity; however, the exact toxicity they experienced was not described. Grade 1–3 toxicities were categorized as gastrointestinal 6/19 (32 %), hematological 1/19 (5 %), hepatic 6/19 (32 %), and other 4/19 (21 %) [35].

5 Image-Guided Percutaneous Thermal Ablation

Recently, several groups of investigators have reported promising early results from percutaneous thermal (radiofrequency or microwave) ablation of small- to moderate-sized, usually solitary, ICCs in patients who are considered poor candidates for surgical resection or who have recurrent disease after resection with curative intent. Table 3 summarizes results of these thermal ablation investigations.

The first moderate-sized series to prove safety and efficacy of microwave ablation for ICC was published in 2011 by Yu et al. who described sonographically guided percutaneous microwave ablation of 24 tumors in 15 patients with biopsy-proven ICC. With a mean of 2.5 treatment sessions per patient, this group achieved median OS of 10 months, similar to many prior series published for transarterial therapies. Their major complication rate was 20 %, with abscesses requiring drainage occurring in 2 patients at 3 and 13 months after ablation, and needle tract seeding occurring in 1 of these patients. The authors speculate that their relatively low survival and high complication rates reflected patient selection factors, such as 25 % of cases involving tumors adjacent critical structures such as bowel or central vessels [37]. Studies yielding more impressive results followed shortly.

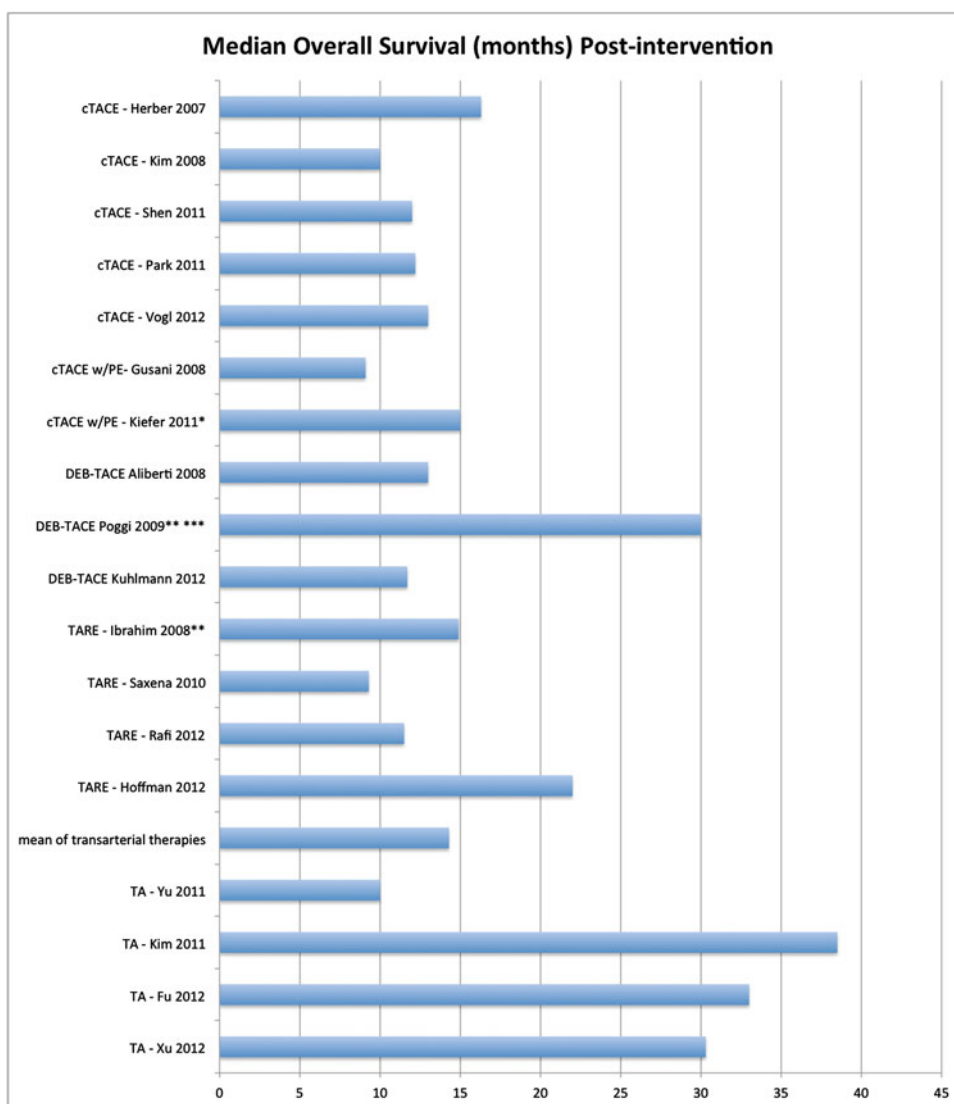
Kim et al. described a series of 13 patients with 17 tumors treated with percutaneous RFA for with median OS and PFS of 38.5 and 32.2 months, respectively. Mean maximum tumor diameter was 3.0 cm. Technical success, defined as complete tumor ablation by contrast CT or MRI 1 month after treatment, was achieved in 15/17 patients. The 2 patients in whom residual tumor was found at 1 month had tumors measuring 7 and 8 cm diameter. One patient (8 %) developed a liver abscess that was treated with antibiotics and percutaneous drainage. This same patient died of sepsis 3.3 months after the ablation. No other severe complications occurred [38].

Fu et al. published a retrospective study of RFA treatment for 26 ICC tumors in 17 patients ranging from 2.1 to 6.9 cm diameter (median 4.4 cm) with technical success in 25/26 (96 %) cases at 1 month follow-up. The 1 case in 26 with local recurrence at 1 month was successfully treated with a second ablation. Median OS was 33 months, and recurrence-free survival was 17 months. Univariate regression analysis revealed pathologic tumor grade ($p = 0.033$) was associated with decreased overall survival. One patient (4 %) suffered a major complication: dyspnea resolved after thoracentesis [39].

Xu et al. reported retrospectively evaluated results of prospectively gathered data on percutaneous RFA or microwave ablation for 25 ICC tumors in 18 patients, 8 with primary and 10 with lesions recurring post-resection. Technical success was achieved in 23/25 (92 %) tumors ranging in size from 0.7 to 4.3 cm diameter, with a mean tumor maximum diameter of 2.8 cm. Residual viable tumor was seen 1 month after treatment for 2/25 tumors with diameters of 6.4 and 6.9 cm. Recurrence after surgical resection was associated with decreased overall survival by univariate regression analysis. OS for the entire cohort was 30.3 % at 60 months; however, for those without prior resection (primary rather than recurrent), OS was 62.5 % at 60 months ($p = 0.001$ by univariate regression analysis).

Fig. 3 Median overall survival post-intervention by studies cited in this chapter.

cTACE conventional transarterial chemoembolization with temporary embolic (Lipiodol and/or gelfoam or starch microspheres), *cTACE w/ PE* *cTACE* with permanent embolic material (tris-acryl gelatin or polyvinyl alcohol); *DEB-TACE* drug-eluting bead TACE, *TARE* transarterial radioembolization with ^{90}Y -bearing microspheres, *TA* thermal ablation (radiofrequency or microwave), *patients included ICC and adenocarcinoma of unknown primary, **includes patients downstaged to resection, ***patients received oxaliplatin DEB-TACE followed by systemic gemcitabine + oxaliplatin



There was 1 major complication (6 %), in which a fever of suspected infectious etiology responded to antibiotic therapy [40].

6 Conclusion

Inoperable ICC continues to have a dismal prognosis. Originally developed for patients with unresectable HCC, the application of transarterial therapies has been shown by numerous small- and medium-sized series to prolong survival in patients with unresectable ICC well beyond a year after intervention. A review of the current literature reveals several interesting observations in the interventional management of this disease. The parity of response to treatment

and of survival outcomes between patients with biopsy-proven cholangiocarcinoma and intrahepatic adenocarcinoma of unknown primary in the study by Kiefer et al. supports the hypothesis that the latter entity may in fact be poorly differentiated cholangiocarcinoma.

While treatment with state-of-the-art dual-agent systemic chemotherapy is associated with overall survival of less than 12 months [36], average OS after transarterial therapy based on the studies reviewed here is over 14 months (Fig. 3). DEB-TACE and TARE are newer transarterial treatment modalities that may further maximize treatment effect while minimizing morbidity from systemic exposure. Of particular interest is the study by Poggi et al. combining DEB-TACE with dual-agent systemic chemotherapy to achieve a median OS of 30 months. Further investigation into the potential

Table 4 Factors related to prolonged survival

Primary author	Year	Rx type	N Pts	Child-Pugh A	ECOG PS 0	Prior chemotherapy	Prior TACE	Primary (vs. recurrent post-op)	Peripheral morphology	Small tumor size	Low tumor grade	Tumor burden < 25 %	Absence of portal thrombus	Hypervascularity	Embolec therapy	Gem-cis dual-agent cTACE	(+) RECIST response
Vogl	2006	TACI/ TACE	12														
Gusani	2008	TACE	42						0.012							0.0005	0.017 SD vs. PD
Kim	2008	TACE/ TACI	42	0.014						0.048				<0.001	0.002*		
Ibrahim	2008	TARE	24		<0.0001	0.0274**		<0.0005					<0.0003				
Saxena	2010	TARE	25		<0.001*				0.004*								
Park	2011	TACE vs. BSC	75														0.001 OR vs. SD or PD
Kiefer	2011	TACE	62			0.02											
Shen	2011	TACI/ TACE vs. BSC***	53												0.045		
Vogl	2012	TACE	115	<0.001													<0.001 OR or SD vs. PD
Hoffman	2012	TARE	33		<0.001							<0.001		<0.001			<0.001 OR or SD vs. PD
Rafi	2012	TARE	19				0.047										
Fu	2012	RFA	17								0.033*						
Xu	2012	RFA and MWA	18					0.001*									

TACI transarterial chemoembolization, TACE transarterial chemoembolization, BSC best supportive care, systemic systemic chemotherapy, TARE transarterial radioembolization, DEB-TACE drug-eluting bead TACE, RFA radiofrequency ablation, MWA microwave ablation, RECIST Response Evaluation Criteria in Solid Tumors (copyright National Cancer Institute), CR RECIST complete response, PR partial response, SD stable disease, PD progression of disease, OR sum of CR + PR, gem-cis gemcitabine + cisplatin

** Prior chemotherapy negatively associated with prolonged survival

*** All patients with recurrence post-resection, variables not found associated with differential survival: solitary tumors and extrahepatic disease

benefit of combined systemic and transarterial therapy is needed to confirm these encouraging initial findings.

Research to date suggests that patient factors associated with prolonged survival include the absence of cirrhosis or the presence of no worse than Child A cirrhosis, normal or near normal (0–1) ECOG performance status, peripheral (rather than periductal) tumor type, tumor hypervascularity, small tumor size, low tumor grade, low tumor burden, the absence of portal thrombus, prior TACE and RECIST imaging evidence of stable disease or response to treatment (Table 4). Several studies have found that transarterial chemoembolic therapy is more effective than transarterial chemoinfusion alone for unresectable ICC; however, no study has been conducted to evaluate whether this difference between TACE and TACI persists in the subpopulation of hypovascular tumors. One study, by Gusani et al., found that, just as has been confirmed for systemic chemotherapy, dual-agent conventional TACE with gemcitabine and cisplatin was more effective than single-agent cTACE. The presence of extrahepatic disease has not been found significantly to impact survival, confirming the high mortality of the primary disease.

Early results of percutaneous RFA and microwave ablation for selected patients with small-to-moderate-sized unresectable ICC are promising. Three recent studies of patients receiving thermal ablation each reported median OS periods of over 30 months post-treatment. One of these studies, by Xu et al., noted that excluding patients treated for recurrence post-resection yielded a median OS of 62.5 months, survival similar to that often cited for resection itself. Prospective studies of transarterial and percutaneous ablative therapies are needed.

References

- Pitt HA, Grochow LB, Abrams RA (1997) Cancer of the biliary tree, 5th edn. Lippincott-Raven, Philadelphia
- Poultides GA, Zhu AX, Choti MA et al (2010) Intrahepatic cholangiocarcinoma. *Surg Clin N Am* 90:817–837
- Brown DB, Fundakowski CE, Lisker-Melman M (2004) Comparison of MELD and Child-Pugh Scores to predict survival after chemoembolization for hepatocellular carcinoma. *J Vasc Interv Radiol* 15:1209–1218
- Basile A, Carrafiello G, Ierardi AM et al (2005) Quality-improvement guidelines for hepatic transarterial chemoembolization. *Cardiovasc Intervent Radiol* 35:765–774
- Brown DB, Cardella JF, Sacks D et al (2009) Quality improvement guidelines for transhepatic arterial chemoembolization, embolization and chemotherapeutic infusion for hepatic malignancy. *J Vasc Interv Radiol* 20:S219–S226
- Rose SC, Kikolski SG, Chomas JE, Downstream hepatic arterial blood pressure changes caused by deployment of the Surefire antireflux expandable tip. *Cardiovasc Interv Radiol*, 19 December 2012 ePub in advance of print
- Shiozawa S, Tsuchiya A, Endo S et al (2003) Transradial approach for transcatheter arterial chemoembolization in patients with hepatocellular carcinoma. *J Clin Gastroenterol* 37:412–417
- Brown KT, Koh BY, Brody LA et al (1999) Particle embolization of hepatic neuroendocrine metastases for control of pain and hormonal symptoms. *J Vasc Interv Radiol* 10:309–403
- Lee SH, Hahn ST, Park SH (2001) Intraarterial lidocaine administration for the relief of pain resulting from transarterial chemoembolization of hepatocellular carcinoma: its effectiveness and optimal timing of administration. *Cardiovasc Interv Radiol* 24:368–371
- Llovet JM, Real MI, Montana X et al (2002) Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* 359:1734–1739
- Lo CM, Ngan H, Tso WK et al (2002) Randomized controlled trial of transarterial Lipiodol chemoembolization for unresectable hepatocellular carcinoma. *Hepatology* 35:1164–1171
- Camma C, Schepis F, Orlando A et al (2002) Transarterial chemoembolization for unresectable hepatocellular carcinoma: meta-analysis of randomized controlled trials. *Radiology* 224:47–54
- Maeda S, Fujiyama S, Tanaka M et al (2002) Survival and local recurrence rates of hepatocellular carcinoma patients treated by transarterial chemolipiodolization with and without embolization. *Hepatol Res* 23:202–210
- Vogl TJ, Schwarz W, Eichler K et al (2006) Hepatic intraarterial chemotherapy with gemcitabine in patients with unresectable cholangiocarcinomas and liver metastases of pancreatic cancer: a clinical study on maximum tolerable dose and treatment efficacy. *J Cancer Res Clin Oncol* 132:745–755
- Kim JH, Yoon H-K, Sung K-B et al (2008) Transcatheter arterial chemoembolization or chemoinfusion for unresectable intrahepatic cholangiocarcinoma: clinical efficacy and factors influencing outcomes. *Cancer* 113:1614–1622
- Shen WF, Zhong W, Liu Q et al (2011) Adjuvant transcatheter arterial chemoembolization for intrahepatic cholangiocarcinoma after curative surgery: retrospective control study. *World J Surg* 35:2083–2091
- Venkatesan AM, Kundu S, Sacks D et al (2010) Practice guidelines for adult antibiotic prophylaxis during vascular and interventional radiology procedures. *J Vasc Interv Radiol* 21:1611–1630
- Geschwind JF, Kaushik S, Ramsey DE et al (2002) Influence of a new prophylactic antibiotic therapy on the incidence of liver abscesses after chemoembolization treatment of liver tumors. *J Vasc Interv Radiol* 13:1163–1166
- Tanaka N, Yamakado K, Nakatsuka A et al (2002) Arterial chemoinfusion therapy through an implanted port system for patients with unresectable intrahepatic cholangiocarcinoma: initial experience. *Eur J Radiol* 41:42–48
- Burger I, Hong K, Schulick R et al (2005) Transcatheter arterial chemoembolization in unresectable cholangiocarcinoma: initial experience in a single institution. *J Vasc Interv Radiol* 16:353–361
- Herber S, Otto G, Schneider J, Manzl N, Kummer I, Kanzler S et al (2007) Transarterial chemoembolization (TACE) for inoperable intrahepatic cholangiocarcinoma. *Cardiovasc Intervent Radiol* 30(6):1156–1165
- Gusani NJ, Balaa FK, Steel JL, Geller DA, Marsh JW, Zajko AB et al (2008) Treatment of unresectable cholangiocarcinoma with gemcitabine-based transcatheter arterial chemoembolization (TACE): a single-institution experience. *J Gastrointest Surg* 12(1):129–137
- Park SY, Kim JH, Yoon HJ et al (2011) Transarterial chemoembolization versus supportive therapy in the palliative

- treatment of unresectable intrahepatic cholangiocarcinoma. *Clin Radiol* 66:322–328
24. Kiefer MV, Albert M, McNally M et al (2011) Chemoembolization of intrahepatic cholangiocarcinoma with cisplatin, doxorubicin, mitomycin C, Ethiodol and polyvinyl alcohol: a 2-center study. *Cancer* 117:1498–1505
 25. Vogl TJ, Naguib NN, Nour-Eldin NE, Bechstein WO, Zeuzem S, Trojan J et al (2012) Transarterial chemoembolization in the treatment of patients with unresectable cholangiocarcinoma: Results and prognostic factors governing treatment success. *Int J Cancer* 131(3):733–740
 26. Knuppel M, Kubicka S, Vogel A, et al (2012) Combination of conservative and interventional therapy strategies for intra- and extrahepatic cholangiocellular carcinoma: a retrospective survival analysis. *Gastroenterol Res Prac* 2012:190708 [Epub 2011 Jun 8]. doi: [10.1155/2012/190708](https://doi.org/10.1155/2012/190708)
 27. Lammer J, Malagari K, Vogl T et al (2010) Prospective randomized study of doxorubicin-eluting-bead embolization in the treatment of hepatocellular carcinoma: results of the PRECISION V study. *Cardiovasc Intervent Radiol* 33:41–52
 28. Aliberti C, Benea G, Tilli M et al (2008) Chemoembolization (TACE) of unresectable intrahepatic cholangiocarcinoma with slow-release doxorubicin-eluting beads: preliminary results. *Cardiovasc Intervent Radiol* 31:883–888
 29. Poggi G, Amatu B, Quaretti P et al (2009) OEM-TACE: A new therapeutic approach in unresectable intrahepatic cholangiocarcinoma. *Cardiovasc Intervent Radiol* 32(6):1187–1192
 30. Kuhlmann JB, Euringer W, Spangenberg HC et al (2012) Treatment of unresectable cholangiocarcinoma: conventional transarterial chemoembolization compared with drug eluting bead-transarterial chemoembolization and systemic chemotherapy. *Eur J Gastroenterol Hepatol* 4:437–443
 31. Schiffman SC, Metzger T, Dubel G et al (2011) Precision hepatic arterial irinotecan therapy in the treatment of unresectable intrahepatic cholangiocellular carcinoma: optimal tolerance and survival. *Ann Surg Oncol* 18:431–438
 32. Ibrahim SM, Mulcahy MF, Lewandowsky RJ et al (2008) Treatment of unresectable cholangiocarcinoma using yttrium-90 microspheres: results from a pilot study. *Cancer* 113:2119–2128
 33. Saxena A, Bester L, Chua TC et al (2010) Yttrium-90 radiotherapy for unresectable intrahepatic cholangiocarcinoma: a preliminary assessment of this novel treatment option. *Ann Surg Oncol* 17:484–491
 34. Hoffman RT, Paprottka PM, Schon A et al (2012) Transarterial hepatic yttrium-90 radioembolization in patients with unresectable intrahepatic cholangiocarcinoma: factors associated with prolonged survival. *Cardiovasc Intervent Radiol* 35:105–116
 35. Rafi S, Piduru SM, El-Rayes B et al (2013) Yttrium-90 radioembolization for unresectable standard-chemorefractory intrahepatic cholangiocarcinoma: survival, efficacy and safety study. *Cardiovasc Intervent Radiol* 36:440–448
 36. Valle J, Wasan H, Palmer D et al (2010) Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. *N Engl J Med* 362:1273–1281
 37. Yu MA, Liang P, Yu XL et al (2011) Sonography guided percutaneous microwave ablation of intrahepatic primary cholangiocarcinoma. *Eu J Radiol* 80:548–552
 38. Kim JH, Won HJ, Shin YM et al (2011) Radiofrequency ablation for the treatment of primary intrahepatic cholangiocarcinoma. *AJR Am J Roentgenol* 196:W205–W209
 39. Fu Y, Yang W, Wu W et al (2012) Radiofrequency ablation in the management of unresectable intrahepatic cholangiocarcinoma. *J Vasc Interv Radiol* 23:642–649
 40. Xu HX, Wang Y, Lu MD et al (2012) Percutaneous ultrasound guided thermal ablation for intrahepatic cholangiocarcinoma. *Br J Radiol* 85:1078–1084

Overview of Emerging Strategies in the Surgical Management of Biliary Tract Tumors

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Abstract

Cholangiocarcinoma can arise anywhere along the biliary system. Patients with biliary tract cancer (intra-hepatic, peri-hilar, extra-hepatic, and gallbladder cancer) tend to have advanced disease at presentation, with a median survival of about 6–9 months from the time of diagnosis [1]. Surgery with clear histologic margins (R0 resection) in combination with appropriate lymph node dissection is the only chance of cure with published five-year survival rates of 24–40 % [2]. Curative surgical resection, however, is only feasible in a minority of patients [3], even in the setting of radical hepatic surgery [4, 5]. The management of patients with biliary tract cancer is complex and has been changing with the development of novel treatment alternatives. Furthermore, more aggressive surgical approaches have emerged over the past decade to treat patients previously considered to have unresectable disease, including portal vein embolization, combined hepatectomy with vascular resection, non-touch technique and, in highly selected patients, liver transplantation [6]. Minimal invasive surgery in hepato-pancreato-biliary diseases and neoadjuvant treatment is also among the last newly strategies added to the biliary tract cancer therapy. Indications, surgical selection, possible benefits, and limitations of each of these treatment alternatives will be discussed in the light of the most recent literature.

Keywords

Cholangiocarcinoma • Gallbladder cancer • Surgery • Hepatectomy • Duodenopancreatectomy • Biliary tract cancer

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1 Introduction

1.1 Gallbladder Cancer

The depth of invasion into the gallbladder wall to the peritoneum and the consequent risk of lymph node spread and distant dissemination determine the treatment of gallbladder cancer. Thus, pT1a lesions are treated by cholecystectomy alone with long-term disease-free survival approaching 90 %. For pT1b and pT2 lesions, however, cholecystectomy should at least be accompanied by portal lymphadenectomy [7, 8]. A recent review of the SEER database (The Surveillance, Epidemiology, and End Results Program of the National Cancer Institute, USA) demonstrates that patients with as few as one lymph node evaluated have a better median overall survival than patients with pT2 lesions and no nodal evaluation (123 vs. 22 months $p < 0.001$) [7, 9]. The SEER database also demonstrates that there is no benefit to radical resection of the gallbladder bed beyond that needed to achieve a margin-negative (R0) resection and lymph node dissection, meaning that patients without direct extension into the liver may not benefit from partial hepatectomy. Nevertheless, one can only unquestionably confirm this information with the pathologic postoperative evaluation.

For more advanced gallbladder cancers, several studies have demonstrated a benefit to an aggressive approach including partial hepatic resection, extended hepatectomy and resection of the extra-hepatic biliary system, when necessary, with the aim of achieving an R0 resection [10, 11] (Fig. 1). A Canadian group evaluated their approach to gallbladder cancer over two time periods (1990–1996 and 1996–2002) and examined their institutional efforts to become more aggressive about clearing the disease by liver resection and resection of the extra-hepatic bile ducts [11]. Portal lymphadenectomy was unchanged over both periods and was routinely used. There was, however, an increased rate of R0 resection in the later time period with an associated statistical improvement in survival (9 months vs. 17 months). In both period intervals, R0 resection showed more than double the length of overall survival from 7 months for R1 and R2 resections to 18 months for R0 resections favoring a more aggressive resection [9].

1.1.1 Hilar Cholangiocarcinoma

Hilar cholangiocarcinomas represent about 60 % of all treated biliary cancers, and aggressive surgery to achieve an R0 resection has long been recognized as the corn stone of therapy (Figs. 2, 3). Jarnagin et al. [3], in a review of the Memorial Sloan-Kettering Cancer Center (MSKCC) experience, from 1991 to 2000 identified 80 patients, 62 of which achieved an R0 resection. The overall survival was 42 months in the R0 group against 21 months in those with

a positive microscopic margin ($P < 0.0075$). A similar association between positive margin of resection and survival was reported in 281 patients treated at Johns Hopkins between 1974 and 2004 [12].

Lymph nodal involvement and number of lymph nodes evaluated also appeared to affect outcomes. In the experience of MSKCC, among patients with hilar malignancy treated with negative resection margins and no nodal involvement, but for whom fewer than 7 portal nodes were evaluated, had a worse survival relative to N0 patients who had >7 nodes evaluated [13].

Whereas vascular involvement has usually been considered a feature of unresectability, reports from Japanese hepatobiliary centers have revealed long-term survival subsequent to isolated portal vein resection [5, 14]. The results of hepatic artery resection have been more disappointing, and most studies recognize no long-term survivors among these patients. Nonetheless, one retrospective report from Nagoya demonstrated a survival advantage with combined portal vein and hepatic artery resection in patients with gross involvement of these vessels [5]. Among 50 treated patients, R0 resection was possible in 30 cases and the 5-year survival rate in this group was 40.7 % against 30.3 % in the entire cohort.

Patients with non-metastatic locally advanced and unresectable disease typically receive palliative chemotherapy and/or radiation therapy (RT). The Mayo clinic, however, has described more promising results with the use of orthotopic liver transplantation (OLT) in selected patients [15].

1.1.2 Intrahepatic Cholangiocarcinoma

Intrahepatic cholangiocarcinoma (IHC) (Fig. 4) is a relatively uncommon disease, and surgical resection seems to be the only therapy associated with long-term survival. Recurrence following resection is, nevertheless, common. Interestingly, retrospective reports demonstrate conflicting result regarding the impact of R0 resection on survival [16, 17]. A review of 44 patients with IHC treated at Johns Hopkins from 1973 to 2004 showed that those with a negative resection margin had a statistically longer median disease-free and overall survival than those undergoing R1/R2 resection or palliative procedures [12]. A similar review at MSKCC from 1990 to 2006, though, demonstrated longer survival in patients treated by surgery (36 months vs. 9 months) but failed to demonstrate any benefit to R0 versus R1 resection [16]. These conflicts were probably due to the small number of patients in these series making them underpowered. In 2011, an international multi-institutional database reviewed data from 449 patients who underwent surgery for ICC between 1973 and 2010 and identified an unquestionable negative impact of positive resection margins on survival, with a hazard ratio of 2.20. Those authors also demonstrated that multinodular

Fig. 1 A Hilar cholangiocarcinoma with portal vein confluence encasement. **a** Abdominal CT showing the conduct of the mass with the portal vein bifurcation and the narrowing of the right anterior branch. **b** Percutaneous trans-hepatic cholangiogram detecting a IIIa tumor. **c** and **d** Intraoperative aspects before and after portal vein resection with end to end anastomosis

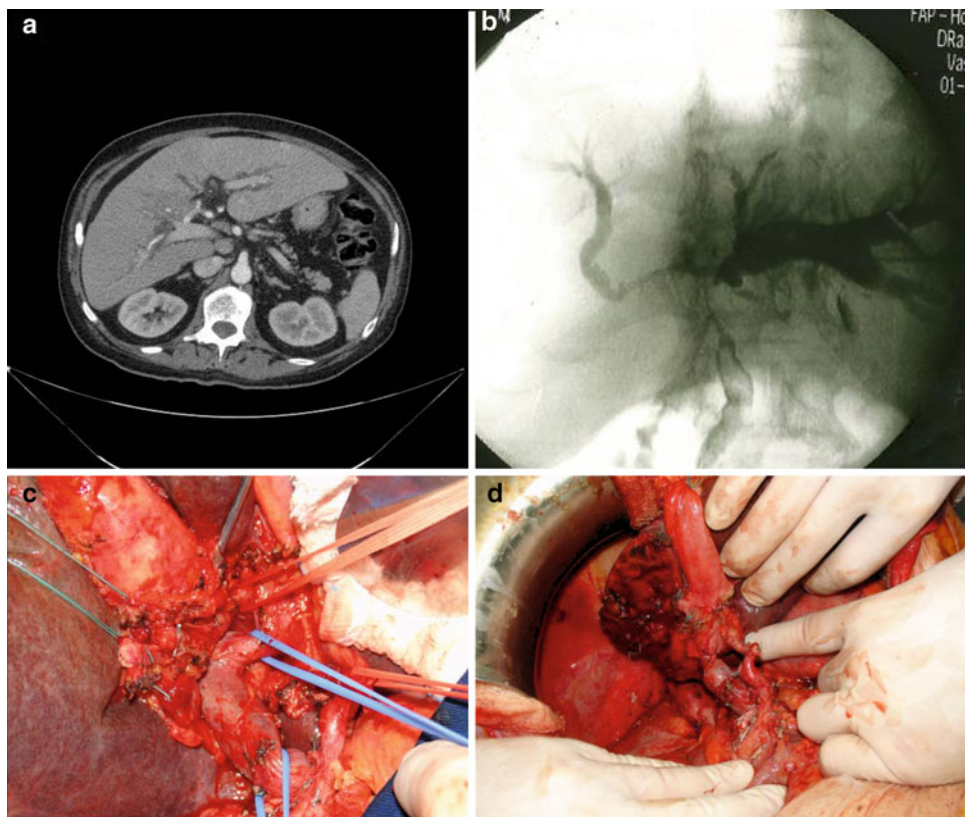


Fig. 2 A Hilar cholangiocarcinoma Bismuth–Corlette type IIIB treated by left lobectomy and caudate resection. **a** Abdominal CT showing the extension of the tumor to the left main duct. **b–d** Intraoperative view after liver transection with hanging liver approach, resection of the caudate lobe, and intrahepatic bilioenteric anastomosis

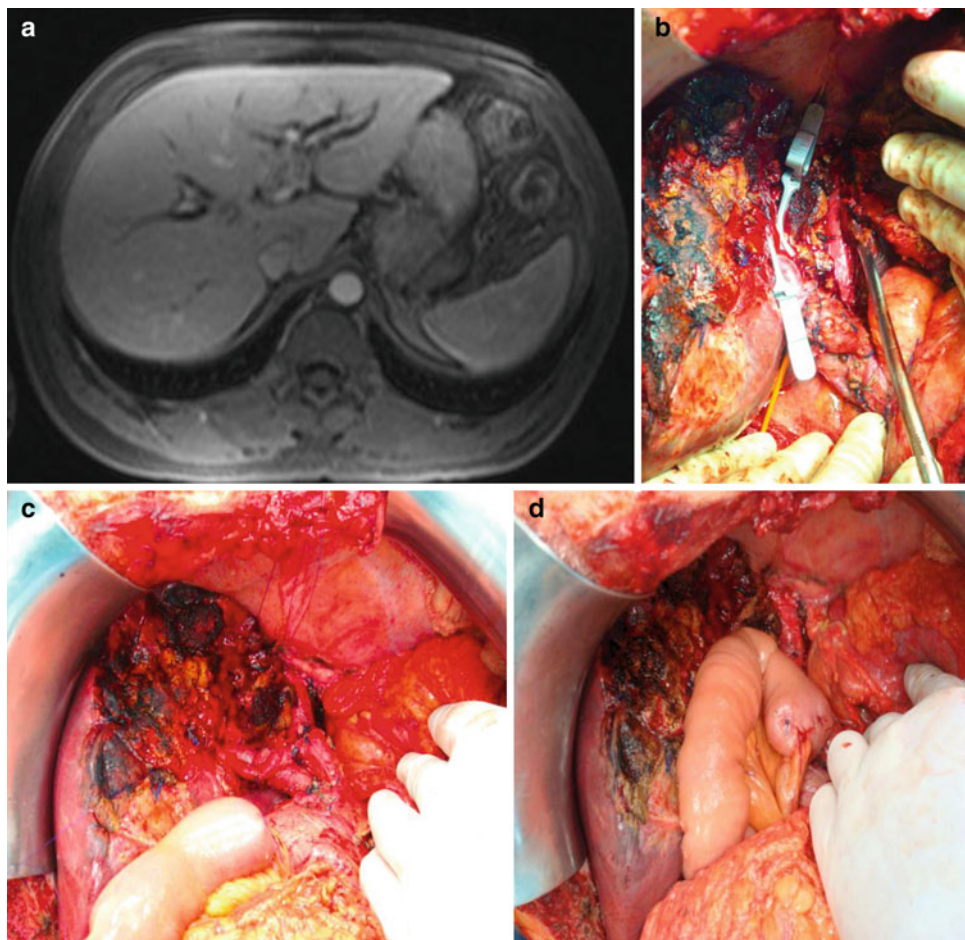


Fig. 3 Radiological and intraoperative aspects of gallbladder cancer. **a** Abdominal CT with a heterogeneous mass in the gallbladder fundus. **b–d** Radical cholecystectomy with *en bloc* resection of segments IVB and V and hilar lymphadenectomy

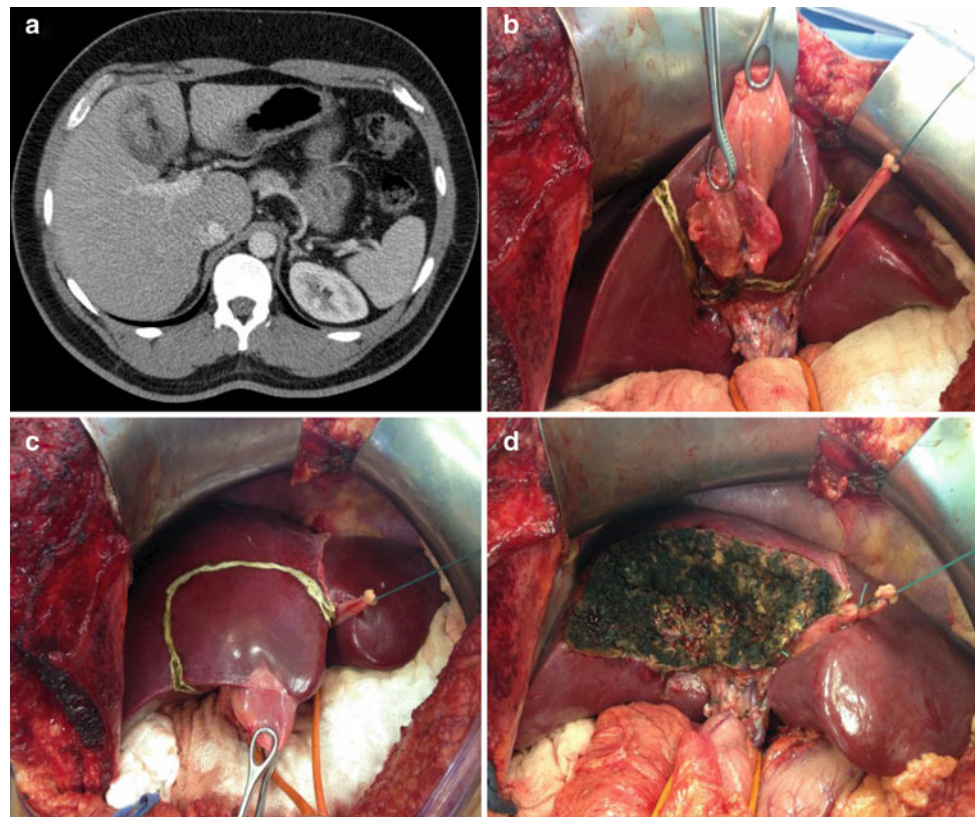


Fig. 4 Radiological aspects on CT and MRI of intrahepatic cholangiocarcinoma. Note the heterogeneous enhancement, left lobe atrophy and biliary dilatation



disease and vascular invasion, but not tumor size, were independent prognostic factors of survival in the absence of lymph node spread. In patients with N positive disease, who correspond to 30 % from those who had had a lymphadenectomy, these factors became the main prognostic factor of survival [18].

Despite the lack of prospective evidence, it seems that achieving a negative margin (R0) at the time of surgery offers a survival advantage over incomplete resection or chemotherapy alone, even if a major hepatic resection is required. For those patients who do experience recurrent disease, some authors have demonstrated that aggressive

treatment of liver recurrences, including re-resections or ablation and further chemotherapy, may provide a survival benefit. A retrospective review from the University of Bologna studied 39 patients with recurrent disease treated between 1988 and 2008 and compared twenty-three cases treated with additional surgery and chemotherapy and 16 patients that received only systemic therapy. The three-year disease-free survival in the first group was 60 % against 0 % among patients with less aggressive approach [17]. The best evidence so far points out that all the efforts to obtain a R0 resection margin are justified in the primary setting and possibly for recurrence.

2 Surgical Results

2.1 Morbidity and Mortality

Due to the extensive surgery that is sometimes necessary that may include biliary and hepatic resections to obtain a R0 procedure, perioperative morbidity and mortality are noteworthy. In the treatment of gallbladder and biliary tract cancers, morbidity and mortality can reach rates of 14–76 and 0–19 %, respectively [19]. The most common complications following these operations are bleeding, biliary fistula, liver failure, and infections, including cholangitis, liver abscess, intra-abdominal abscess, wound infection, and pneumonia. They account for over 50 % of all adverse events [3, 20, 21]. Due to the high perioperative risk, the complexity of surgical procedures required and the rarity of these tumors, these patients should be managed only in tertiary cancer centers. Postoperative hepatic failure and its associated mortality have been associated with the extent of liver resection [22, 23], and various efforts have been employed in order to reduce its incidence.

2.2 Recurrence

Unfortunately, recurrence rates after resection of biliary tract cancer are high, reaching levels of 50–75 %. The most common sites of recurrence following resection include the hepatic pedicle, liver, and peritoneum [20, 24, 25]. Median disease-free survival ranges from 12 to 43 months. Prognostic factors for recurrence include histologic grade, pathologic T and N stage, and margin status [20, 24, 25]. Recently, a multi-institutional database analysis of 301 patients who underwent surgery for ICC pointed the liver as the most common site of recurrence, followed by the combination of intra- and extra-hepatic disease. Furthermore, factors associated with increased risk of recurrence were macrovascular invasion, nodal metastases, unknown nodal status, and tumor size [26]. Owing to the fact that the majority of patients with recurrent disease are not amenable to curative intent, progress in adjuvant therapy is necessary to improving long-term outcome.

2.3 Outcomes

The 5-year survival rates for these patients vary from 25 to 40 % in recent series [19]. Many clinic pathological factors have been shown to have an impact on long-term outcome, including negative histologic margin status, concomitant hepatic resection, absence of nodal involvement, lower AJCC T stage, well-differentiated tumor grade, papillary tumor morphology, and lack of perineural spread [3, 20, 22, 27].

Among these, a complete resection with histologically negative margins is the only modifiable factor and is therefore the primary goal of surgical therapy. Although some authors have identified a benefit of a R1 resection compared with no surgical therapy, a “planned” R1 procedure is still not recommended [20, 28, 29].

3 Emerging Strategies

3.1 Combined Treatment

Along with the better understanding of molecular characteristics and staging of gallbladder and biliary tract cancer, new multimodal treatment strategies to improve the survival have begun to emerge. Hopefully, with more effective systemic therapies, this will allow for more effective selection of patients with loco regional advanced disease suitable for subsequent surgical resection. However, up till now, the role of the combination of non-operative therapies and surgery is not clear.

In the setting of metastatic disease, treatment options include palliative chemotherapy with gemcitabine and platinum-based regimens. Recently, the addition of erlotinib to gemcitabine and oxaliplatin doublet has demonstrated an improved response rate, but no impact on progression-free survival [30]. Many groups have investigated the possibility of using targeted therapies, especially in patients with advanced IHC, but there is no definitive data on to support their use at the current time [31]. Typically, there is no role for surgery for patients with systemic recurrence of biliary tumors, with few cases reported in the literature of patients undergoing surgery for metastatic disease after tumor control with systemic treatment [32].

The liver is the main recurrence site of resected biliary tumors, often as multinodular spread. For IHC, whether this clinical presentation corresponds to primary tumor satellite lesions or foci of intrahepatic metastasis, or even multiple primary tumor foci, is sometimes impossible to determine [33]. Regardless, multi-focal disease—especially multifocal recurrent disease—reflects an aggressive disease biology and a relative contraindication for surgery.

Furthermore, the disappointing results particularly in the setting of locally advanced disease and/or lymph node metastases have fostered the idea of neoadjuvant treatment, in order to increase the rate of complete resections and select the best candidates for surgical approach [34]. Although the literature is very scarce regarding this topic, the same rationale has been used in other tumors with advanced disease and poor prognostic factors, including adenocarcinomas of the esophagus and pancreas, for example.

In a retrospective analysis, this approach did not show any benefit, leading to a possible delay in the surgical

therapy, which may be associated with worse survival [35]. It is noteworthy that, in this series, the patients with the most advanced disease should have been referred for neoadjuvant treatment, which already biases this group to having a worse prognosis thereby perhaps explaining, in part, the inferior results in this group. The current recommendation is the surgical approach for all patients when resection with negative margins can be expected, as long as one can preserve adequate liver volume. The choice of preoperative systemic and loco regional therapy should be reserved only for unresectable cases, including chemotherapy and radiotherapy and/or intra-arterial chemotherapy.

The use of trans-arterial therapy for biliary tract tumors, especially IHCs, has been investigated both for unresectable tumors and in the adjuvant setting. The most frequently used technique consists in the administration of microspheres carried with chemotherapeutic agents, including doxorubicin and gemcitabine, either in an embolization procedure or as continuous infusion chemotherapy. The data are largely from Asian and demonstrate advantage in terms of response rate and progression-free survival in uncontrolled retrospective series for patients with locally advanced disease or metastases confined to the liver [36]. The lesion's size, underlying liver function and tumor vasculature on imaging studies could predict the results obtained with this therapeutic modality.

After a R0 resection, the arterial chemoembolization was related to a longer relapse-free survival in patients with objective worse prognostic factors in a single series; however, it still needs stronger evidence [37].

Other treatment options for unresectable cases are radioembolization with Yttrium and photodynamic therapy, the latter with positive results in terms of progression-free survival in two small prospective and randomized studies [38].

Recently, a multi-institutional analyses from five major American centers compiled data of 198 patients with advanced ICC treated with intra-arterial therapies between 1992 and 2012. The majority of patients received conventional transarterial chemoembolization (cTACE–64.7 %), although 23.2 % had Yttrium-90 radioembolization. Complication rates demonstrated that this is a safe procedure and most patients experienced stable disease or partial response. There was no difference in survival rates regarding the type of intra-arterial therapy, and the results were better for those who had response on image exams.

The supporting data for the addition of systemic therapy and/or radiotherapy after surgical resection of these tumors are heterogeneous, as most of the studies are from single institutions and retrospective. The number of nodes, angiovascular invasion, and lymph node metastases were identified as the main prognostic factors for overall and recurrence-free survival for IHC, with greater impact than other

historical prognostic variables used previously for these neoplasms, as tumor size, for example. Such features should then guide the selection of patients for systemic treatment in research protocols.

The use of chemotherapy alone comes from the extrapolation of results from prospective studies for adjuvant treatment of pancreato-biliary cancer, with different proportions of patients with gallbladder and biliary tumors included in the study population [39]. Likewise, the use of radiation and chemotherapy after surgical resection is based on retrospective analyzes that demonstrated benefits in terms of progression-free and overall survival [40, 41].

3.2 Preoperative Management

3.2.1 Preoperative Biliary Drainage

The impact of preoperative biliary drainage on outcome is controversial [24, 42, 43]. Preoperative biliary drainage is associated with an increased risk of cholangitis, lengthened postoperative hospital stay, and may hamper the ability to determine the extent of the tumor during surgery. Conversely, unrelieved biliary obstruction is correlated with hepatic and renal dysfunction and coagulopathy [24, 44, 45].

Some authors attest that patients with hilar cholangiocarcinoma will benefit from biliary drainage of the anticipated remnant liver to enhance its capacity for post-resection hypertrophy. Because of potential difficulties in effective endoscopic stent insertion, and to optimally define the intrahepatic biliary anatomy, biliary drainage for hilar cholangiocarcinoma is often performed percutaneously. Described morbidity of percutaneous trans-hepatic catheter location includes the following: hemobilia, hepatic artery pseudoaneurysm, intrahepatic arteriovenous fistula, and catheter tract dissemination [46–48].

3.2.2 Portal Vein Embolization

Resection of >80 % of the total liver volume is correlated with major complications and prolonged hospital stay for patients with normal liver function [49, 50], and resection of >60 % of the total liver volume is associated with increased major complications, postoperative liver failure, and mortality in patients with compromised liver function secondary to chronic liver disease, chronic biliary obstruction, or high-dose chemotherapy [51]. Preoperative portal vein embolization (PVE) was first described in 1986 and is currently used to increase the volume and function of the future liver remnant (FLR) [52]. This strategy has been used prior to major hepatic resection for hilar cholangiocarcinoma, hepatocellular carcinoma, and hepatic resection of colorectal metastases [50].

Numerous studies have found that portal vein embolization accelerates hepatic mitochondrial function and

induces hepatocyte proliferation in the non-embolized segments [53, 54]. The potential benefits of PVE are its capacity to induce hypertrophy in the FLR, thereby reducing the risk of postoperative liver failure, and its ability to permit curative resection for patients who otherwise would be considered unresectable due to insufficient FLR.

Prospective randomized trials and single institutional series support the safety and efficacy of preoperative PVE [55, 56]. A potential disadvantage of performing PVE is that it is sometimes difficult to determine preoperatively whether a right or left hemihepatectomy will be required if the tumor is located centrally at the hilus. Currently, there is no evidence to support routine use of PVE for hilar cholangiocarcinoma, but PVE should be considered for potentially resectable patients with normal liver function when anticipated FLR <20 % of the total liver volume, or patients with compromised liver function when anticipated FLR <40 % of the total liver volume. Most patients with hilar cholangiocarcinoma present with jaundice and are considered to have cholestasis-induced compromised liver function.

Recently, a German group [57] has introduced a rescue technique of accelerated liver regeneration to perform extended hepatectomy by right portal vein ligation combined with in situ splitting the liver. This new two-staged procedure, known as “Associating Liver Partition and Portal Vein Ligation for Staged Hepatectomy (ALPPS)”, may offer a new approach to treat patients with peri-hilar cholangiocarcinoma [58]. However, a call for caution has been made with the new ALPPS approach because of a high postoperative morbidity (70–90 %) and even mortality. Further data are necessary before ALPPS can be recommended for the treatment of patients with PHC [57–59].

3.3 Minimally Invasive Surgery

There is very little evidence in the literature regarding laparoscopy procedures to treat biliary tract cancer. Performing major laparoscopic hepatectomy remains a challenge among most hepatobiliary surgeons. The technical complexity and infrequent approach to this tumor and technique, along with the fact of unanswered questions about potential worse oncological results in good prognosis patients discourages most surgeons. Laparoscopic liver resection has become an acceptable and safe alternative to open procedure for the treatment of benign and selected malignant liver tumors, though. Recent studies show morbidity and mortality rates similar to conventional open procedures. Technical experience in liver surgery and laparoscopy are necessary for performing laparoscopic liver resections successfully. Lobectomies should only be

accomplished laparoscopically after gaining experience with smaller laparoscopic liver resections.

Kazaryan et al. [60] published their 10 year experience from a center in Norway with laparoscopic liver resections for benign and malignant liver tumors. One hundred and thirteen patients underwent 121 procedures, the vast majority of the sample corresponding to liver metastases of colorectal adenocarcinoma; there were only two cases of laparoscopic resection for cholangiocarcinoma. The rate of conversion to laparotomy surgery was 3.4 %, with a mean operative time of 164 min (50–488) and estimated blood loss of 350 mL (<50–4,000). There were ten intraoperative complications (10 %) and 18 postoperative complications (12.6 %). Only one patient died (0.7 %). The mean hospital stay was 3 days (1–42), and the need for opioids for pain control was 1 day (0–11). Free margins of resection determined by pathological examination were successful in 140 of the 149 procedures performed (94 %).

Gumbs et al. [61] recently, in a review showing the experience of three centers with expertise in hepatobiliary laparoscopic surgery, analyzed all patients with tumors of the gallbladder, hilar, and IHC undergoing laparoscopic surgery with curative intent; they excluded patients with distal cholangiocarcinoma undergoing duodenopancreatectomy. Fifteen patients underwent laparoscopic surgery for gallbladder cancer, with the average number of dissected nodes being 4 (1–11) and all patients had an R0 resection. Only one case was converted to open procedure (7 %). No patients developed biliary fistula, percutaneous drainage, and/or passages of drains/stents in the biliary tract. There was local recurrence in one case, despite the free surgical margins, and distant recurrence another one after 20 months of follow-up.

Nine patients underwent hepatectomy for IHC, and all bilio-enteric anastomoses were performed laparoscopically. Two patients developed biliary leakage, one of them died after percutaneous biliary drainage due to intracavitary hemorrhage secondary to uncontrolled bleeding even after laparotomy. A third patient developed pulmonary thromboembolism. The morbidity rate was 33 and 11 % mortality. Only one case was converted to open surgery, and six patients remain alive after a mean follow-up of 22 months [61].

Five patients with hilar cholangiocarcinoma underwent minimally invasive liver resections, and two of them were in need of major liver resections. The estimated blood loss was 240 ml (0–400), and mean hospital stay was 15 days (11–21). All patients were alive after a median follow-up of 11 months; no recurrences were detected in any portal sites operated in 29 cases [61]. There is nothing significant to mention about robotic surgery for cholangiocarcinoma in the literature, except a few case reports so far.

3.4 Staging Laparoscopy

Despite exhaustive preoperative imaging studies, a significant proportion of patients is found to have unresectable disease at the time of laparotomy [3, 20]. Of the patients who are explored with curative intent only 40–50 % are ultimately resectable, which has motivated close evaluation of the role of staging laparoscopy for patients with hilar cholangiocarcinoma. The yield and accuracy of laparoscopy for patients with hilar cholangiocarcinoma are between 25 to 42 and 42 to 53 %, respectively [62]. Laparoscopic ultrasonography can also be used and has been shown to increase the yield by up to 17 % [63]. To use this technology selectively in patients with a higher likelihood of harboring occult metastases, the MSKCC staging system has been used to predict findings of occult metastases at laparoscopy. In patients with T2/T3 tumors, 36 % had occult metastases detected at laparoscopy versus 9 % in patients with T1 tumors ($p = 0.02$) [64], suggesting that laparoscopy should be used for patients with T2/T3 tumors.

3.5 Extension of Resection

3.5.1 Hepatic Resection

Over the past 20 years, there has been an increase in the use of hepatic resection in patients with hilar cholangiocarcinoma. Major hepatic resection addresses both direct hepatic invasion and intraductal extension of hilar cholangiocarcinoma to achieve negative radial and longitudinal resection margins. The inclusion of major hepatic resection as a fundamental surgical strategy for this disease has improved the proportion of R0 resections [3, 20, 22, 27], enhanced recurrence-free survival outcomes, and decreased the prevalence of hepatic recurrences [20]. Interestingly, many reports have shown improved survival with major hepatic resection even in the patients undergoing R0 resections, and poor outcome associated with extra-hepatic bile duct excision alone [3, 20, 22, 65].

However, there is still some debate about the impact of hepatic resection in survival of patients with Bismuth and Corlette type I or II tumors, specially type I. Ikeyama et al. [66] retrospectively assessed surgical outcome of 54 patients with Bismuth and Corlette type I and II hilar cholangiocarcinoma and showed survival benefit from right hepatectomy with caudate lobectomy for nodular and sclerosing tumors, but not for papillary tumors. Other group has reported no significant difference in survival between hepatectomy and bile duct resection alone for B–C type I and II tumors [67]. This needs to be further weighed in larger studies with longer follow-up, but for now, most experts

agree that liver resection is necessary for hilar cholangiocarcinoma. Moreover, extra-hepatic bile duct resection was associated with a greater risk of positive resection margins and worse lymph node clearance in a recent multi-institutional series [68].

For T1a gallbladder tumors, there is no need for hepatic resection. However, to T1b, when the muscularis is invaded, most centers agree with that. In published series, the five-year survival rate for patients with T1b gallbladder cancer having undertaken radical resection averages 87.5 %, whereas it averages only 61.3 % in patients who have been submitted only to cholecystectomy [69]. A recent published decision analysis suggests that radical surgery for T1b tumor, just as stage II, is related to improved survival if compared with cholecystectomy alone [69]. Tumors greater than T1a, therefore, must be treated with standardized liver resection and lymphadenectomy. The hepatic resection must include segments V and IVb in most series. It is still controversial whether the anatomical resection of segments V and IVb is superior to gallbladder bed resection. In a recent analysis of a nationwide data from the Japanese Biliary Tract Cancer Registry, the data of 85 patients with pT2 gallbladder cancer patients were retrospectively compared: fifty-five treated with gallbladder bed resection, and 30 with S4a + 5 hepatectomy. The five-year survival rate did not differ significantly between the two groups. Recurrence occurred most frequently in both lobes than in S4a + 5 of the liver following gallbladder bed resection. They concluded that S4a + 5 hepatectomy was not superior to gallbladder bed resection alone for those cases [70].

In the case of direct invasion of right pedicle or deep liver invasion, an extended right trisectionectomy, in a fit patient with localized disease, must be performed. In this situation, also adjacent structures, as hepatic flexure of the colon, should be resected *en bloc*. Long-term survival has been reported from some centers, ranging from 15 to 63 %, with these extended procedures [71].

3.5.2 Caudate Lobe Resection

The caudate lobe ducts join the left and right hepatic ducts near their confluence, although the primary drainage is to the left hepatic duct [20, 72]. This intimate anatomical relationship explains the remark that the caudate lobe is involved by hilar cholangiocarcinoma in 40–98 % of patients [20, 72–74]. Retrospective studies have shown a decrease in local recurrence and improvement in five-year survival when concomitant caudate lobe resection is performed [24, 75, 76]. Routine caudate lobe resection, without direct invasion, however, remains controversial. Most institutions perform caudate lobe resection selectively, depending on tumor location, preferably with left-sided tumor.

3.5.3 Vascular resection

Portal vein resection and reconstruction have been performed for hilar cholangiocarcinoma with conflicting results [13, 22, 77, 78]. Although several retrospective series have shown no difference in operative mortality between patients undergoing portal vein resection and patients who did not [22, 77, 78], the influence of portal vein resection on long-term survival is less clear. Neuhaus et al. [22] proposed portal vein resection as part of the “no-touch” technique for the management of tumor and adjacent tissue. Portal vein resections were identified as an independent positive prognostic factor in their multivariate analysis of patients undergoing R0 resection, when initial 60-day mortality was excluded. However, overall 60-day mortality after portal vein resection was 17 % as compared with 5 % for patients without portal vein resection, and all of these deaths occurred after noncurative surgeries. Many other studies have shown equivalent or worse survival in patient undergoing *en bloc* resection of the portal vein [78–80]. Recently, de Jong et al. [68] identified 51 patients who underwent portal vein resection in an international multi-institutional database of 305 cases of hilar cholangiocarcinoma. In these series, PVR was most likely associated to a right-side hepatectomy and allowed a better lymph node clearance (median number of lymph node resected—6 vs. 4 in patients who underwent only bile duct and liver resection, $p = 0.03$). The incidence of R0 resection was not different between patients who had or not PVR, as well as the five-year survival rate. The 30-day mortality was, however, significantly higher in the group of PVR (6.7 vs. 11.8 %, $p = 0.03$). It is noteworthy that those rates were no longer significantly different when the 90-day mortality was analyzed. Those authors could also demonstrate that the survival rate in the group of patients who had disease that grossly involved the main portal vein and necessitate PVR was comparable with the five-year survival rate for the patients who underwent a “non-touch” technique, it means those who probably received a prophylactic PVR, as proposed by the German group. It is likely that the role of routine resection of the portal vein will not be directly defined unless a randomized clinical trial can be completed.

3.5.4 Pancreas resection

Distal tumors are often considered to be periampullar tumors, as it is difficult to characterize their origins before resection. Most cases are treated with classical pancreaticoduodenectomies with or without pylorus preserving and standard lymphadenectomy [12, 81, 82]. During the past decade, more aggressive approaches, combining pancreaticoduodenectomy with portal vein resection, have gained wider appreciation. Pancreatic fistula is still the Achilles’ heel of this surgery and varies from 3 to 30 % depending on

the expertise of the surgical team and the stiffness of the pancreas, whereas perioperative mortality is presently less than 5 % [82, 83]. The five-year survival rate is about 30 % [12, 84]. The most important prognostic factor is a negative resection margin [12, 83, 85].

3.5.5 Hepato-pancreato-duodenectomy

Major hepatectomy can sometimes be combined with pancreaticoduodenectomy in the presence of positive proximal bile duct margin when extending to only one hemiliver [86]. This procedure, sometimes labeled as hepatopancreato-duodenectomy (HPD), remains controversial and performed in only a few centers because of a high rate of mortality (15–60 %). Two recent series have shown that R0 resection was possible in between 73 and 85 % of the cases [87, 88]. The largest series, presented by Ebata et al. [88], reported on 26 cases of distal cholangiocarcinoma with increasing R0 resection after HPD and five-year patient survival rate of 37.5 %. The main drawback of such an approach was high morbidity of 77 %, but low mortality of 2.4 %.

Another recent study shows a similar experience, but with a higher mortality rate of 13 % [87]. The incidence of severe complications must be anticipated with this type of surgery, ranging from 31 to 100 % [86, 88]. In summary, this aggressive approach must be restricted to a few large-volume centers, and only for the purpose of a R0 resection. Perioperative mortality rates should ideally not exceed 5 %, and no longer be as high as 50 % as in historical series [89]. The approach warrants further investigation including the role of neoadjuvant or adjuvant chemotherapies.

3.6 Lymph Node Dissection

Metastasis to regional lymph nodes in hilar cholangiocarcinoma is common and is an important prognostic factor influencing survival after resection for hilar cholangiocarcinoma and gallbladder cancer [20, 90, 91]. Kitagawa et al. [92] evaluated 110 patients who underwent surgical resection with lymph node dissection counting both the regional and para-aortic nodes for hilar cholangiocarcinoma and found that 47 % had no involved nodes, 35 % had regional nodal metastases, and 17 % had regional and para-aortic nodal metastases. The five-year survival was 30 % for node-negative patients, 15 % for the patients with regional nodal metastases, and 12 % for those with para-aortic nodal metastases. Other studies have shown poor survival for those with nodal involvement beyond the hepatoduodenal ligament with five-year survival of 0–6 % [20, 29, 90, 91]. Consequently, routine lymph node dissection beyond the hepatoduodenal ligament is not recommended. Patients with grossly involved lymph nodes beyond the hepatoduodenal ligament are considered to have unresectable disease.

In the setting of gallbladder cancer, extended lymph node dissection is well established and should always be performed with T1b or greater and must include porta-hepatis, gastro-hepatic ligament, and retro-duodenal region. Many studies have evaluated the importance of portal lymph node dissection in the survival of gallbladder cancer. Recently, Jensen et al. [7] analyzed the data from the SEER neoplasm registry to identify patients who had an operation for gallbladder cancer between 1988 and 2004. Patients were classified by stage of disease, operative procedure performed (cholecystectomy alone or radical resection), number of LNs evaluated (0, 1, >1), and receipt of radiation (RT). They observed that LN evaluation was still associated with a decrease in mortality compared with no LN evaluated (HR = 0.611; 95 % CI = 0.484, 0.770). The pathologic evaluation of additional LN (>1) did not provide any additional benefit compared with the evaluation of a single node (HR = 0.795; 95 % CI = 0.571, 1.107). Radical resection alone (without LN evaluation) did not provide any benefit over cholecystectomy alone (HR = 1.098; 95 % CI = 0.971, 1.241).

Lymph node toilette in intrahepatic cholangiocarcinoma used to be more controversial. However, recent data are pushing in this direction, showing its importance not only on staging but also in the treatment of this disease. In 2011, the first large retrospective cohort of patients with ICC dedicated to analyze prognostic factors and lymph node assessment [18] demonstrated that only 55 % of cases had a lymphadenectomy performed. In this group, an incidence of 30 % of positive lymph nodes was found with a significant worse survival rate. More remarkable was the fact that no preoperative accessible characteristics could predict the occurrence of lymph node metastases, such as size, multiplicity of the nodules, and gross morphology. Thus, the authors conclude that lymph node dissection should be routinely performed in patients with ICC who underwent surgical treatment. Ribero et al. in a recent publication (2012), evaluating 434 patients with IHC, from sixteen tertiary referrals center in Italy, also demonstrated that only two-thirds of patients received a lymphadenectomy as part of surgical procedure. Although the incidence of lymph node metastases (overall, 36.9 %) increased with tumor size, 24.4 % of patients with a small ICC (diameter ≤ 3 cm) had N1 disease. Lymph node metastases were an independently prognostic factor (hazard ratio, 2.21; $p = 0.001$), and the potential survival benefit of a lymphadenectomy was assessed with the therapeutic value index, which was calculated to be 5.9 points. These new emerging data are supporting the trend of many specialized centers to routinely consider lymphadenectomy for all patients [93, 94].

3.7 Liver Transplantation

In the treatment of hilar cholangiocarcinoma, OLT offers the advantage of resection of all structures that may be involved by tumor, including portal vein, bilateral hepatic ducts, and atrophic liver lobes. Thus, total hepatectomy may permit R0 resection even in very locally advanced tumors that are beyond the criteria for resection. Unfortunately, the early experience with OLT for hilar cholangiocarcinoma was disappointing [95–97].

The Cincinnati Transplant Tumor Registry reported 28 % five-year survival with a 51 % tumor recurrence rate [96]. Spanish liver transplant centers reported similar results of 30 % five-year survival and 53 % tumor recurrence rate for 36 patients with non-disseminated, unresectable hilar cholangiocarcinoma [98]. Consequently, in reason of these early results and the limited availability of organs, hilar cholangiocarcinoma was felt to be a relative contraindication to OLT.

Recently, the so-called “Mayo protocol” has been developed with the intent of treating a highly selected group of patients with hilar cholangiocarcinoma with a strict regimen of preoperative staging and neoadjuvant treatment followed by OLT [99]. This protocol was developed at the Mayo Clinic to treat selected patients with unresectable hilar cholangiocarcinoma or hilar cholangiocarcinoma arising in a setting of primary sclerosing cholangitis. Inclusion criteria include the following: locally advanced unresectable disease with, positive intraluminal brush cytology, positive intraluminal biopsy, or CA19-9 >100 in the setting of a radiographic malignant stricture; primary sclerosing cholangitis with resectable disease and absence of medical contraindications for OLT. Since 2003, biliary aneuploidy as demonstrated by digital image analysis and fluorescent in situ hybridization has been considered equivalent to cytology. Exclusion criteria include the following: extra-hepatic disease including regional lymph node involvement; uncontrolled infection; prior attempt at resection; prior treatment with radiation or chemotherapy, and; previous malignancy within 5 years. In this protocol, patients receive external beam radiotherapy to a target dose of 4,500 cGy with concomitant fluorouracil (5-FU). Following this, transcatheter Iridium-192 brachytherapy, with a target dose of 2,000–3,000 cGy, is administered. Thereafter, patients receive oral capecitabine as tolerated until transplantation. Importantly, prior to transplantation, patients undergo a staging laparotomy, at which time biopsy of perihilar lymph nodes as well as any lymph nodes or nodules suspicious for tumor is performed. Only patients with negative staging operations remain eligible for transplantation.

Therefore, patients eligible for OLT under this protocol have locally advanced tumors but no pathologic nodal

disease. Moreover, the prolonged course of neoadjuvant therapy, staging laparotomy, and time on the OLT waiting list provides an opportunity to exclude patients demonstrating disease progression. This highly rigorous selection bias in favor of patients with biologically favorable disease is reflected in the early outcomes published from the Mayo group. In 38 patients who underwent this protocol, 82 % 5-year survival was reported [15] (as compared with 21 % 5-year survival after resection, which included patients with nodal disease, $p < 0.022$). The patients who ultimately underwent OLT were generally young (mean age 48 years). Pathologic analysis of resected specimens confirmed N0 and R0 status in all patients. However, only 58 % patients had histologically proven cancer. Later outcomes on 65 patients under this protocol showed a one-year survival of 91 and 76 % at 5 years (mean FU 32 months) [100].

Cholangiocarcinoma complicating primary sclerosing cholangitis often results in the discovery of tumors at an advanced stage, which preclude effective therapy. Rosen et al. [101] investigated 70 patients with primary sclerosing cholangitis prospectively for an average of 30 months, and found that cholangiocarcinoma was present in at least 7 % of the patients and 42 % of the autopsied patients. Another report showed 10 % of patients with primary sclerosing cholangitis undergoing liver transplantation had an unsuspected cholangiocarcinoma [102].

Recent data from 12 large-volume transplant centers in the United States with perihilar cholangiocarcinoma using neoadjuvant therapy followed by liver transplantation confirm these results. Analyzed from 1993 to 2010 were 287 patients with variable neoadjuvant protocols. The patients completed external radiation (99 %), brachytherapy (75 %), radiosensitizing therapy (98 %), and/or maintenance chemotherapy (65 %). Seventy-one patients dropped out before liver transplantation (rate, 11.5 % in 3 months). Intent-to-treat survival rates were 68 and 53 %, 2 and 5 years after therapy, respectively; post-transplant, recurrence-free survival rates were 78 and 65 %, respectively [103].

In June 2009, the United Network of Organ Sharing/Organ Procurement and Transplantation Network (UNOS/OPTN) approved the allocation of a standard model of end-stage liver disease (MELD) exception score for patients with perihilar cholangiocarcinoma who completed an approved neoadjuvant therapy protocol. The MELD score was set to equal the current standard assigned score for hepatocellular carcinoma [104].

Because of neoadjuvant RT, particular attention needs to be paid to late effects of radiation injury. There is an increased incidence of late vascular complications in this patient population. As for the frequent hepatic artery

thrombosis when the hepatic artery is used for deceased donor arterial reconstruction, some transplant groups preferentially performs arterial reconstruction with a donor iliac artery interposition graft to the infra-renal aorta. When an interposition graft is used, the arterial complication rate in patients transplanted for CCA is significantly decreased. These patients remain at risk of late portal venous stenosis, and this complication has been successfully treated by transhepatic transportal angioplasty and intraluminal stent insertion. However, there are technical challenges given the short vessels of a living donor allograft and vascular complications occur at a higher than deceased donor allograft. If detected in time by a Doppler study, the stenting of the artery can often prevent early graft loss or the late sequelae of chronic biliary injury. When pancreatoduodenectomy is required, there is a high risk of a pancreatic leak or a vascular complication because of the proximity of the pancreatic and vascular anastomosis.

Despite the great advances in survival in this specific population, at present, OLT cannot be considered a standard form of therapy for hilar cholangiocarcinoma for patients with resectable disease, but it does offer a potential option for patients with underlying primary sclerosing cholangitis.

Currently, data suggest that highly selected patients with unresectable early stage hilar CCA may benefit from OLT, with notably improved outcomes and renewed interest in OLT over the past decade. Therefore, the challenges for the future still remain and should be based on continuing understanding of the tumor biology and reducing wait-list drop-out and post-transplant recurrence either by further refinements in patient selection, immunosuppression, or, ideally, by more effective chemo radiotherapy. Further studies are needed to fully define the role of OLT in this setting.

4 Conclusion

While there has been substantial progress in the understanding of the biology of these tumors and in their classification and sub-categorization, this knowledge has yet to be translated into curative therapy for all but the most fortunate patients with early stage disease. Surgical resection remains the mainstay of treatment of biliary tract cancer. Negative resection margins enhanced by major hepatic resections are associated with improved outcomes. One should always be considered for resection if R0 margins can be anticipated. Lymph node hilum dissection should be accomplished in all curative cases. Pre-resectional management with biliary drainage, portal vein embolization, and staging laparoscopy should be considered in selected

patients. Additional evidence is needed to fully define the role of OLT in biliary tumors, but the most recent protocol seems to help selecting good outcome cluster of patients. Improvements in adjuvant therapy are necessary for improving long-term outcome, and multidisciplinary care of each of these complex patients must be encouraged.

References

- Khan SA, Davidson BR, Goldin R, Pereira SP, Rosenberg WM, Taylor-Robinson SD et al (2002) Guidelines for the diagnosis and treatment of cholangiocarcinoma: consensus document. *Gut* 51(suppl VI):i1–i9. doi:10.1136/gut.51.suppl_6.vi1
- Seyama Y, Makuuchi M (2007) Current surgical treatment for bile duct cancer. *World J Gastroenterol* 13:1505–1515
- Jarnagin WR, Fong Y, DeMatteo RP, Gonen M, Burke EC, Bodniewicz BSJ et al (2001) Staging, resectability, and outcome in 225 patients with hilar cholangiocarcinoma. *Ann Surg* 234:507–517
- Neuhaus P, Jonas S, Settmacher U, Thelen A, Benckert C, Lopez-Hänninen E et al (2003) Surgical management of proximal bile duct cancer: extended right lobe resection increases resectability and radicality. *Langenbecks Arch Surg* 388:194–200. doi:10.1007/s00423-003-0383-5
- Nagino M, Nimura Y, Nishio H, Ebata T, Igami T, Matsushita M et al (2010) Hepatectomy with simultaneous resection of the portal vein and hepatic artery for advanced perihilar cholangiocarcinoma: an audit of 50 consecutive cases. *Ann Surg* 252:115–123. doi:10.1097/SLA.0b013e3181e463a7
- Rea DJ, Rosen CB, Nagorney DM, Heimbach JK, Gores GJ (2009) Transplantation for cholangiocarcinoma: when and for whom? *Surg Oncol Clin N Am* 18:325–337. doi:10.1016/j.soc.2008.12.008
- Jensen EH, Abraham A, Jarosek S, Habermann EB, Al-Refaie WB, Vickers SA et al (2009) Lymph node evaluation is associated with improved survival after surgery for early stage gallbladder cancer. *Surgery* 146:706–711. doi:10.1016/j.surg.2009.06.056 (discussion 711–713)
- Singh S, Agarwal AK (2007) Gallbladder cancer: the role of laparoscopy and radical resection. *Ann Surg* 250:494–495. doi:10.1097/SLA.0b013e3181b490d9 (author reply 495)
- Marsh Rde W, Alonzo M, Bajaj S, Baker M, Elton E, Farrell TA et al (2012) Comprehensive review of the diagnosis and treatment of biliary tract cancer 2012. Part II: multidisciplinary management. *J Surg Oncol* 106:339–345. doi:10.1002/jso.23027
- Hueman MT, Vollmer CM Jr, Pawlik TM (2009) Evolving treatment strategies for gallbladder cancer. *Ann Surg Oncol* 16:2101–2115. doi:10.1245/s10434-009-0538-x
- Dixon E, Vollmer CM Jr, Sahajpal A, Cattral M, Grant D, Doig C et al (2005) An aggressive surgical approach leads to improved survival in patients with gallbladder cancer: a 12-year study at a North American Center. *Ann Surg* 241:385–394. doi:10.1097/01.sla.0000154118.07704.ef
- DeOliveira ML, Cunningham SC, Cameron JL, Kamangar F, Winter JM, Lillemoe KD et al (2007) Cholangiocarcinoma: thirty-one-year experience with 564 patients at a single institution. *Ann Surg* 245:755–762. doi:10.1097/01.sla.0000251366.62632.d3
- Miyazaki M, Kato A, Ito H, Kimura F, Shimizu H, Ohtsuka M, Yoshidome H et al (2007) Combined vascular resection in operative resection for hilar cholangiocarcinoma: does it work or not? *Surgery* 141:581–588. doi:10.1016/j.surg.2006.09.016
- Ito K, Ito H, Allen PJ, Gonen M, Klimstra D, D'Angelica MI et al (2010) Adequate lymph node assessment for extrahepatic bile duct adenocarcinoma. *Ann Surg* 251:675–681. doi:10.1097/SLA.0b013e3181d3d2b2
- Rea DJ, Heimbach JK, Rosen CB, Haddock MG, Alberts SR, Kremers WK et al (2005) Liver transplantation with neoadjuvant chemoradiation is more effective than resection for hilar cholangiocarcinoma. *Ann Surg* 242:451–458 (discussion 458–461)
- Endo I, Gonen M, Yopp AC, Dalal KM, Zhou Q, Klimstra D et al (2008) Intrahepatic cholangiocarcinoma: Rising frequency, improved survival, and determinants of outcome after resection. *Ann Surg* 248:84–96. doi:10.1097/SLA.0b013e318176c4d3
- Ercolani G, Vetrone G, Grazi GL, Aramaki O, Cescon M, Ravaioli M et al (2010) Intrahepatic cholangiocarcinoma: primary liver resection and aggressive multimodal treatment of recurrence significantly prolong survival. *Ann Surg* 252:107–114. doi:10.1097/SLA.0b013e3181e462e6
- de Jong MC, Nathan H, Sotiropoulos G, Paul A, Alexandrescu S, Marques H et al (2011) Intrahepatic cholangiocarcinoma: an international multi-institutional analysis of prognostic factors and lymph node assessment. *J Clin Oncol* 29:3140–3145
- Ito F, Cho CS, Rikkers LF, Weber SM (2009) Hilar cholangiocarcinoma: current management. *Ann Surg* 250:210–218. doi:10.1097/SLA.0b013e3181afe0ab
- Ito F, Agni R, Rettammel RJ, Been MJ, Cho CS, Mahvi DM et al (2008) Resection of hilar cholangiocarcinoma: concomitant liver resection decreases hepatic recurrence. *Ann Surg* 248:273–279. doi:10.1097/SLA.0b013e31817f2bfd
- Nimura Y, Kamiya J, Kondo S, Nagino M, Uesaka K, Oda K et al (2000) Aggressive preoperative management and extended surgery for hilar cholangiocarcinoma: nagoya experience. *J Hepatobiliary Pancreat Surg* 7:155–162
- Neuhaus P, Jonas S, Bechstein WO, Lohmann R, Radke C, Kling N et al (1999) Extended resections for hilar cholangiocarcinoma. *Ann Surg* 230:808–818
- Gerhards MF, van Gulik TM, de Wit LT, Obertop H, Gouma DJ (2000) Evaluation of morbidity and mortality after resection for hilar cholangiocarcinoma—a single center experience. *Surgery* 127:395–404. doi:10.1067/msy.2000.104250
- Rea DJ, Munoz-Juarez M, Farnell MB, Donohue JH, Que FG, Crownhart B et al (2004) Major hepatic resection for hilar cholangiocarcinoma: analysis of 46 patients. *Arch Surg* 139:514–523. doi:10.1001/archsurg.139.5.514
- Park SW, Park YS, Chung JB, Kang JK, Kim KS, Choi JS et al (2004) Patterns and relevant factors of tumor recurrence for extrahepatic bile duct carcinoma after radical resection. *Hepatogastroenterology* 51:1612–1618
- Hyder O, Hatszaras I, Sotiropoulos G, Paul A, Alexandrescu S, Marques H et al (2013) Recurrence after operative management of intrahepatic cholangiocarcinoma. *Surgery* 153:8–18
- Lee SG, Lee YJ, Park KM, Hwang S, Min PC (2000) One hundred and eleven liver resections for hilar bile duct cancer. *J Hepatobiliary Pancreat Surg* 7:135–141
- Hemming AW, Reed AI, Fujita S, Foley DP, Howard RJ (2005) Surgical management of hilar cholangiocarcinoma. *Ann Surg* 241:693–699
- Todoroki T, Kawamoto T, Koike N, Takahashi H, Yoshida S, Kashiwagi H et al (2000) Radical resection of hilar bile duct carcinoma and predictors of survival. *Br J Surg* 87:306–313. doi:10.1046/j.1365-2168.2000.01343.x
- Lee J, Park SH, Chang HM, Kim JS, Choi HJ, Lee MA et al (2012) Gemcitabine and oxaliplatin with or without erlotinib in advanced biliary-tract cancer: a multicentre, open-label, randomised, phase 3 study. *Lancet Oncol* 13:181–188. doi:10.1016/S1470-2045(11)70301-1

31. Faris JE, Zhu AX (2012) Targeted therapy for biliary tract cancers. *J Hepatobiliary Pancreat Sci* 19:326–336. doi:[10.1007/s00534-011-0496-0](https://doi.org/10.1007/s00534-011-0496-0)
32. Sanada T, Baba H, Baba H, Wakabayashi M, Nakamura H, Kuwabara H et al (2011) Curative resection of gallbladder cancer with simultaneous liver metastasis (abstract). *Gan To Kagaku Ryoho* 38:2433–2435
33. Tsai S, Nathan H, Pawlik TM (2010) Primary liver cancer: intrahepatic cholangiocarcinoma emerges from the shadows. *Updates Surg* 62:5–9. doi:[10.1007/s13304-010-0011-1](https://doi.org/10.1007/s13304-010-0011-1)
34. Nelson JW, Ghafoori AP, Willett CG, Tyler DS, Pappas TN, Clary BM et al (2009) Concurrent chemoradiotherapy in resected extrahepatic cholangiocarcinoma. *Int J Radiat Oncol Biol Phys* 73:148–153. doi:[10.1016/j.ijrobp.2008.07.008](https://doi.org/10.1016/j.ijrobp.2008.07.008)
35. Glazer ES, Liu P, Abdalla EK, Vauthey JN, Curley SA (2012) Neither neoadjuvant nor adjuvant therapy increases survival after biliary tract cancer resection with wide negative margins. *J Gastrointest Surg* 16:1666–1671. doi:[10.1007/s11605-012-1935-1](https://doi.org/10.1007/s11605-012-1935-1)
36. Kim JH, Yoon HK, Sung KB, Ko GY, Gwon DI, Shin JH et al (2008) Transcatheter arterial chemoembolization or chemoinfusion for unresectable intrahepatic cholangiocarcinoma: clinical efficacy and factors influencing outcomes. *Cancer* 113:1614–1622. doi:[10.1002/cncr.23787](https://doi.org/10.1002/cncr.23787)
37. Wu ZF, Zhang HB, Yang N, Zhao WC, Fu Y, Yang GS (2012) Postoperative adjuvant transcatheter arterial chemoembolisation improves survival of intrahepatic cholangiocarcinoma patients with poor prognostic factors: results of a large monocentric series. *Eur J Surg Oncol* 38:602–610. doi:[10.1016/j.ejso.2012.02.185](https://doi.org/10.1016/j.ejso.2012.02.185)
38. Zoepf T, Jakobs R, Arnold JC, Apel D, Riemann JF (2005) Palliation of nonresectable bile duct cancer: improved survival after photodynamic therapy. *Am J Gastroenterol* 100:2426–2430. doi:[10.1111/j.1572-0241.2005.00318.x](https://doi.org/10.1111/j.1572-0241.2005.00318.x)
39. Takada T, Amano H, Yasuda H, Nimura Y, Matsushiro T, Kato H et al (2002) Is postoperative adjuvant chemotherapy useful for gallbladder carcinoma? A phase III multicenter prospective randomized controlled trial in patients with resected pancreaticobiliary carcinoma. *Cancer* 95:1685–1695. doi:[10.1002/cncr.10831](https://doi.org/10.1002/cncr.10831)
40. Anderson C, Kim R (2009) Adjuvant therapy for resected extrahepatic cholangiocarcinoma: a review of the literature and future directions. *Cancer Treat Rev* 35:322–327. doi:[10.1016/j.ctrv.2008.11.009](https://doi.org/10.1016/j.ctrv.2008.11.009)
41. Shinohara ET, Mitra N, Guo M, Metz JM (2008) Radiation therapy is associated with improved survival in the adjuvant and definitive treatment of intrahepatic cholangiocarcinoma. *Int J Radiat Oncol Biol Phys* 72:1495–1501. doi:[10.1016/j.ijrobp.2008.03.018](https://doi.org/10.1016/j.ijrobp.2008.03.018)
42. Cherqui D, Benoist S, Malassagne B, Humeres R, Rodriguez V, Fagniez PL (2000) Major liver resection for carcinoma in jaundiced patients without preoperative biliary drainage. *Arch Surg* 135:302–308. doi:[10.1001/archsurg.135.3.302](https://doi.org/10.1001/archsurg.135.3.302)
43. Figueras J, Llado L, Valls C, Serrano T, Ramos E, Fabregat J et al (2000) Changing strategies in diagnosis and management of hilar cholangiocarcinoma. *Liver Transpl* 6:786–794. doi:[10.1053/jlts.2000.18507](https://doi.org/10.1053/jlts.2000.18507)
44. Pitt HA, Gomes AS, Lois JF, Mann LL, Deutsch LS, Longmire WP Jr (1985) Does preoperative percutaneous biliary drainage reduce operative risk or increase hospital cost? *Ann Surg* 201:545–553
45. Hochwald SN, Burke EC, Jarnagin WR, Fong Y, Blumgart LH (1999) Association of preoperative biliary stenting with increased postoperative infectious complications in proximal cholangiocarcinoma. *Arch Surg* 134:261–266. doi:[10.1001/archsurg.134.3.261](https://doi.org/10.1001/archsurg.134.3.261)
46. Savader SJ, Trerotola SO, Merline DS, Venbrux AC, Osterman FA (1992) Hemobilia after percutaneous transhepatic biliary drainage: treatment with transcatheter embolotherapy. *J Vasc Interv Radiol* 3:345–352
47. Chapman WC, Sharp KW, Weaver F, Sawyers JL (1989) Tumor seeding from percutaneous biliary catheters. *Ann Surg* 209:708–713
48. Sakata J, Shirai Y, Wakai T, Nomura T, Sakata E, Hatakeyama K (2005) Catheter tract implantation metastases associated with percutaneous biliary drainage for extrahepatic cholangiocarcinoma. *World J Gastroenterol* 11:7024–7027
49. Shoup M, Gonen M, D'Angelica M, Jarnagin WR, DeMatteo RP, Schwartz LH et al (2003) Volumetric analysis predicts hepatic dysfunction in patients undergoing major liver resection. *J Gastrointest Surg* 7:325–330. doi:[10.1016/S1091-255X\(02\)00370-0](https://doi.org/10.1016/S1091-255X(02)00370-0)
50. Vauthey JN, Chaoui A, Do KA, Bilimoria MM, Fenstermacher MJ, Charnsangavej C et al (2000) Standardized measurement of the future liver remnant prior to extended liver resection: methodology and clinical associations. *Surgery* 127:512–519. doi:[10.1067/msy.2000.105294](https://doi.org/10.1067/msy.2000.105294)
51. Shirabe K, Shimada M, Gion T, Hasegawa H, Takenaka K, Utsunomiya T et al (1999) Postoperative liver failure after major hepatic resection for hepatocellular carcinoma in the modern era with special reference to remnant liver volume. *J Am Coll Surg* 188:304–309. doi:[10.1016/S1072-7515\(98\)00301-9](https://doi.org/10.1016/S1072-7515(98)00301-9)
52. Kinoshita H, Sakai K, Hirohashi K, Igawa S, Yamasaki O, Kubo S (1986) Preoperative portal vein embolization for hepatocellular carcinoma. *World J Surg* 10:803–808. doi:[10.1007/BF01655244](https://doi.org/10.1007/BF01655244)
53. Shimizu Y, Suzuki H, Nimura Y, Onoue S, Nagino M, Tanaka M et al (1995) Elevated mitochondrial gene expression during rat liver regeneration after portal vein ligation. *Hepatology* 22:1222–1229. doi:[10.1002/hep.1840220430](https://doi.org/10.1002/hep.1840220430)
54. Takeuchi E, Nimura Y, Mizuno S, Nagino M, Shoji-Kawaguchi M, Izuta S et al (1996) Ligation of portal vein branch induces DNA polymerases alpha, delta, and epsilon in nonligated lobes. *J Surg Res* 65:15–24. doi:[10.1006/jsre.1996.0337](https://doi.org/10.1006/jsre.1996.0337)
55. Nagino M, Kamiya J, Nishio H, Ebata T, Arai T, Nimura Y (2006) Two hundred forty consecutive portal vein embolizations before extended hepatectomy for biliary cancer: surgical outcome and long-term follow-up. *Ann Surg* 243:364–372. doi:[10.1097/01.sla.0000201482.11876.14](https://doi.org/10.1097/01.sla.0000201482.11876.14)
56. Vauthey JN, Pawlik TM, Abdalla EK, Arens JF, Nemr RA, Wei SH et al (2004) Is extended hepatectomy for hepatobiliary malignancy justified? *Ann Surg* 239:722–730. doi:[10.1097/01.sla.0000124385.83887.d5](https://doi.org/10.1097/01.sla.0000124385.83887.d5)
57. Schnitzbauer AA, Lang SA, Goessmann H, Nadalin S, Baumgart J, Farkas SA et al (2012) Right portal vein ligation combined with in situ splitting induces rapid left lateral liver lobe hypertrophy enabling 2-staged extended right hepatic resection in small-for-size settings. *Ann Surg* 255:405–414. doi:[10.1097/SLA.0b013e31824856f5](https://doi.org/10.1097/SLA.0b013e31824856f5)
58. De Santibañes E, Clavien PA (2012) Playing Play-Doh to prevent postoperative liver failure: the 'ALPPS' approach. *Ann Surg* 255:415–417. doi:[10.1097/SLA.0b013e318248577d](https://doi.org/10.1097/SLA.0b013e318248577d)
59. Li J, Girotti P, Königsrainer I, Ladurner R et al (2013) ALPPS in right trisectionectomy: a safe procedure to avoid postoperative liver failure? *J Gastrointest Surg* 17:956–961. doi:[10.1007/s11605-012-2132-y](https://doi.org/10.1007/s11605-012-2132-y)
60. Kazaryan AM, Pavlik Marangos I, Rosseland AR, Røsok BI, Mala T, Villanger O et al (2010) Laparoscopic liver resection for malignant and benign lesions: ten-year Norwegian single-center experience. *Arch Surg* 145:34–40. doi:[10.1001/archsurg.2009.229](https://doi.org/10.1001/archsurg.2009.229)
61. Gumbs AA, Jarufe N, Gayet B (2013) Minimally invasive approaches to extrapancreatic cholangiocarcinoma. *Surg Endosc* 27:406–414. doi:[10.1007/s00464-012-2489-8](https://doi.org/10.1007/s00464-012-2489-8)

62. Goere D, Wagholikar GD, Pessaux P, Carrère N, Sibert A, Vilgrain V et al (2006) Utility of staging laparoscopy in subsets of biliary cancers: laparoscopy is a powerful diagnostic tool in patients with intrahepatic and gallbladder carcinoma. *Surg Endosc* 20:721–725. doi:[10.1007/s00464-005-0583-x](https://doi.org/10.1007/s00464-005-0583-x)
63. Connor S, Barron E, Wigmore SJ, Madhavan KK, Parks RW, Garden OJ (2005) The utility of laparoscopic assessment in the preoperative staging of suspected hilar cholangiocarcinoma. *J Gastrointest Surg* 9:476–480. doi:[10.1016/j.gassur.2004.10.009](https://doi.org/10.1016/j.gassur.2004.10.009)
64. Weber SM, DeMatteo RP, Fong Y, Blumgart LH, Jarnagin WR (2002) Staging laparoscopy in patients with extrahepatic biliary carcinoma. Analysis of 100 patients. *Ann Surg* 235:392–399
65. Capussotti L, Muratore A, Polastri R, Ferrero A, Massucco P (2002) Liver resection for hilar cholangiocarcinoma: in-hospital mortality and long-term survival. *J Am Coll Surg* 195:641–647. doi:[10.1016/S1072-7515\(02\)01481-3](https://doi.org/10.1016/S1072-7515(02)01481-3)
66. Ikeyama T, Nagino M, Oda K, Ebata T, Nishio H, Nimura Y (2007) Surgical approach to bismuth type I and II hilar cholangiocarcinomas: audit of 54 consecutive cases. *Ann Surg* 246:1052–1057. doi:[10.1097/SLA.0b013e318142d97e](https://doi.org/10.1097/SLA.0b013e318142d97e)
67. Jang JY, Kim SW, Park DJ, Ahn YJ, Yoon YS, Choi MG et al (2005) Actual long-term outcome of extrahepatic bile duct cancer after surgical resection. *Ann Surg* 241:77–84. doi:[10.1097/01.sla.0000150166.94732.88](https://doi.org/10.1097/01.sla.0000150166.94732.88)
68. de Jong MC, Marques H, Clary BM, Bauer T, Marsh JW et al (2012) The impact of portal vein resection on outcomes for hilar cholangiocarcinoma. *Cancer* 118:4737–4747
69. Abramson MA, Pandharipande P, Ruan D, Gold JS, Whang EE (2009) Radical resection for T1b gallbladder cancer: a decision analysis. *HPB (Oxford)* 11:656–663. doi:[10.1111/j.1477-2574.2009.00108.x](https://doi.org/10.1111/j.1477-2574.2009.00108.x)
70. Horiguchi A, Miyakawa S, Ishihara S, Miyazaki M, Ohtsuka M, Shimizu H, et al (2013) Gallbladder bed resection or hepatectomy of segments 4a and 5 for pT2 gallbladder carcinoma: analysis of Japanese registration cases by the study group for biliary surgery of the Japanese Society of Hepato-Biliary-Pancreatic Surgery. *J Hepatobiliary Pancreat Sci* 22 Feb 2013. (Epub ahead of print)
71. Ito H, Matros E, Brooks DC, Osteen RT, Zinner MJ, Swanson RS et al (2004) Treatments outcomes associated with surgery for gallbladder cancer: a 20-year experience. *J Gastrointest Surg* 8:183–190. doi:[10.1016/j.gassur.2003.10.006](https://doi.org/10.1016/j.gassur.2003.10.006)
72. Nimura Y, Hayakawa N, Kamiya J, Kondo S, Shionoya S (1990) Hepatic segmentectomy with caudate lobe resection for bile duct carcinoma of the hepatic hilus. *World J Surg* 14:535–543. doi:[10.1007/BF01658686](https://doi.org/10.1007/BF01658686)
73. Ogura Y, Mizumoto R, Tabata M, Matsuda S, Kusuda T (1993) Surgical treatment of carcinoma of the hepatic duct confluence: analysis of 55 resected carcinomas. *World J Surg* 17:85–92. doi:[10.1007/BF01655714](https://doi.org/10.1007/BF01655714)
74. Sugiura Y, Nakamura S, Iida S, Hosoda Y, Ikeuchi S, Mori S et al (1994) Extensive resection of the bile ducts combined with liver resection for cancer of the main hepatic duct junction: a cooperative study of the Keio Bile Duct Cancer Study Group. *Surgery* 115:445–451
75. Gazzaniga GM, Ciferri E, Bagarolo C, Filauro M, Bondanza G, Fazio S et al (1993) Primitive hepatic hilum neoplasm. *J Surg Oncol Suppl* 3:140–146. doi:[10.1002/jso.2930530537](https://doi.org/10.1002/jso.2930530537)
76. Gazzaniga GM, Filauro M, Bagarolo C, Mori L (2000) Surgery for hilar cholangiocarcinoma: an Italian experience. *J Hepatobiliary Pancreat Surg* 7:122–127. doi:[10.1007/s005340050165](https://doi.org/10.1007/s005340050165)
77. Imamura H, Shimada R, Kubota M, Matsuyama Y, Nakayama A, Miyagawa S et al (1999) Preoperative portal vein embolization: an audit of 84 patients. *Hepatology* 29:1099–1105. doi:[10.1051/S0270913999001718](https://doi.org/10.1051/S0270913999001718)
78. Hemming AW, Kim RD, Mekeel KL, Fujita S, Reed AI, Foley DP, et al (2006) Portal vein resection for hilar cholangiocarcinoma. *Am Surg* 72:599–604 (NÃO TEM DOI)
79. Ebata T, Nagino M, Kamiya J, Uesaka K, Nagasaka T, Nimura Y (2003) Hepatectomy with portal vein resection for hilar cholangiocarcinoma: audit of 52 consecutive cases. *Ann Surg* 238:720–727. doi:[10.1097/01.sla.0000094437.68038.a3](https://doi.org/10.1097/01.sla.0000094437.68038.a3)
80. Muñoz L, Roayaie S, Maman D, Fishbein T, Sheiner P, Emre S et al (2002) Hilar cholangiocarcinoma involving the portal vein bifurcation: long-term results after resection. *J Hepatobiliary Pancreat Surg* 9:237–241. doi:[10.1007/s005340200025](https://doi.org/10.1007/s005340200025)
81. Nakeeb A, Pitt HA, Sohn TA, Coleman J, Abrams RA, Piantadosi S et al (1996) Cholangiocarcinoma. A spectrum of intrahepatic, perihilar, and distal tumors. *Ann Surg* 224:463–473
82. Blechacz B, Gores GJ (2008) Cholangiocarcinoma: advances in pathogenesis, diagnosis, and treatment. *Hepatology* 48:308–321. doi:[10.1002/hep.22310](https://doi.org/10.1002/hep.22310)
83. DeOliveira ML, Winter JM, Schafer M, Cunningham SC, Cameron JL, Yeo CJ et al (2006) Assessment of complications after pancreatic surgery: a novel grading system applied to 633 patients undergoing pancreatoduodenectomy. *Ann Surg* 244:931–937. doi:[10.1097/01.sla.0000246856.03918.9a](https://doi.org/10.1097/01.sla.0000246856.03918.9a)
84. Hong S-M, Pawlik TM, Cho H, Aggarwal B, Goggins M, Hruban RH et al (2009) Depth of tumor invasion better predicts prognosis than the current American Joint Committee on Cancer T classification for distal bile duct carcinoma. *Surgery* 146:250–257. doi:[10.1016/j.surg.2009.02.023](https://doi.org/10.1016/j.surg.2009.02.023)
85. Murakami Y, Uemura K, Hayashidani Y, Sudo T, Hashimoto Y, Ohge H et al (2007) Prognostic significance of lymph node metastasis and surgical margin status for distal cholangiocarcinoma. *J Surg Oncol* 95:207–212. doi:[10.1002/jso.20668](https://doi.org/10.1002/jso.20668)
86. Ebata T, Nagino M, Nishio H, Arai T, Nimura Y (2007) Right hepatopancreatoduodenectomy: improvements over 23 years to attain acceptability. *J Hepatobiliary Pancreat Surg* 14:131–135. doi:[10.1007/s00534-006-1106-4](https://doi.org/10.1007/s00534-006-1106-4)
87. Lim CS, Jang JY, Lee SE, Kang MJ, Kim SW (2012) Reappraisal of hepatopancreatoduodenectomy as a treatment modality for bile duct and gallbladder cancer. *J Gastrointest Surg* 16:1012–1018. doi:[10.1007/s11605-012-1826-5](https://doi.org/10.1007/s11605-012-1826-5)
88. Ebata T, Yokoyama Y, Igami T, Sugawara G, Takahashi Y, Nimura Y et al (2012) Hepatopancreatoduodenectomy for cholangiocarcinoma: a single-center review of 85 consecutive patients. *Ann Surg* 256:297–305. doi:[10.1097/SLA.0b013e31826029ca](https://doi.org/10.1097/SLA.0b013e31826029ca)
89. Nimura Y, Hayakawa N, Kamiya J, Maeda S, Kondo S, Yasui A et al (1991) Hepatopancreatoduodenectomy for advanced carcinoma of the biliary tract. *Hepatogastroenterology* 38:170–175
90. Kosuge T, Yamamoto J, Shimada K, Yamasaki S, Makuuchi M (1999) Improved surgical results for hilar cholangiocarcinoma with procedures including major hepatic resection. *Ann Surg* 230:663–671
91. Klempnauer J, Ridder GJ, von Wasielewski R, Werner M, Weimann A, Pichlmayr R (1997) Resectional surgery of hilar cholangiocarcinoma: a multivariate analysis of prognostic factors. *J Clin Oncol* 15:947–954
92. Kitagawa Y, Nagino M, Kamiya J, Uesaka K, Sano T, Yamamoto H et al (2001) Lymph node metastasis from hilar cholangiocarcinoma: audit of 110 patients who underwent regional and para-aortic node dissection. *Ann Surg* 233:385–392. doi:[10.1097/00000658-200103000-00013](https://doi.org/10.1097/00000658-200103000-00013)
93. Ribero D, Pinna AD, Guglielmi A, Ponti A, Nuzzo G, Giulini SM et al (2012) Surgical approach for long-term survival of patients

- with intrahepatic cholangiocarcinoma: a multi-institutional analysis of 434 patients. *Arch Surg* 147:1107–1113. doi:[10.1001/archsurg.2012.1962](https://doi.org/10.1001/archsurg.2012.1962)
94. Choi SB, Kim KS, Choi JY, Park SW, Choi JS, Lee WJ et al (2009) The prognosis and survival outcome of intrahepatic cholangiocarcinoma following surgical resection: association of lymph node metastasis and lymph node dissection with survival. *Ann Surg Oncol* 16:3048–3056. doi:[10.1245/s10434-009-0631-1](https://doi.org/10.1245/s10434-009-0631-1)
 95. Goldstein RM, Stone M, Tillery GW, Senzer N, Levy M, Husberg BS et al (1993) Is liver transplantation indicated for cholangiocarcinoma? *Am J Surg* 166:768–771 (discussion 771–772)
 96. Meyer CG, Penn I, James L (2000) Liver transplantation for cholangiocarcinoma: Results in 207 patients. *Transplantation* 69:1633–1637
 97. Goss JA, Shackleton CR, Farmer DG, Arnaout WS, Seu P, Markowitz JS et al (1997) Orthotopic liver transplantation for primary sclerosing cholangitis: a 12-year single center experience. *Ann Surg* 225:472–481
 98. Robles R, Figueras J, Turrion VS, Margarit C, Moya A, Varo E et al (2004) Spanish experience in liver transplantation for hilar and peripheral cholangiocarcinoma. *Ann Surg* 239:265–271. doi:[10.1097/01.sla.0000108702.45715.81](https://doi.org/10.1097/01.sla.0000108702.45715.81)
 99. Shimoda M, Farmer DG, Colquhoun SD, Rosove M, Ghobrial RM, Yersiz H et al (2001) Liver transplantation for cholangiocellular carcinoma: analysis of a single-center experience and review of the literature. *Liver Transpl* 7:1023–1033. doi:[10/S1527646501398751](https://doi.org/10/S1527646501398751)
 100. Heimbach JK, Gores GJ, Haddock MG, Alberts SR, Pedersen R, Kremers W et al (2006) Predictors of disease recurrence following neoadjuvant chemoradiotherapy and liver transplantation for unresectable perihilar cholangiocarcinoma. *Transplantation* 82:1703–1707. doi:[10.1097/01.tp.0000253551.43583.d1](https://doi.org/10.1097/01.tp.0000253551.43583.d1)
 101. Rosen CB, Nagorney DM, Wiesner RH, Coffey RJ Jr, LaRusso NF (1991) Cholangiocarcinoma complicating primary sclerosing cholangitis. *Ann Surg* 213:21–25
 102. Marsh JW Jr, Iwatsuki S, Makowka L, Esquivel CO, Gordon RD, Todo S et al (1988) Orthotopic liver transplantation for primary sclerosing cholangitis. *Ann Surg* 207:21–25
 103. Darwish Murad S, Kim WR, Harnois DM, Douglas DD, Burton J et al (2012) Transplantation, for perihilar cholangiocarcinoma at 12 US Centers. *Gastroenterology* 143:88–98. doi:[10.1053/j.gastro.2012.04.008](https://doi.org/10.1053/j.gastro.2012.04.008)
 104. Gores GJ, Gish RG, Sudan D, Rosen CB, MELD Exception Study Group (2006) Model for end-stage liver disease (MELD) exception for cholangiocarcinoma or biliary dysplasia. *Liver Transpl* 12(Suppl3):S95–S97. doi:[10.1002/lt.20965](https://doi.org/10.1002/lt.20965)
 105. Hyder O, Marsh JW, Salem R, Petre EN, Kalva S et al (2013) Intra-arterial therapy for advanced intrahepatic cholangiocarcinoma: a multi-institutional analysis. *Annals of Surgical Oncology*. doi:[10.1245/s10434-013-3127-y](https://doi.org/10.1245/s10434-013-3127-y). Published online 12 Jul 2013

Surgical Management of Intra-Hepatic Cholangiocarcinoma

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Abstract

Intra-hepatic cholangiocarcinoma (ICC) arises from the biliary epithelium of secondary bile ducts or beyond. Many patients present with advanced, often unresectable disease due to vague or absent symptoms. Staging is based on tumor number, vascular invasion, extra-hepatic spread, lymph node involvement, and distant metastatic disease. When feasible, complete surgical resection offers the best hope of long-term survival, and may be approached via open or minimally invasive techniques depending on tumor location and surgeon expertise. Extended hepatic resection, vascular resection, and/or biliary-enteric reconstruction may be required for complete tumor resection. Mortality rates in most modern surgical series are 1–5 %. Five-year overall survival following resection ranges from 17 to 44 %. The role of liver transplant is limited to select centers with clinical trials including rigorous neoadjuvant therapy. The role of adjuvant therapy is still being explored as newer, potentially more effective systemic agents are developed.

1 Introduction

Cholangiocarcinoma is the second most common primary liver cancer after hepatocellular carcinoma (HCC). It arises from the epithelial lining of bile ducts and is anatomically categorized as intra-hepatic or extra-hepatic. Intra-hepatic cholangiocarcinoma (ICC) originates from the secondary or more peripheral bile ducts and does not involve the hepatic duct confluence. Some series in the literature refer to them as peripheral cholangiocarcinomas. These tumors are the least common, representing 5–10 % of cholangiocarcinomas, compared with tumors arising in the peri-hilar extra-hepatic duct (50–60 %) or distal bile duct (20–25 %) [1]. The majority of patients have no identifiable risk factor for ICC, which contributes to the problem of developing an effective screening process. Risk factors that have been

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associated with ICC relate to chronic inflammation within the bile ducts and include primary sclerosing cholangitis (PSC), choledochal cyst, chronic bile duct stones, exposure to Thorotrast contrast agent, smoking, liver fluke infestation, and chronic typhoid carriers [1–4]. Approximately 6,000 new cases of cholangiocarcinoma are diagnosed in the United States each year; the incidence of ICC in the US and worldwide has been increasing, along with the mortality rate [5–7]. The reasons for these increases are a subject of debate. Some authors argue it is influenced by recent changes in the classification system [6], while others point to hepatitis C as an emerging risk factor for cholangiocarcinoma [8].

Patients with ICC present more often with abdominal pain, constitutional symptoms, or an incidental mass, and less commonly with jaundice, as compared with extra-hepatic cholangiocarcinoma [9, 10]. Historically, staging systems for cholangiocarcinoma were derived from data on HCC patients [11], but in the American Joint Committee on Cancer (AJCC)/International Union Against Cancer (UICC) 7th edition staging manual, a unique staging system for ICC was introduced, based on an analysis of SEER data performed by Nathan et al. [12, 13]. Prognostic variables for T classification include vascular invasion, multiple tumors, extra-hepatic extension, and periductal infiltration (Table 1). Noteworthy is that tumor size, which had been a factor in the prior AJCC staging system, was not associated with survival and is not included in the updated edition. The AJCC 7th edition TNM staging correlated well with survival (Fig. 1). This staging system was subsequently found to accurately discriminate outcomes in patients who underwent resection for ICC in a multi-institution European study [14] and was also validated in an international multi-institutional analysis [15].

In addition to TNM staging, macroscopic histologic subtypes of ICC have been associated with prognosis. The main subtypes identified are mass-forming, periductal-infiltrating, and intraductal, although tumors may also have features of more than one subtype, such as mass-forming plus periductal-infiltrating (Fig. 2). These subtypes have different biological behaviors and are associated with different outcomes in Japanese studies [16, 17]. Mass-forming is the most common subtype in Western series, and independent influence on prognosis has not been established in this population [15].

Surgery is the only modality associated with long-term survival; unfortunately, the majority of patients are unresectable at the time of presentation, either due to local invasion or distant metastasis [18, 19]. The remainder of this chapter will address the preoperative preparation, intra-operative technical and decision-making considerations for resection, and postoperative outcomes. The role of adjuvant therapy and liver transplantation will also be discussed.

Table 1 Staging classification for ICC (adapted from AJCC 7th edition cancer staging manual)

Classification	Description
T1	Solitary tumor without vascular invasion ^a
T2a	Solitary tumor with vascular invasion ^a
T2b	Multiple tumors, with or without vascular invasion ^a
T3	Tumor perforating visceral peritoneum or involving local extra-hepatic structures by direct invasion
T4	Tumor with periductal invasion ^b
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis ^c
M0	No distant metastasis
M1	Distant metastasis
Stage groupings	
Stage I	T1 N0 M0
Stage II	T2 N0 M0
Stage III	T3 N0 M0
Stage IVA	T4 N0 M0, Any T N1 M0
Stage IVB	Any T Any N M1

^a Includes major vascular invasion (portal vein or hepatic vein) and microvascular invasion

^b Includes tumors with periductal-infiltrating or mixed mass-forming and periductal-infiltrating growth pattern

^c Nodal involvement of the celiac, periaortic or caval lymph nodes is considered to be distant metastasis (M1)

2 Preoperative Considerations

ICC is commonly identified on ultrasound or cross-sectional imaging, which may be performed for symptoms such as abdominal pain, or for an unrelated indication. Once a liver mass has been identified, an appropriate workup to evaluate for other potential diagnoses such as metastasis should be undertaken. History and physical examination, blood work including hepatitis panel, CA-19-9, CEA, and AFP, and upper and lower endoscopy can help narrow the differential diagnosis. Imaging for preoperative planning is accomplished with contrast-enhanced helical computed tomography (CT) or magnetic resonance imaging (MRI). The goal is to evaluate the local extent of disease to determine resectability and to identify metastatic disease that would preclude resection. A CT of the chest should also be included in the preoperative evaluation to rule out pulmonary metastases.

The definition of a resectable liver tumor in a medically fit candidate is determined by tumor size, number, and location. For ICC, resectability is defined as being able to completely excise a tumor with negative margins, with at least two contiguous segments of liver remaining, and with adequate arterial and portal venous inflow, hepatic venous

Fig. 1 Kaplan–Meier survival curve for ICC, stratified by AJCC 7th edition stage (with permission, from Ref [13])

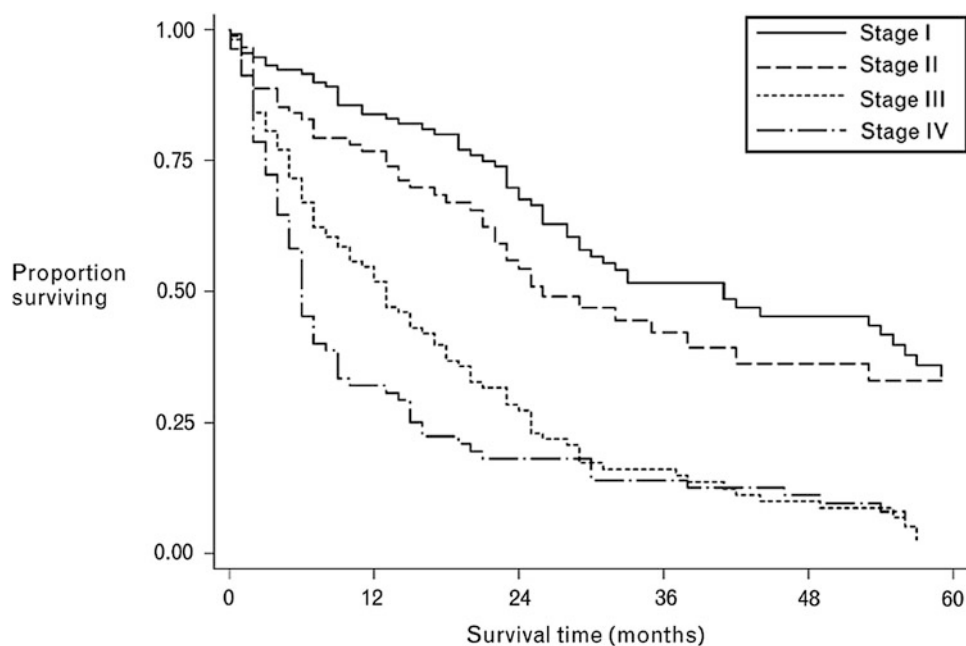
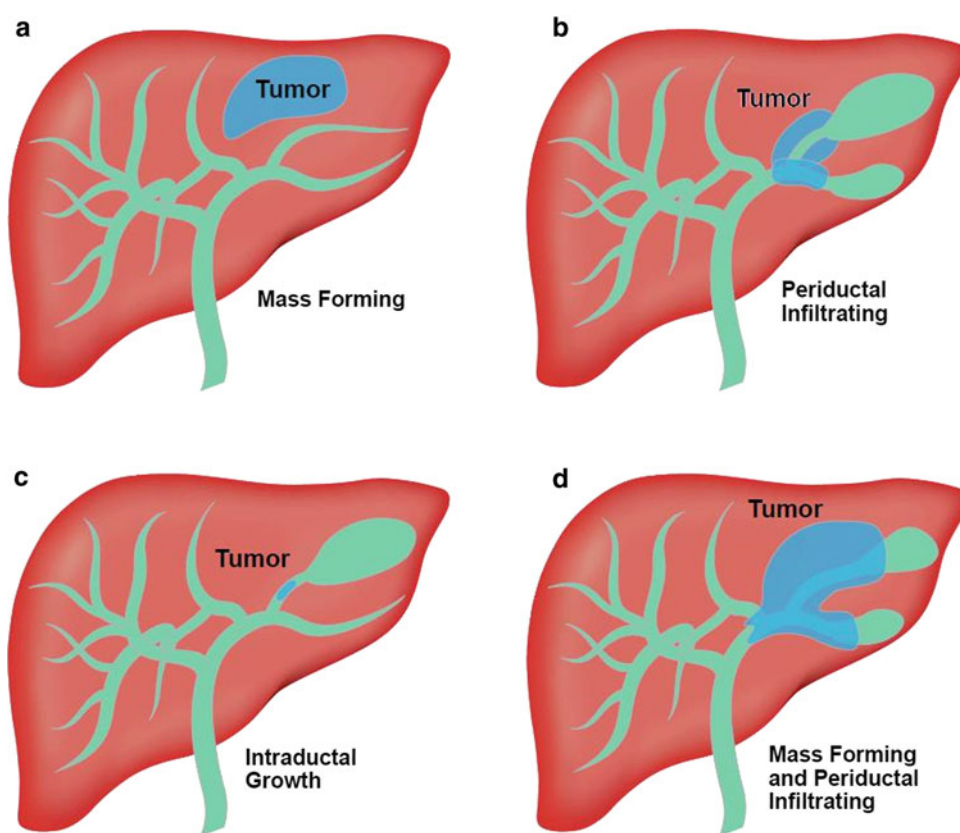


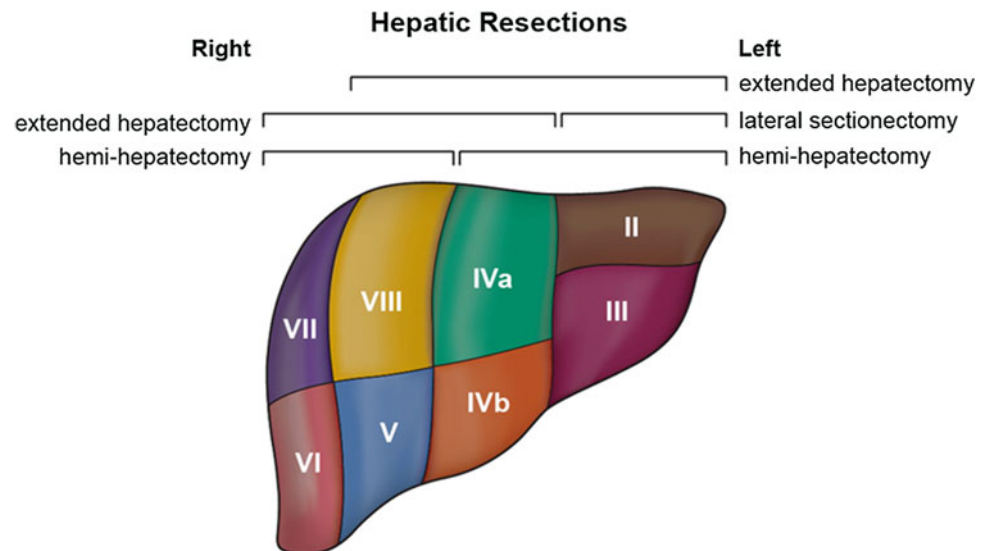
Fig. 2 Macroscopic subtypes of ICC



outflow, and biliary drainage. The amount of sufficient parenchymal future liver remnant required ranges from 20 to 40 %, depending on the health of the background liver [20]. The presence of multiple intra-hepatic tumors and/or grossly involved lymph nodes beyond the porta hepatis influence the likelihood of recurrence and should be

considered when determining if a patient will benefit from resection. In addition, there should be no extra-hepatic metastatic disease [1]. ICC is associated with advanced stage at presentation, and thus, a significant number of patients will not be candidates for resection. In one series of 238 patients diagnosed with ICC over a 16-year period, 128

Fig. 3 Anatomic basis for liver resection procedures, according to Brisbane terminology



patients (54 %) were initially unresectable, based on the presence of multiple tumors (70), locally advanced intra-hepatic disease (37), or metastatic disease (32); 20 patients had more than one indication for unresectability [19].

Hepatic resections are described in terms of the anatomic segments removed. Couinaud [21] defined the hepatic segments based on internal vascular anatomy (Fig. 3). The main portal vein divides into a left and right branch, supplying each hemi-liver. The right portal vein branches into anterior and posterior sections, which supply segments 5/8 and 6/7, respectively. The left portal vein divides into lateral and medial sections, supplying segments 2/3 and 4a/4b, respectively. In 2000, the International Hepato-Pancreato-Biliary Association (IHPBA) published a standardized terminology for liver resections at the World Congress of the IHPBA in Brisbane, Australia, referred to as the Brisbane terminology of liver anatomy and resections, which follows internal vascular anatomic terms [22].

In surgical series of ICC patients, a hemi-hepatectomy is required in 20–70 % of cases to resect the tumor, while extended resections (>4 Couinaud segments) are required in up to 60 % of cases. In 5–25 % of cases, tumor clearance can be accomplished by removal of less than a hemi-liver (segmentectomy, bisegmentectomy, or non-anatomic resection) [10, 15, 19, 23–29]. The utility of subjecting patients to extended resections has been studied; one series of 27 patients undergoing at least an extended hepatectomy demonstrated overall 1- and 3-year survivals of 69 and 55 %, which is in the range of published series for ICC. In patients achieving a complete margin-negative resection (R0), the median survival was 46 months, with 1- and 3-year survivals of 94 and 82 %, respectively [30]. Thus, the need for an extended resection should not deter operative planning in experienced hands. The appearance of grossly involved regional lymph nodes on preoperative imaging portends a

poor prognosis; however, nodal involvement outside of the regional lymph node basin is considered metastatic disease and a contra-indication to exploration [19].

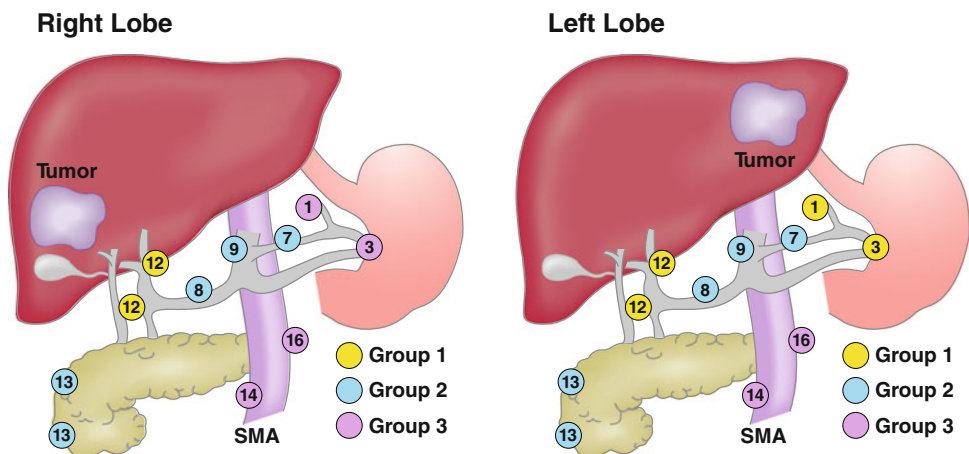
3 Preoperative Portal Venous Embolization

Postoperative liver failure is a potential complication of hepatic resection for any indication. Risk factors for postoperative liver failure include the ratio of the size of the future liver remnant (FLR) to the total estimated liver volume, and the degree of liver dysfunction, which may be a result of cholestasis, chemotherapy, non-alcoholic steatohepatitis (NASH), or fibrosis/cirrhosis. There are varying criteria for determining a safe FLR; some authors advocate for no less than 40 % FLR in a healthy liver [31], while others use 20–25 % FLR as a cutoff in normal liver [20, 32, 33].

Portal vein embolization (PVE) is a process of inducing compensatory hypertrophy in the FLR preoperatively by interrupting portal venous flow to the portion of the liver that will be resected. First introduced in 1990 for patients with biliary tract cancers, the rationale for PVE prior to surgery is to reduce the risk of complications including postoperative liver failure in patients with marginal FLR by increasing the FLR before resection [34]. The technique is performed by accessing the portal vein on the tumor-bearing side of the liver, usually by a percutaneous trans-hepatic or trans-ileocolic route, and delivering embolic microcoils, and polyvinyl particles, and/or alcohol to the segments of liver that will be resected [35].

In one series of 240 patients undergoing PVE before planned resection for biliary tract cancers, the future liver remnant increased significantly from 33 ± 8 % to 43 ± 8 %. Among patients who ultimately underwent

Fig. 4 Patterns of lymphatic spread for *left-sided* and *right-sided* hepatic tumors



resection, the degree of hypertrophy was not different between those who died in the perioperative period ($n = 17$) and those who did not ($n = 176$), but the function of the FLR, as measured by indocyanine green clearance, was significantly worse in non-survivors [36]. This series was recently updated and now includes 353 patients with cholangiocarcinoma and 141 patients with gallbladder cancer [31]. The operative mortality was 6.5 %, and 5-year survival of patients with cholangiocarcinoma (mostly hilar) was 39 %, which is comparable with other published studies. The number of patients with ICC in PVE studies is very low [37, 38], which is likely a reflection of the higher incidence of hilar tumors and the less frequent need for extensive resections in ICC compared with hilar cholangiocarcinoma.

There is evidence to suggest that PVE stimulates tumor growth in an animal model of colorectal cancer liver metastases [39]. The oncologic impact of this is unknown, but an emerging strategy that may address this concern is the addition of intra-arterial therapy (IAT) in a sequential fashion with PVE [40]. This approach has not been shown to cause more hypertrophy than PVE alone, but more tumor necrosis was seen. The role for combined preoperative IAT and PVE remains to be defined.

4 Operative Considerations

4.1 Laparoscopic Staging

Staging laparoscopy with or without laparoscopic ultrasonography at the time of planned surgical resection for hepatobiliary-pancreatic malignancy was originally introduced to spare patients with occult metastatic disease, a non-therapeutic laparotomy [41]. In 62 patients explored for ICC without laparoscopy, metastatic disease was identified in 14 (23 %), suggesting that laparoscopy could benefit up to 1 in 4 ICC patients [42]. Early studies of laparoscopy in

mixed hepatobiliary-pancreatic tumors found that laparoscopy identified unresectable disease in up to 46 % of patients [41, 43, 44]. One small study of 11 patients with ICC found that occult metastatic disease was detected at laparoscopy in four patients, for a yield of 36 %. An additional two patients were found to be unresectable at laparotomy, making the sensitivity 67 % (4/6) [45]. A larger review of 53 ICC patients, of whom 22 underwent staging laparoscopy, reported unresectable disease in six of 22 (27 %). Peritoneal metastases (4) and intra-hepatic metastases (2) were the findings precluding resection. At laparotomy, five additional patients had metastatic disease identified in celiac lymph nodes, making the sensitivity of laparoscopy 6/11 or 55 % [28].

As cross-sectional imaging has improved, the efficacy and cost-effectiveness of diagnostic laparoscopy in liver tumors have come into question [46, 47]. This is particularly true for cholangiocarcinoma, which tends to invade locally, such that the determination of resectability may be made only after dissection of biliary or vascular structures, compared with tumors such as gallbladder carcinoma, which demonstrate earlier peritoneal dissemination. Data specific to the utility of laparoscopy in patients with ICC remain sparse.

Studies reporting resectability at laparotomy offer rates of 62–83 %, which varies in part by the era of study, the use of laparoscopy, and the approach to tumors with lymph node or vascular involvement [9, 10, 19, 24, 26, 28].

4.2 Lymphadenectomy

The lymphatic drainage for liver tumors has been described, based on tumor location within the liver (Fig. 4). Left-sided tumors tend to spread toward the gastro-hepatic ligament to the lesser curve and cardia of the stomach, while right-sided tumors drain to the hepatoduodenal ligament [48]. However, left-sided ICC has been shown to follow “right-sided” drainage patterns in half of cases subjected to systematic

lymphadenectomy [49]. The incidence of lymph node metastasis in published series of ICC ranges from 16 to 81 %; the higher rates reflect the tendency in many Western centers to perform lymphadenectomy only when suspicious lymph nodes are encountered [9, 10, 15, 19, 23, 24, 26, 29, 50]. In centers where lymphadenectomy is routinely performed, the incidence is 20–50 % [42, 49, 50].

Lymph node metastases are associated with poor prognosis in numerous studies [9, 10, 15, 26, 28]; thus, lymphadenectomy may help select patients for adjuvant therapy. However, routine lymphadenectomy has not been performed in many large Western series [15, 19, 24, 28, 51], with the rationale that routine lymphadenectomy has not been shown to influence survival [52].

4.3 Vascular Resection

Cholangiocarcinoma tends to display locally aggressive growth, which may involve major vascular structures such as the inferior vena cava (IVC) or the main or contra-lateral portal vein. Some form of vascular resection is required in 9–14 % of resections for ICC in large series [19, 23–28]. The impact of major vascular resection on outcomes for ICC patients was studied in a single institution review [23]. In a series of 121 patients, 14 underwent vascular resection. Of the vascular resection group, three of 12 patients with lymph nodes removed had lymph node metastases, and R0 resection was achieved in 86 %. There was no difference in overall survival at 1, 3, or 5 years between patients with (85, 56, 44 %) and without (85, 45, 23 %) vascular resection. Median overall survival was 32 versus 49 months, which was not statistically different. Thus, there is evidence that vascular resection is feasible and should be performed if necessary to achieve complete tumor excision, as outcomes are comparable in experienced hands.

4.4 Minimally Invasive Approach

Minimally invasive liver resections have been performed in over 3,000 patients worldwide for a variety of benign and malignant indications [53]. In the largest review of laparoscopic liver cases, <13 % of malignant cases were for the indication of ICC; overall morbidity and mortality for minimally invasive resection in all patients were 10.5 and 0.3 % [54]. The benefits of a laparoscopic approach, when technically feasible and performed by appropriately trained and experienced surgeons, include shorter length of stay, less pain medication requirements, less blood loss, quicker

resumption of oral intake, with equivalent rates of complications [55]. While operating room costs may be higher in a laparoscopic procedure compared with a matched open procedure, the total hospital costs are equivalent or reduced [55, 56].

Peripherally located tumors are most amenable to a laparoscopic approach; the ideal candidate would have no tumor near the planned transection plane. Vascular resections and extra-hepatic bile duct resections are more technically demanding in the minimally invasive setting, and most experienced surgeons would approach those tumors in an open fashion. The use of robotic assistance facilitates the more complex dissection and suturing required in extended hepatectomies, and has been employed in a variety of benign and malignant tumors, including ICC [57, 58]. Preliminary evidence demonstrates feasibility from a technologic and oncologic perspective, and the role of robotic-assisted minimally invasive hepatectomy continues to be explored [59].

4.5 Transplant in ICC

As ICC frequently presents at an advanced stage that precludes complete resection, total hepatectomy with orthotopic liver transplant (OLT) has been explored as a potential solution to this clinical problem. Reports on early experience with OLT for cholangiocarcinoma include a review of 54 patients with ICC who underwent resection (34) or OLT (20) at the University of Pittsburgh Medical Center [60]. OLT was performed for unresectable disease in 12 patients and concurrent advanced cirrhosis in 8. Overall survival at 1, 3, and 5 years for resection was 60, 37, and 31 %, which was similar to survival after OLT (70, 29 and 18 %), and comparable with the authors' outcomes for OLT in patients with HCC [61].

While these authors concluded that the comparable survival outcomes support the application of transplant to ICC, subsequent reports documented high rates of tumor recurrence in patients undergoing OLT for ICC and questioned the appropriateness of transplant in patients with ICC. Using data from the Cincinnati tumor registry, Meyer et al. [62] reported a 51 % rate of tumor recurrence in 207 patients transplanted for cholangiocarcinoma or mixed HCC-cholangiocarcinoma, including both peri-hilar and intra-hepatic tumors. The median time to recurrence was 9.7 months (range <1–64 months), and the median time between recurrence and death was 2 months (range <1–53 months). These data, and other studies with similar findings [63–66], led to a general consensus that cholangiocarcinoma should

Table 2 Perioperative outcomes in surgical series of ICC

First author	Year	N	Resectability (%)	LN pos (%)	R0 (%)	Mortality (%)	Morbidity (%)
Madariaga	1998	34	–	18	71	14	32
Weber	2001	33	62	15	88	3	19
Nakagawa	2005	44	83	47	75	5.7	–
DeOliveira	2007	44	66	30	45	4.5	35
Paik	2007	97	64	24	93	0	–
Shimada	2007	76	n/a	16	67	1	–
Endo	2008	77	70	16	85	1.2	38
Konstadoulakis	2008	54	75	82	78	7	11
Gugliemi	2009	62	–	18	90	–	–
Lang	2009	83	52	33	64	7.1	44
Nathan	2009	598	–	21	–	–	–
Shen	2009	429	–	20	74	1.2	6
de Jong	2011	449	–	17	81	–	–
Ali	2012	121	–	28	96	1	43

not be considered an appropriate indication for liver transplant outside of clinical trials focusing on neoadjuvant and/or adjuvant therapy to improve outcomes.

For ICC, investigators at UCLA have developed a protocol using preoperative radiation—either external beam or short-course stereotactic body radiation therapy (SBRT)—followed by 5-FU-based chemotherapy until the time of transplant [67]. In a review of 40 patients (26 intra-hepatic, 13 hilar), the 5-year recurrence-free survival was 29 %, with a median time to recurrence of 11 months. Multivariate analysis identified seven pathologic and treatment factors independently associated with prognosis: multi-focal disease, perineural invasion, infiltrative subtype, lympho-vascular invasion, hilar location, history of PSC, and use of adjuvant and/or neoadjuvant therapy. A scoring system based on these risk factors was created, and the lowest-risk patients' 5-year recurrence-free survival was 78 %, compared with 19 % for intermediate risk and 0 for high risk.

In another study, the same group compared OLT to radical bile duct resection and partial hepatectomy in 57 patients (37 with intra-hepatic tumors and 20 hilar) [68]. Twenty-five patients with ICC underwent OLT, and 12 underwent resection. The overall 5-year survival for all ICC patients was 34 %. 3- and 5-year recurrence-free survival for all patients was 39 and 6 % for OLT compared with 33 % and 0 ($p = 0.05$). For intra-hepatic tumors, the improved survival with OLT was not statistically significant. On multivariate analysis, resection versus OLT, hilar versus intra-hepatic location, perineural invasion and multi-focal tumors were factors associated with diminished survival. However, given the global shortage of organ donors, most transplant centers consider ICC a contraindication for OLT, and should only be performed in the setting of a clinical trial or protocol.

4.6 Perioperative Outcomes

Over the past several decades, the rates of postoperative death and complications following hepatic resection have decreased, such that most modern series from high-volume centers report less than 3 % mortality [69–72]. One review of 30-day outcomes of hepatectomies from the National Surgical Quality Improvement Project (NSQIP) database found a 30-day mortality of 2.5 % and morbidity of 19.6 % [73]. Improvements in surgical and anesthetic techniques, better patient selection, and innovations in hemostatic equipment are thought to contribute to these trends. Unfortunately, there is significant disparity in outcomes between high-volume and low-volume institutions performing hepatectomy, with lower mortality at high-volume centers (5.8 vs. 8.9 % in low-volume centers) [74].

In series of ICC patients, surgical mortality ranges from 1 to 14 % [9, 19, 23–25, 27–29, 50], and when reported, the most common causes for mortality are liver failure, sepsis, and cardiac events [19, 24, 27–29]. Several authors anecdotally note an association between extended liver resections, extra-hepatic bile duct resections and/or vascular resections and reconstructions with mortality, but this has not been formally demonstrated.

There is significant variability in how surgical complications are defined and reported; thus, the observed incidences of complications for resection of ICC have a wide range from 6 to 43 % [9, 19, 23–25, 27–29, 50]. The more common complications include intra-abdominal abscess, transient hepatic failure, infections (wound infections, pneumonia or sepsis), other pulmonary complications (pleural effusions or symptomatic atelectasis, ARDS), bile leak, and cholangitis [9, 19, 24, 27–29, 50]. Perioperative outcomes in recent series are summarized in Table 2.

Table 3 Factors influencing survival after resection of ICC

First author	Year	R0 resection	LN+	Tumor size	Multiple tumors	Vascular invasion
Madariaga	1998	Yes-OS	No	No	Yes	No
Weber	2001	Yes	No	Yes-DFS	No	Yes-OS
Nakagawa	2005	Yes-OS	Yes-OS	No	Yes-OS	No
DeOliveira	2007	Yes	Yes	No	No	No
Paik	2007	Yes-DFS	Yes-DFS	Yes-DFS	Yes-DFS	No
Endo	2008	No	Yes-RFS	Yes	Yes-RFS	No
Lang	2009	Yes	No	No	No	No
Nathan	2009	n/a	Yes	No	Yes	Yes
Shen	2009	Yes-OS	Yes-OS	Yes-OS	No	No
de Jong	2011	Yes-OS	Yes-OS	No	Yes	Yes
Farges	2011	Yes-OS in N0 pts	No	No	Yes-OS	No

OS overall survival; DFS disease-free survival; RFS recurrence-free survival

The goal of surgical resection of ICC is the complete removal of all gross and microscopic disease (R0 resection). This was achieved in 45–96 % of attempted curative resections [9, 10, 15, 19, 23, 24, 26–28, 50, 75]. Many studies have found R0 resection to be associated with more favorable outcomes [9, 10, 15, 25–28, 75]. In one recent multi-institutional review of 449 patients with resected ICC, the influence of resection margins on overall survival was significant only in patients with node-negative disease; those with positive lymph nodes had no other factors independently associated with survival [15]. This relationship was also seen in a European multi-institutional study of 212 patients undergoing resection for ICC; patients with N0 disease demonstrated resection margin to be associated with survival, but node-positive patients did not have additional factors influencing survival [75].

While the practice of routine lymphadenectomy is variable, there is consistency across centers that lymph node involvement with ICC is a poor prognostic factor [9, 10, 15, 19, 26, 27]. However, in the absence of more effective treatment, surgical resection with lymphadenectomy is still advocated for patients with ICC and regional nodal disease [18]. Tumor size [10, 19, 27, 28], multiple tumors [10, 15, 19, 25, 26, 75], and vascular invasion [12, 15, 28] have also been associated with survival. Factors associated with survival in modern series are summarized in Table 3. Median overall survival following resection ranges from 12.4 to 52.9 months [9, 10, 15, 23, 25, 27, 28, 50]. Some series report overall survival for a cohort that contains unresected or R2 patients [27]. Overall survival at one year ranges from 51 to 85 %, 3 years 22–66 %, and 5 years 17–44 % [9, 10, 15, 23–28, 50]. Survival outcomes are summarized in Table 4.

5 Adjuvant Therapy

Given the overall poor prognosis of ICC, even following a potentially curative resection, multimodality therapy is an attractive strategy to improve outcomes. The study of adjuvant and/or neoadjuvant therapy in ICC is limited by the relative rarity of ICC compared with other tumors such as HCC. However, studies of systemic therapy in unresectable cholangiocarcinoma may offer some insights.

For many years, 5-FU-based regimens were the only option for biliary tract cancers, with little efficacy [76, 77]. Single-agent gemcitabine was subsequently investigated in small studies of advanced biliary tract cancer, after promising results in pancreatic cancers [78–80]. Most recently, multi-agent gemcitabine-based regimens have been studied in phase II and phase III trials. One of the largest such studies enrolled 410 patients with unresectable biliary tract cancers, including 241 cholangiocarcinomas, which were not stratified by anatomic tumor location. Patients were randomized to receive gemcitabine (1,000 mg/m²) on days 1, 8 and 15 of a 4-week cycle, or cisplatin (25 mg/m²) and gemcitabine (1,000 mg/m²) on days 1 and 8 of a 3-week cycle. Overall survival in the cisplatin–gemcitabine group was significantly longer at 11.7 months, compared with 8.1 months in the gemcitabine-only group, and progression-free survival was also significantly improved in the cisplatin–gemcitabine group, at 8.0 versus 5.0 months [81].

It is not clear whether these modest but promising results will be applicable in the patients undergoing resection. In a retrospective review, Glazer et al. analyzed 157 patients with biliary tract cancer, 54 of whom had ICC. These patients were treated with a variety of adjuvant and/or neoadjuvant treatments (gemcitabine/platinum based or

Table 4 Median and overall survival after resection for ICC

First author	Year	N	Median survival (mos)	1-year OS (%)	3-year OS (%)	5-year OS (%)
Madariaga	1998	34	19	67	40	35
Weber	2001	33	37		55	31
Nakagawa	2005	44	22	66	38	26
DeOliveira	2007	44	28			40
Paik	2007	97	53	75	52	31
Endo	2008	77	36 (DSS)			
Konstadoulakis	2008	54		80	49	25
Gugliemi	2009	62	41		55	26
Lang	2009	83	26	71	38	21
Nathan	2009	598	21		31	18
Shen	2009	429	12	51	22	17
de Jong	2011	449	27	78	44	31
Ali	2012	121	43	85	66	44

OS overall survival; DSS disease-specific survival

5-FU neoadjuvant regimens, 5-FU or capecitabine-based adjuvant regimens). On univariate analysis, chemotherapy was associated with diminished survival, but on multivariate analysis, neither adjuvant nor neo-adjuvant treatment had any impact on survival [82].

6 Conclusions

ICC is increasing in incidence for unclear reasons, but perhaps related to hepatitis C as a risk factor. Over 50 % of patients are unresectable at presentation, most often due to locally advanced disease. Staging laparoscopy may spare some patients a non-therapeutic laparotomy, but will not identify all patients with locally advanced, unresectable disease. Surgical resection is the only possibility for long-term survival, and extended resection and/or vascular resection with reconstruction should be undertaken by an experienced team if needed to achieve complete tumor clearance. Effective neo-adjuvant or adjuvant treatments have not been demonstrated, but this remains the subject of ongoing investigation.

References

- Khan SA, Davidson BR, Goldin R, Pereira SP, Rosenberg WM, Taylor-Robinson SD, Thillainayagam AV, Thomas HC, Thursz MR, Wasan H (2002) Guidelines for the diagnosis and treatment of cholangiocarcinoma: consensus document. *Gut* 51 Suppl 6:VI1–VI9
- Shaib Y, El-Serag HB (2004) The epidemiology of cholangiocarcinoma. *Semin Liver Dis* 24:115–125
- Chapman RW (1999) Risk factors for biliary tract carcinogenesis. *Ann Oncol Off J Eur Soc Med Oncol (ESMO)* 10 Suppl 4:308–311
- Shaib YH, El-Serag HB, Davila JA, Morgan R, McGlynn KA (2005) Risk factors of intrahepatic cholangiocarcinoma in the United States: a case-control study. *Gastroenterology* 128:620–626
- Patel T (2001) Increasing incidence and mortality of primary intrahepatic cholangiocarcinoma in the United States. *Hepatology* 33:1353–1357
- Khan SA, Emadossadaty S, Lade NG, Thomas HC, Elliott P, Taylor-Robinson SD, Toledano MB (2012) Rising trends in cholangiocarcinoma: is the ICD classification system misleading us? *J Hepatol* 56:848–854
- Patel T (2002) Worldwide trends in mortality from biliary tract malignancies. *BMC Cancer* 2:10
- Sempoux C, Jibara G, Ward SC, Fan C, Qin L, Roayaie S, Fiel MI, Schwartz M, Thung SN (2011) Intrahepatic cholangiocarcinoma: new insights in pathology. *Semin Liver Dis* 31:49–60
- DeOliveira ML, Cunningham SC, Cameron JL, Kamangar F, Winter JM, Lillemoe KD, Choti MA, Yeo CJ, Schulick RD (2007) Cholangiocarcinoma: thirty-one-year experience with 564 patients at a single institution. *Ann Surg* 245:755–762
- Paik KY, Jung JC, Heo JS, Choi SH, Choi DW, Kim YI (2008) What prognostic factors are important for resected intrahepatic cholangiocarcinoma? *J Gastroenterol Hepatol* 23:766–770
- Vauthey JN, Lauwers GY, Esnaola NF, Do KA, Belghiti J, Mirza N, Curley SA, Ellis LM, Regimbeau JM, Rashid A, Cleary KR, Nagorney DM (2002) Simplified staging for hepatocellular carcinoma. *J Clin Oncol: Off J Am Soc Clin Oncol* 20:1527–1536
- Nathan H, Aloia TA, Vauthey JN, Abdalla EK, Zhu AX, Schulick RD, Choti MA, Pawlik TM (2009) A proposed staging system for intrahepatic cholangiocarcinoma. *Ann Surg Oncol* 16:14–22
- Nathan H, Pawlik TM (2010) Staging of intrahepatic cholangiocarcinoma. *Curr Opin Gastroenterol* 26:269–273
- Farges O, Fuks D, Le Treut YP, Azoulay D, Laurent A, Bachellier P, Nuzzo G, Belghiti J, Pruvot FR, Regimbeau JM (2011) AJCC 7th edition of TNM staging accurately discriminates outcomes of patients with resectable intrahepatic cholangiocarcinoma: by the AFC-IHCC-2009 study group. *Cancer* 117:2170–2177
- de Jong MC, Nathan H, Sotiropoulos GC, Paul A, Alexandrescu S, Marques H, Pulitano C, Barroso E, Clary BM, Aldrighetti L, Ferrone CR, Zhu AX, Bauer TW, Walters DM, Gamblin TC,

- Nguyen KT, Turley R, Popescu I, Hubert C, Meyer S, Schulick RD, Choti MA, Gigot JF, Mentha G, Pawlik TM (2011) Intrahepatic cholangiocarcinoma: an international multi-institutional analysis of prognostic factors and lymph node assessment. *J Clin Oncol Off J Am Soc Clin Oncol* 29:3140–3145
16. Hirohashi K, Uenishi T, Kubo S, Yamamoto T, Tanaka H, Shuto T, Kinoshita H (2002) Macroscopic types of intrahepatic cholangiocarcinoma: clinicopathologic features and surgical outcomes. *Hepato-Gastroenterology* 49:326–329
 17. Shimada K, Sano T, Sakamoto Y, Esaki M, Kosuge T, Ojima H (2007) Surgical outcomes of the mass-forming plus periductal infiltrating types of intrahepatic cholangiocarcinoma: a comparative study with the typical mass-forming type of intrahepatic cholangiocarcinoma. *World J Surg* 31:2016–2022
 18. Nguyen KT, Steel J, Vanounou T, Tsung A, Marsh JW, Geller DA, Gamblin TC (2009) Initial presentation and management of hilar and peripheral cholangiocarcinoma: is a node-positive status or potential margin-positive result a contraindication to resection? *Ann Surg Oncol* 16:3308–3315
 19. Endo I, Gonen M, Yopp AC, Dalal KM, Zhou Q, Klimstra D, D'Angelica M, DeMatteo RP, Fong Y, Schwartz L, Kemeny N, O'Reilly E, Abou-Alfa GK, Shimada H, Blumgart LH, Jarnagin WR (2008) Intrahepatic cholangiocarcinoma: rising frequency, improved survival, and determinants of outcome after resection. *Ann Surg* 248:84–96
 20. Vauthey JN, Chaoui A, Do KA, Bilimoria MM, Fenstermacher MJ, Charnsangavej C, Hicks M, Alsasser G, Lauwers G, Hawkins IF, Caridi J (2000) Standardized measurement of the future liver remnant prior to extended liver resection: methodology and clinical associations. *Surgery* 127:512–519
 21. Couinaud C (1954) Lobes de segments hepatiques: notes sur l'architecture anatomique et chirurgicale de foie. *La Presse medicale* 62:709–712
 22. Strasberg S, Gelghiti J, Clavien PA, Gadzijev E, Garden J, Lau W-Y, Makuuchi M, Strong R (2000) The Brisbane 2000 terminology of liver anatomy and resections. *HPB* 2:333–339
 23. Ali SM, Clark CJ, Zaydfudim VM, Que FG, Nagorney DM (2012) Role of major vascular resection in patients with intrahepatic cholangiocarcinoma. *Annals of surgical oncology* 20:1–6
 24. Konstadoulakis MM, Roayaie S, Gornat IP, Labow D, Fiel MI, Miller CM, Schwartz ME (2008) Fifteen-year, single-center experience with the surgical management of intrahepatic cholangiocarcinoma: operative results and long-term outcome. *Surgery* 143:366–374
 25. Madariaga JR, Iwatsuki S, Todo S, Lee RG, Irish W, Starzl TE (1998) Liver resection for hilar and peripheral cholangiocarcinomas: a study of 62 cases. *Ann Surg* 227:70–79
 26. Nakagawa T, Kamiyama T, Kurauchi N, Matsushita M, Nakanishi K, Kamachi H, Kudo T, Todo S (2005) Number of lymph node metastases is a significant prognostic factor in intrahepatic cholangiocarcinoma. *World J Surg* 29:728–733
 27. Shen WF, Zhong W, Xu F, Kan T, Geng L, Xie F, Sui CJ, Yang JM (2009) Clinicopathological and prognostic analysis of 429 patients with intrahepatic cholangiocarcinoma. *World J Gastroenterol (WJG)* 15:5976–5982
 28. Weber SM, Jarnagin WR, Klimstra D, DeMatteo RP, Fong Y, Blumgart LH (2001) Intrahepatic cholangiocarcinoma: resectability, recurrence pattern, and outcomes. *J Am Coll Surg* 193:384–391
 29. Lang H, Sotiropoulos GC, Sgourakis G, Schmitz KJ, Paul A, Hilgard P, Zopf T, Trarbach T, Malago M, Baba HA, Broelsch CE (2009) Operations for intrahepatic cholangiocarcinoma: single-institution experience of 158 patients. *J Am Coll Surg* 208:218–228
 30. Lang H, Sotiropoulos GC, Fruhauf NR, Domland M, Paul A, Kind EM, Malago M, Broelsch CE (2005) Extended hepatectomy for intrahepatic cholangiocellular carcinoma (ICC): when is it worthwhile? Single center experience with 27 resections in 50 patients over a 5-year period. *Ann Surg* 241:134–143
 31. Ebata T, Yokoyama Y, Igami T, Sugawara G, Takahashi Y, Nagino M (2012) Portal vein embolization before extended hepatectomy for biliary cancer: current technique and review of 494 consecutive embolizations. *Dig Surg* 29:23–29
 32. Abdalla EK, Barnett CC, Doherty D, Curley SA, Vauthey JN (2002) Extended hepatectomy in patients with hepatobiliary malignancies with and without preoperative portal vein embolization. *Arch Surg* 137:675–680 (discussion 671–680)
 33. Kishi Y, Abdalla EK, Chun YS, Zorzi D, Madoff DC, Wallace MJ, Curley SA, Vauthey JN (2009) Three hundred and one consecutive extended right hepatectomies: evaluation of outcome based on systematic liver volumetry. *Ann Surg* 250:540–548
 34. Makuuchi M, Thai BL, Takayasu K, Takayama T, Kosuge T, Gunven P, Yamazaki S, Hasegawa H, Ozaki H (1990) Preoperative portal embolization to increase safety of major hepatectomy for hilar bile duct carcinoma: a preliminary report. *Surgery* 107:521–527
 35. Madoff DC, Hicks ME, Vauthey JN, Charnsangavej C, Morello FA Jr, Ahrar K, Wallace MJ, Gupta S (2002) Transhepatic portal vein embolization: anatomy, indications, and technical considerations. *Radiographics: a review publication of the Radiological Society of North America, Inc* 22:1063–1076
 36. Nagino M, Kamiya J, Nishio H, Ebata T, Arai T, Nimura Y (2006) Two hundred forty consecutive portal vein embolizations before extended hepatectomy for biliary cancer: surgical outcome and long-term follow-up. *Ann Surg* 243:364–372
 37. Hemming AW, Reed AI, Howard RJ, Fujita S, Hochwald SN, Caridi JG, Hawkins IF, Vauthey JN (2003) Preoperative portal vein embolization for extended hepatectomy. *Ann Surg* 237:686–691
 38. Iida H, Aihara T, Ikuta S, Yoshie H, Yamanaka N (2012) Comparison of percutaneous transhepatic portal vein embolization and unilateral portal vein ligation. *World J Gastroenterol (WJG)* 18:2371–2376
 39. Simoneau E, Aljiffry M, Salman A, Abualhassan N, Cabrera T, Valenti D, El Baage A, Jamal M, Kavan P, Al-Abbad S, Chaudhury P, Hassanain M, Metrakos P (2012) Portal vein embolization stimulates tumour growth in patients with colorectal cancer liver metastases. *Off J Int Hepatol Pancreatol Biliary Assoc (HPB)* 14:461–468
 40. Peng PD, Hyder O, Bloomston M, Marques H, Corona-Villalobos C, Dixon E, Pulitano C, Hirose K, Schulick RD, Barroso E, Aldrighetti L, Choti M, Shen F, Kamel I, Geschwind JF, Pawlik TM (2012) Sequential intra-arterial therapy and portal vein embolization is feasible and safe in patients with advanced hepatic malignancies. *Off J Int Hepatol Pancreatol Biliary Assoc (HPB)* 14:523–531
 41. John TG, Greig JD, Crosbie JL, Miles WF, Garden OJ (1994) Superior staging of liver tumors with laparoscopy and laparoscopic ultrasound. *Ann Surg* 220:711–719
 42. Ohtsuka M, Ito H, Kimura F, Shimizu H, Togawa A, Yoshidome H, Miyazaki M (2002) Results of surgical treatment for intrahepatic cholangiocarcinoma and clinicopathological factors influencing survival. *Br J Surg* 89:1525–1531
 43. Callery MP, Strasberg SM, Doherty GM, Soper NJ, Norton JA (1997) Staging laparoscopy with laparoscopic ultrasonography: optimizing resectability in hepatobiliary and pancreatic malignancy. *J Am Coll Surg* 185:33–39
 44. Tillemann EH, de Castro SM, Busch OR, Bemelman WA, van Gulik TM, Obertop H, Gouma DJ (2002) Diagnostic laparoscopy and laparoscopic ultrasound for staging of patients with malignant proximal bile duct obstruction. *J Gastrointest Surg Off J Soc Surg Aliment Tract* 6:426–430 (discussion 421–430)

45. Goere D, Wagholikar GD, Pessaux P, Carrere N, Sibert A, Vilgrain V, Sauvanet A, Belghiti J (2006) Utility of staging laparoscopy in subsets of biliary cancers: laparoscopy is a powerful diagnostic tool in patients with intrahepatic and gallbladder carcinoma. *Surg Endosc* 20:721–725
46. Gaujoux S, Allen PJ (2010) Role of staging laparoscopy in peripancratic and hepatobiliary malignancy. *World J Gastrointest Surg* 2:283–290
47. Hoekstra LT, Bieze M, Busch OR, Gouma DJ, van Gulik TM (2012) Staging laparoscopy in patients with hepatocellular carcinoma: is it useful? *Surg Endosc* 27(3):826–831
48. Shirabe K, Shimada M, Harimoto N, Sugimachi K, Yamashita Y, Tsujita E, Aishima S (2002) Intrahepatic cholangiocarcinoma: its mode of spreading and therapeutic modalities. *Surgery* 131:S159–S164
49. Okami J, Dono K, Sakon M, Tsujie M, Hayashi N, Fujiwara Y, Nagano H, Umeshita K, Nakamori S, Monden M (2003) Patterns of regional lymph node involvement in intrahepatic cholangiocarcinoma of the left lobe. *J Gastrointest Surg Off J Soc Surg Aliment Tract* 7:850–856
50. Guglielmi A, Ruzzenente A, Campagnaro T, Pachera S, Valdegamberi A, Nicoli P, Cappellani A, Malfermoni G, Iacono C (2009) Intrahepatic cholangiocarcinoma: prognostic factors after surgical resection. *World J Surg* 33:1247–1254
51. Nathan H, Pawlik TM, Wolfgang CL, Choti MA, Cameron JL, Schulick RD (2007) Trends in survival after surgery for cholangiocarcinoma: a 30-year population-based SEER database analysis. *J Gastrointest Surg Off J Soc Surg Aliment Tract* 11:1488–1496 (discussion 1487–1496)
52. Morine Y, Shimada M, Utsunomiya T, Imura S, Ikemoto T, Mori H, Hanaoka J, Kanamoto M, Miyake H (2012) Clinical impact of lymph node dissection in surgery for peripheral-type intrahepatic cholangiocarcinoma. *Surg Today* 42:147–151
53. Reddy SK, Tsung A, Geller DA (2011) Laparoscopic liver resection. *World J Surg* 35:1478–1486
54. Nguyen KT, Gamblin TC, Geller DA (2009) World review of laparoscopic liver resection-2,804 patients. *Ann Surg* 250:831–841
55. Nguyen KT, Marsh JW, Tsung A, Steel JJ, Gamblin TC, Geller DA (2011) Comparative benefits of laparoscopic vs open hepatic resection: a critical appraisal. *Arch Surg* 146:348–356
56. Vanounou T, Steel JL, Nguyen KT, Tsung A, Marsh JW, Geller DA, Gamblin TC (2010) Comparing the clinical and economic impact of laparoscopic versus open liver resection. *Ann Surg Oncol* 17:998–1009
57. Packiam V, Bartlett DL, Tohme S, Reddy S, Marsh JW, Geller DA, Tsung A (2012) Minimally invasive liver resection: robotic versus laparoscopic left lateral sectionectomy. *J Gastrointest Surg Off J Soc Surg Aliment Tract* 16:2233–2238
58. Choi GH, Choi SH, Kim SH, Hwang HK, Kang CM, Choi JS, Lee WJ (2012) Robotic liver resection: technique and results of 30 consecutive procedures. *Surg Endosc* 26:2247–2258
59. Kitisin K, Packiam V, Bartlett DL, Tsung A (2011) A current update on the evolution of robotic liver surgery. *Minerva Chir* 66:281–293
60. Casavilla FA, Marsh JW, Iwatsuki S, Todo S, Lee RG, Madariaga JR, Pinna A, Dvorchik I, Fung JJ, Starzl TE (1997) Hepatic resection and transplantation for peripheral cholangiocarcinoma. *J Am Coll Surg* 185:429–436
61. Iwatsuki S, Starzl TE, Sheahan DG, Yokoyama I, Demetris AJ, Todo S, Tzakis AG, Van Thiel DH, Carr B, Selby R et al (1991) Hepatic resection versus transplantation for hepatocellular carcinoma. *Ann Surg* 214:221–228 (discussion 228–229)
62. Meyer CG, Penn I, James L (2000) Liver transplantation for cholangiocarcinoma: results in 207 patients. *Transplantation* 69:1633–1637
63. Ghali P, Marotta PJ, Yoshida EM, Bain VG, Marleau D, Peltekian K, Metrakos P, Deschenes M (2005) Liver transplantation for incidental cholangiocarcinoma: analysis of the Canadian experience. *Liver Transplant* 11:1412–1416 (Official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society)
64. Goldstein RM, Stone M, Tillery GW, Senzer N, Levy M, Husberg BS, Gonwa T, Klintmalm G (1993) Is liver transplantation indicated for cholangiocarcinoma? *Am J Surg* 166:768–771 (discussion 762–771)
65. Becker NS, Rodriguez JA, Barshes NR, O'Mahony CA, Goss JA, Aloia TA (2008) Outcomes analysis for 280 patients with cholangiocarcinoma treated with liver transplantation over an 18-year period. *J Gastrointest Surg* 12:117–122 (the official journal of the Society for Surgery of the Alimentary Tract)
66. Brandsaeter B, Isoniemi H, Broome U, Olausson M, Backman L, Hansen B, Schrupf E, Oksanen A, Ericzon BG, Hockerstedt K, Makisalo H, Kirkegaard P, Friman S, Bjoro K (2004) Liver transplantation for primary sclerosing cholangitis; predictors and consequences of hepatobiliary malignancy. *J Hepatol* 40:815–822
67. Hong JC, Petrowsky H, Kaldas FM, Farmer DG, Durazo FA, Finn RS, Saab S, Han SH, Lee P, Markovic D, Lassman C, Hiatt JR, Busuttil RW (2011) Predictive index for tumor recurrence after liver transplantation for locally advanced intrahepatic and hilar cholangiocarcinoma. *J Am Coll Surg* 212:514–520 (discussion 511–520)
68. Hong JC, Jones CM, Duffy JP, Petrowsky H, Farmer DG, French S, Finn R, Durazo FA, Saab S, Tong MJ, Hiatt JR, Busuttil RW (2011) Comparative analysis of resection and liver transplantation for intrahepatic and hilar cholangiocarcinoma: a 24-year experience in a single center. *Arch Surg* 146:683–689
69. Imamura H, Seyama Y, Kokudo N, Maema A, Sugawara Y, Sano K, Takayama T, Makuuchi M (2003) One thousand fifty-six hepatectomies without mortality in 8 years. *Arch Surg* 138:1198–1206 (discussion 1206)
70. Belghiti J, Hiramatsu K, Benoist S, Massault P, Sauvanet A, Farges O (2000) Seven hundred forty-seven hepatectomies in the 1990s: an update to evaluate the actual risk of liver resection. *J Am Coll Surg* 191:38–46
71. Kamiyama T, Nakanishi K, Yokoo H, Kamachi H, Tahara M, Yamashita K, Taniguchi M, Shimamura T, Matsushita M, Todo S (2010) Perioperative management of hepatic resection toward zero mortality and morbidity: analysis of 793 consecutive cases in a single institution. *J Am Coll Surg* 211:443–449
72. Geller DA, Tsung A, Maheshwari V, Rutstein LA, Fung JJ, Marsh JW (2005) Hepatic resection in 170 patients using saline-cooled radiofrequency coagulation. *HPB* 7:208–213 (official journal of the International Hepato Pancreato Biliary Association)
73. Aloia TA, Fahy BN, Fischer CP, Jones SL, Duchini A, Galati J, Gaber AO, Ghobrial RM, Bass BL (2009) Predicting poor outcome following hepatectomy: analysis of 2313 hepatectomies in the NSQIP database. *HPB* 11:510–515 (the official journal of the International Hepato Pancreato Biliary Association)
74. Dimick JB, Wainess RM, Cowan JA, Upchurch GR Jr, Knol JA, Colletti LM (2004) National trends in the use and outcomes of hepatic resection. *J Am Coll Surg* 199:31–38
75. Farges O, Fuks D, Boleslawski E, Le Treut YP, Castaing D, Laurent A, Ducerf C, Rivoire M, Bachellier P, Chiche L, Nuzzo G, Regimbeau JM (2011) Influence of surgical margins on outcome in patients with intrahepatic cholangiocarcinoma: a multicenter study by the AFC-IHCC-2009 study group. *Ann surg* 254:824–829 (discussion 830)
76. Ducreux M, Van Cutsem E, Van Laethem JL, Gress TM, Jeziorski K, Rougier P, Wagener T, Anak O, Baron B, Nordlinger B (2005) A randomised phase II trial of weekly high-dose 5-fluorouracil

- with and without folinic acid and cisplatin in patients with advanced biliary tract carcinoma: results of the 40955 EORTC trial. *Eur J Cancer* 41:398–403
77. Choi CW, Choi IK, Seo JH, Kim BS, Kim JS, Kim CD, Um SH, Kim JS, Kim YH (2000) Effects of 5-fluorouracil and leucovorin in the treatment of pancreatic-biliary tract adenocarcinomas. *Am J Clin Oncol* 23:425–428
78. Raderer M, Hejna MH, Valencak JB, Kornek GV, Weinlander GS, Barek E, Lenauer J, Brodowicz T, Lang F, Scheithauer W (1999) Two consecutive phase II studies of 5-fluorouracil/leucovorin/mitomycin C and of gemcitabine in patients with advanced biliary cancer. *Oncology* 56:177–180
79. Lin MH, Chen JS, Chen HH, Su WC (2003) A phase II trial of gemcitabine in the treatment of advanced bile duct and perianapillary carcinomas. *Chemotherapy* 49:154–158
80. Tsavaris N, Kosmas C, Gouveris P, Gennatas K, Polyzos A, Mouratidou D, Tsipras H, Margaris H, Papastratis G, Tzima E, Papadoniou N, Karatzas G, Papalambros E (2004) Weekly gemcitabine for the treatment of biliary tract and gallbladder cancer. *Invest New Drugs* 22:193–198
81. Valle J, Wasan H, Palmer DH, Cunningham D, Anthoney A, Maraveyas A, Madhusudan S, Iveson T, Hughes S, Pereira SP, Roughton M, Bridgewater J (2010) Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. *New Engl J Med* 362:1273–1281
82. Glazer ES, Liu P, Abdalla EK, Vauthey JN, Curley SA (2012) Neither neoadjuvant nor adjuvant therapy increases survival after biliary tract cancer resection with wide negative margins. *Journal of Gastrointest Surg* 16:1666–1671 (official journal of the Society for Surgery of the Alimentary Tract)

Surgical Techniques for Extrahepatic Biliary Tract Cancers

Junichi Shindoh, Giuseppe Zimmitti, and Jean-Nicolas Vauthey

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Abstract

For patients with extrahepatic biliary tract cancer, surgical resection is the only therapeutic option offering a chance of cure. However, with its specific location surrounded by multiple viscera and major vascular structures, surgical resection for biliary tract cancer frequently requires advanced surgical techniques. To secure the safety of radical surgery in high risk patients, optimization of the condition of patient and adequate selection of surgical procedure is necessary.

1 Introduction

Complete surgical resection is the single most effective treatment for patients diagnosed with extrahepatic biliary tract cancer [38, 40, 55]. However, because of its specific location surrounded by multiple viscera and complex vascular structures, resection of extrahepatic biliary cancer frequently requires combined hepatic resection or Whipple procedure to secure the surgical margin. In addition, extrahepatic biliary cancers are often complicated by jaundice and impaired hepatic function due to obstruction of the biliary tract. Therefore, meticulous preoperative assessment and preparations are needed to achieve this ‘high-risk’ surgical resection. In this chapter, surgical strategies and technical refinements to improve the treatment outcomes of extrahepatic biliary cancer will be described.

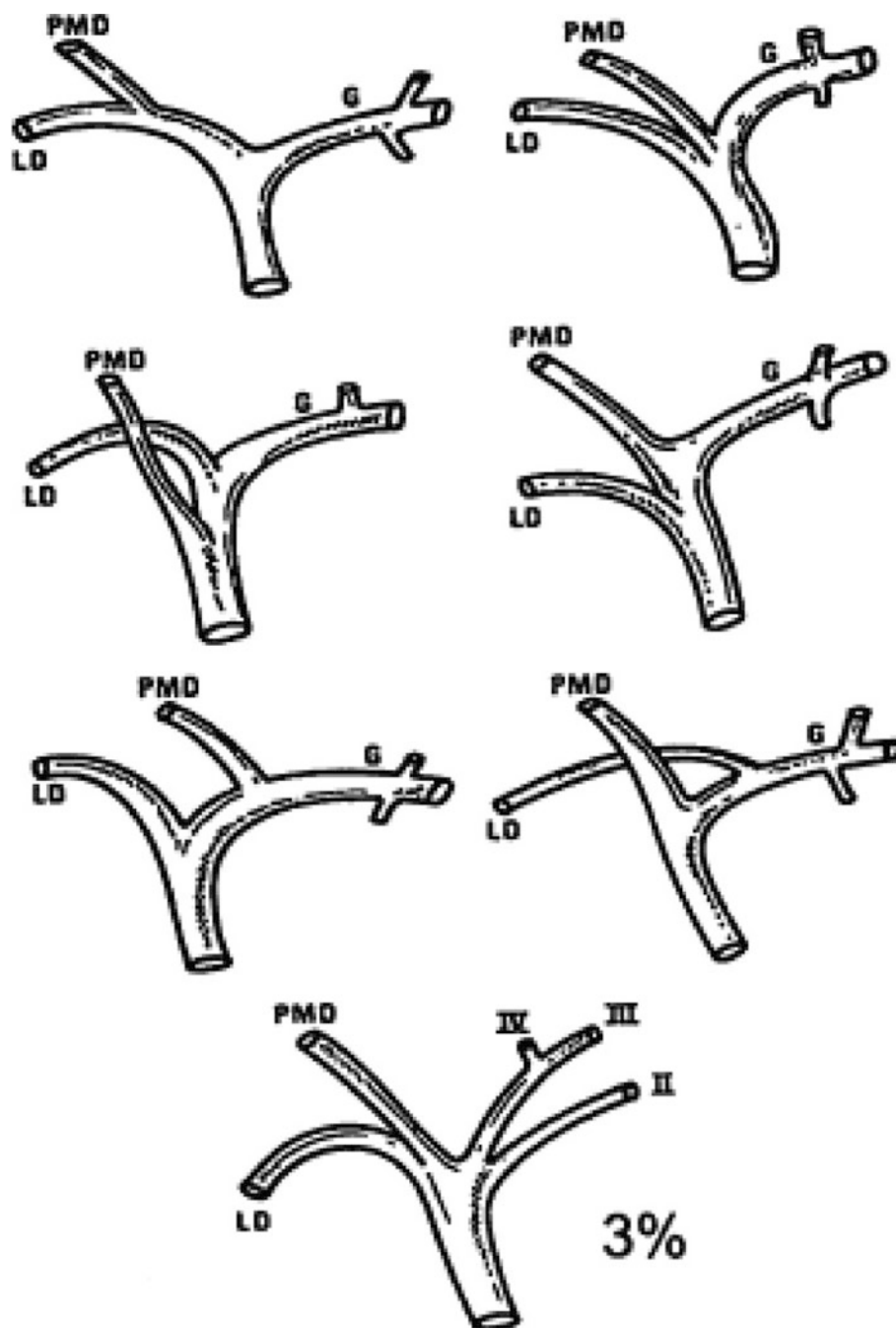
2 Anatomic Basis for the Surgical Resection of Extrahepatic Biliary Cancer

2.1 Variations in Biliary Anatomy

In 1957, Couinaud [10] described major variations of biliary anatomy in adult liver (Fig. 1). One of the most important observations in his work is that the left hepatic duct is

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Fig. 1 Variations in biliary anatomy. The left hepatic duct is long and present in 97 % of patients. In types I, II, and IIIa hilar cholangiocarcinomas, an extended right hepatectomy permits placement of a safe and single anastomosis away from the confluence, minimizing the probability of positive margins. *G* left; *LD* right lateral ; *PMD* right paramedian (from [10] pp 469–479, with permission.)



present in 97 % of patients, while the biliary branches for the right side of the liver vary considerably. It is also noteworthy that the left hepatic duct is mostly extrahepatic, located strategically at the base of Segment IV, and extends to the left for a length of 1–5 cm. These characteristics aid in the extension of the resection toward the left, away from the biliary confluence, with minimizing the likelihood of positive margins and facilitating the biliary reconstruction [61].

2.2 Three-dimensional Relationship between the Extrahepatic Biliary Tract and Surrounding Vascular Structures

Figure 2 shows a typical three-dimensional relationship among the biliary tract, the portal vein, and the hepatic artery at the hepatic hilum. The common hepatic duct bifurcates into the right and left branches at the very cephalad part of the hepatoduodenal ligament, and these

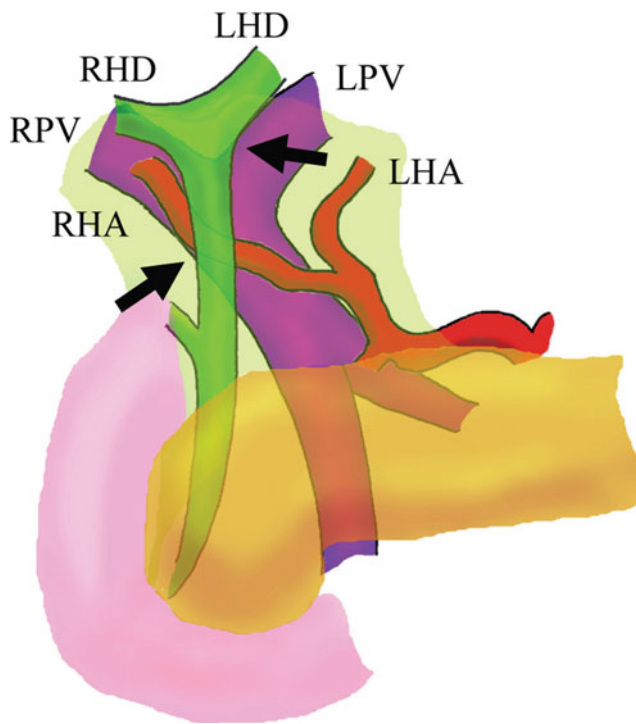


Fig. 2 Vascular anatomy at the hepatic hilum. Right hepatic artery and portal bifurcation are located just behind the extrahepatic biliary tract, and they are subjected to invasion by extrahepatic biliary cancers at these points (arrows). LHA left hepatic artery; LHD left hepatic duct; LPV left portal vein; RHA right hepatic artery; RHD right hepatic duct; RPV right portal vein

branches run within the dense connective tissue called *hilar plate* before entering the hepatic parenchyma. Two oncologically important anatomic features regarding the three-dimensional vascular relationship are that (1) the right hepatic artery runs just behind the common hepatic duct and (2) the portal bifurcation is located very close to the confluence of the hepatic ducts. Because these structures are prone to invasion by extrahepatic biliary cancers, right-sided hepatic resection is preferred in most of the surgical resection for hilar cholangiocarcinoma.

2.3 Peribiliary Lymphatic Systems

Patterns of peribiliary lymphatic drainage have been actively investigated, and two major lymphatic drainage routes have been described [34, 78]: the right-sided route from biliary/portal nodes (No. 12b/12p) to para-aortic nodes (No. 16) and the left-sided route from hepatic arterial nodes (No. 12a) to para-aortic nodes (No. 16) via common hepatic arterial nodes (No. 8a) and celiac nodes (No. 9) (Fig. 3). Although the detailed anatomy in biliary lymphatic systems has not been fully understood, groups of

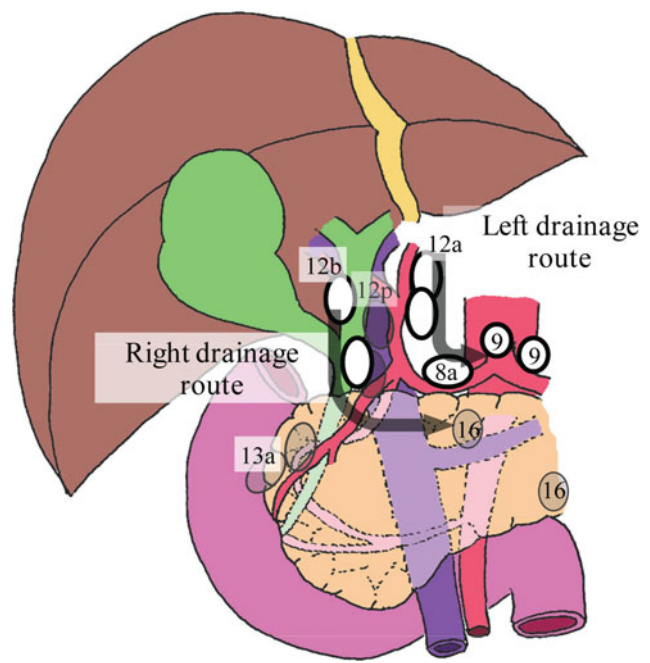


Fig. 3 Schematic drawing of the biliary lymphatic system and supposed major drainage routes (Adapted from [29])

regional lymph node have been defined and lymphadenectomy has been recommended according to each location of tumor [39, 68].

3 Assessment of Tumor Extension and Surgical Planning

3.1 Diagnosis and Assessment of Tumor Extension

For diagnosis of extrahepatic malignancy, histologic assessment is required to rule out other benign cause for biliary obstruction. Extent of disease is assessed using several imaging modalities including computed tomography (CT), magnetic resonance imaging/cholangiopancreatography (MRI/MRCP), endoscopic ultrasonography (EUS), fluorodeoxyglucose positron emission tomography, or direct cholangiography via endoscopic or percutaneous routes. Among these, direct contrast cholangiography may yield the most important anatomic data regarding the location and morphologic characteristics of biliary obstruction. However, cholangiography may place the patients at risk for cholangitis and hepatic abscess by introducing enteric/cutaneous flora to the biliary system. Also, subsequently placed biliary stent would interfere with further evaluation of resectability by the other imaging modalities. Aloia et al. [3] have reported that the use of high-resolution CT is an

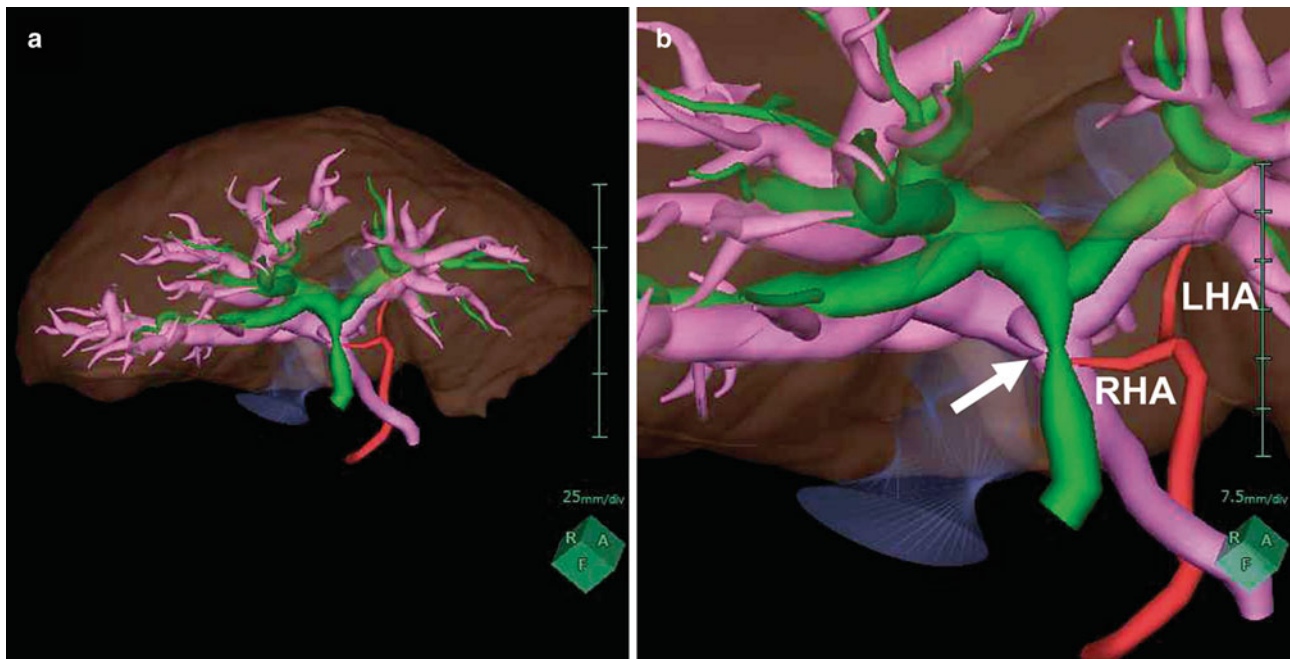


Fig. 4 3-D visualization of anatomic structures for surgical planning of hilar cholangiocarcinoma (courtesy to Dr. Yoshihiro Mise). **a** 3-D intrahepatic vascular structures (*pink* portal vein; *green* biliary tract; *red* hepatic artery). **b** Magnified view of hepatic hilum. Cancerous

stricture of the common hepatic duct (*arrow*) and its relation with the right hepatic artery are clearly visualized. *RHA* right hepatic artery; *LHA* left hepatic artery

oblique coronal plane with three-dimensional reformation that accurately predicts resectability of hilar cholangiocarcinoma with sensitivity of 94 % and specificity of 79 %. Thin-slice high-resolution CT scan with an adequate enhancement protocol may offer sufficient anatomic and oncological data for surgery non-invasively. It may also enable simultaneous assessment of distant metastasis through single scanning from thorax to pelvis.

3.2 Surgical Planning with Advanced Imaging Modalities

Recently, a novel three-dimensional (3-D) simulation technique has been introduced and broadly used in anatomic confirmation and/or surgical planning for complex hepatobiliary surgery [45, 64, 65, 72]. The major advantages of this technique are individualized inflow/outflow analysis and accurate volume calculation that enables surgical planning through various virtual hepatectomies simulated on a computer. For surgical planning of extrahepatic biliary cancer, a 3-D simulator offers accurate visualization of 3-D vascular relationships and it may help to avoid misunderstandings of complex anatomy and secure surgical margin (Fig. 4).

4 Preparation for Safe Surgical Resection

4.1 Biliary Drainage

Because biliary obstruction and jaundice have been reported to impair hepatic function, [16, 25, 35], biliary drainage either by endoscopic or percutaneous approach is preferable for patients with small future liver remnant. Empirically, portal vein embolization and surgical resections can be safely performed when serum bilirubin levels reach less than 5 mg/dL and less than 2 mg/dL, respectively [41, 71]. However, biliary drainage may also increase the risk of cholangitis or hepatic abscess due to introduction of enteric/cutaneous flora. Therefore, adequate management of drainage tubes (e.g., checking the patency of the stent by daily counting drainage volume) and close monitoring of signs of cholangitis are required to minimize preoperative cholangitis and optimize surgical outcome.

4.2 Portal Vein Embolization

Portal vein embolization (PVE) is a safe and minimally invasive procedure that leads to atrophy in the liver to be resected and compensatory hypertrophy of the future liver

Fig. 5 Incidences of major complication, hepatic insufficiency, and liver failure death after resection of hilar cholangiocarcinoma (MD Anderson Cancer Center 1997–2011, $n = 47$)

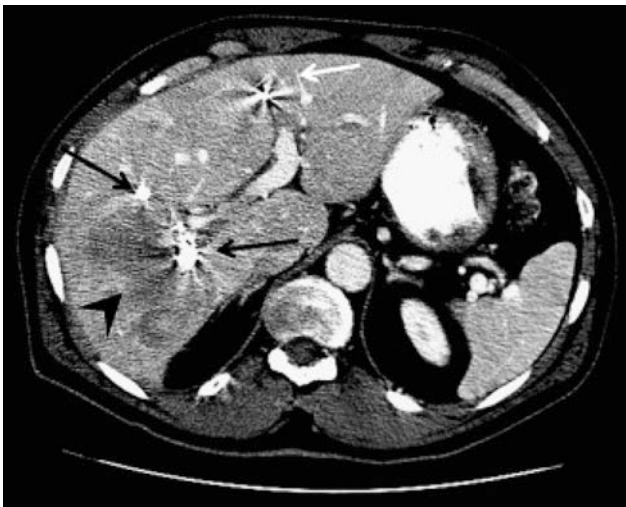
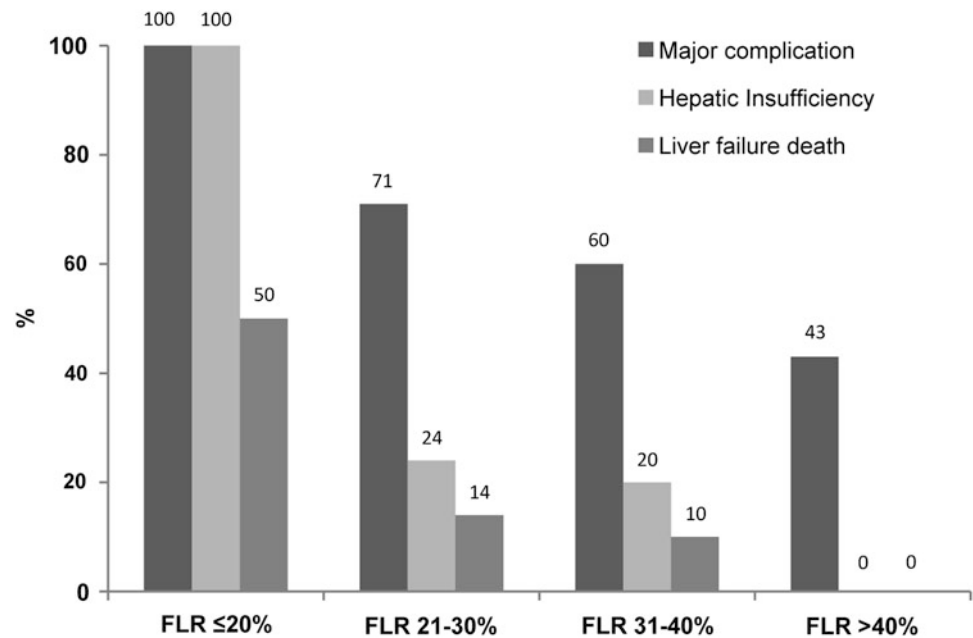


Fig. 6 CT image after right PVE + Segment IV embolization. Note that major S4 branches, in addition to right portal vein branches, were distally embolized (white arrow). Black arrows, coils used to embolized right portal vein branches; black arrowhead, tumor (From [37], with permission)

remnant (FLR). Although proposed minimum requirement of FLR volume in patients with normal liver varies among the authors and optimal FLR volume for extrahepatic biliary cancer is still controversial, [25, 35, 36, 53, 71, 81], many patients have complications such as jaundice, cholangitis, or malnutrition before surgery, and therefore, at least 30–40 % of FLR volume may be needed especially in patients with hilar cholangiocarcinoma [35, 53, 71] (Fig. 5).

To maximize regeneration of the FLR in PVE, selection of embolic materials [44] and concurrent embolization of

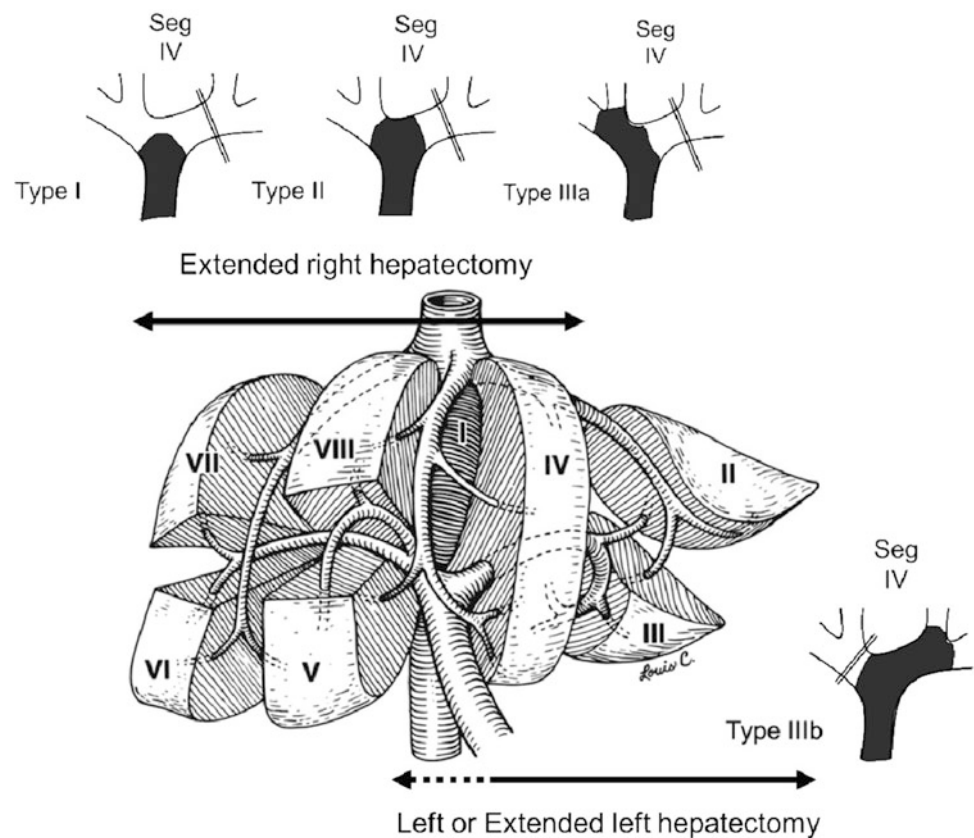
Segment IV portal veins [37, 52] have been recommended. Our previous work comparing right PVE with and without Segment IV embolization revealed significant difference in volume increase rates in Segment II + III (median, 26 % vs. 54 %; $p = 0.021$) [37] (Fig. 6). Recently, European groups have introduced associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) approach consisting of right portal vein ligation and in situ splitting of the liver at the umbilical fissure, and reported rapid and significant regeneration of FLR after the procedure. However, considering the very high morbidity and mortality rates of current ALPPS approach, [70], routine use of this technique should be avoided in patients with extrahepatic biliary cancer who are at high risk of postoperative hepatic insufficiency.

5 Surgical Principles for Hilar Cholangiocarcinoma

5.1 Classification and Surgical Approach

Surgical procedure for hilar cholangiocarcinoma is selected according to the Bismuth–Corlette classification (Fig. 7) [6]. For types I, II, and IIIa hilar cholangiocarcinoma, an extended right hepatectomy is usually performed, whereas for type IIIb hilar cholangiocarcinoma, a left or extended left hepatectomy is performed. In both surgical approaches, Segment IV must be completely or partially resected because most hilar cholangiocarcinomas extend to involve the base of Segment IV. Isolated bile duct resections or central resections for these tumors are not recommended

Fig. 7 Extent of hepatic resection according to Bismuth–Corlette classification of hilar cholangiocarcinoma (adapted from [62] with permission)



because of the limited margins and multiple bile duct anastomoses, both of which increase the risk of bile leaks and recurrences [61].

5.2 Significance of Caudate Lobe Resection

Mizumoto et al. [49] first emphasized the importance of resection of the caudate lobe based on their observation that 11 out of 26 patients had tumor invasion to the caudate lobe or its biliary branches and that curative resection rate was higher in patients with combined caudate lobectomy. Sugiura et al. [74] indicated that the survival rate was superior in patients with combined caudate lobectomy in a multi-institutional study. Type I or II hilar cholangiocarcinoma can theoretically be resected without hepatectomy. However, caudate branches usually drain into proximal part of the hepatic ducts, and prognostic superiority of hepatectomy with combined caudate lobectomy for types I and II hilar cholangiocarcinoma has also been reported [24].

Anatomic structure of the caudate lobe and distribution of its biliary ducts are rather complex. However, in some cases, partial preservation of the caudate lobe can be feasible especially on the left side (Spiegel lobe) as long as tumor invasion to this part or to its biliary branch can be excluded (Fig. 8). Couinaud [11] reported that Spiegel

branch drains into left hepatic duct in 90 % of the cases, while drainage patterns of right caudate branch vary considerably. If the Spiegel duct is sufficiently distant from the biliary bifurcation and drains into left hepatic duct, Spiegel lobe can be preserved in extended right hepatectomy.

5.3 Role of Vascular Resection

Due to proximity to major vascular structures (Fig. 2), portal bifurcation is often involved and combined resection is required for R0 resection in some cases. Recently, with introduction of vascular surgery techniques, relatively favorable outcomes in *en bloc* portal vein resection have been reported [54, 56, 57]. However, morbidity/mortality rates associated with this procedure is relatively high (Table 1), and its prognostic impact is still controversial. Therefore, vascular resection should be considered only in patients with definite vascular invasion.

5.4 Long-term Surgical Outcomes

Surgical outcomes of hilar cholangiocarcinoma reported from high-volume centers during the last decade have shown that 3- and 5-year overall survival rates ranged from

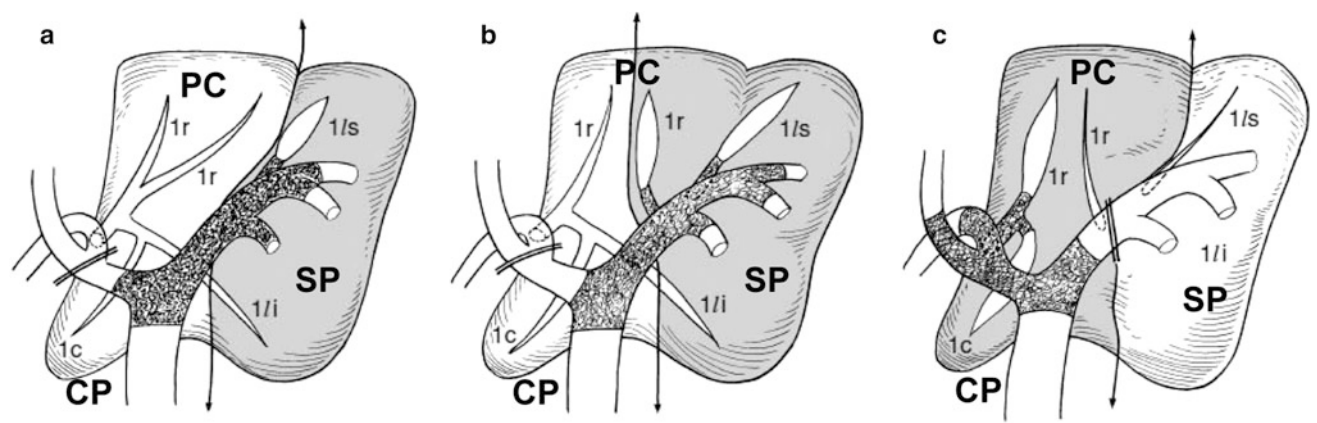


Fig. 8 Partial preservation of the caudate lobe based on the biliary anatomy and tumor invasion (Adapted from [59] Tan-to-sui 26:309–314 with permission). **a** Preservation of right caudate lobe in extended left hepatectomy for type IIIb tumor without invasion to 1r and 1c. **b** Partial preservation of right caudate lobe in extended left hepatectomy for type IIIb tumor with suspected invasion to one of right

caudate branch (1r). **c** Preservation of the Spiegel lobe in extended right hepatectomy for type IIIa tumor without invasion to Spiegel branches. Areas with *black dot* represent tumor invasion. *Gray* areas represent part of the caudate lobe to be resected. *SP* Spiegel lobe; *PC* paracaval portion; *CP* caudate process; 1r, right caudate branch; 1c, caudate process branch; 1/s, left superior branch; 1/i left inferior branch

Table 1 Curative resection rate and operative mortality in patients who underwent vascular resection

Authors	Year	N	R0 resection (%)	Mortality (%)
Nimura et al. [57]	1999	29	–	17.2
Neuhaus et al. [56]	1999	23	60.9	17.4
Ebata et al. [14]	2003	52	–	9.6
Miyazaki et al. [47]	2007	41	55.9	8.8
Hirano et al. [20]	2009	64	96.9	4.7
Nagino et al. [54]	2010	50	66.7	2.0
Hemming et al. [18]	2011	42	–	5.0

37 to 60 % and 20 to 42 %, respectively [2, 5, 8, 13, 17, 19, 21, 23, 26, 32, 43, 48, 51, 56, 58, 67, 71, 77, 80, 86]. A recent report from Japanese Biliary Tract Cancer Statistics Registry revealed that the overall 3- and 5-year survival rates after curative surgery for hilar cholangiocarcinoma (*n* = 255) were 47 and 39 %, respectively [46].

6 Surgical Principles for Distal Bile Duct Cancer

6.1 Tumor Distribution and Selection of Surgical Procedure

Distal bile duct cancer comprises 20–30 % of all cholangiocarcinoma [1]. Because of its specific location at the terminal part of the biliary tract, most patients present jaundice at relatively early stage of the disease, and accordingly, resection rate of distal bile duct cancer is usually higher than hilar cholangiocarcinoma.

Pancreaticoduodenectomy (PD), including pylorus-preserving PD (PPPD) coupled with lymphadenectomy, is the standard treatment of choice for the complete removal of distal bile duct cancer. Simple extrahepatic bile duct resection is feasible only in 10 % of patients [15, 82]. For patients with distal bile duct cancers, PPPD has been investigated with the expectation of functional preservation of the stomach. However, randomized controlled trial and meta-analysis have revealed that PD and PPPD provide equal short-term and long-term outcomes for pancreaticobiliary malignancies. Therefore, selection of surgical procedure should depend on the results of preoperative assessment including potential nodal involvement and/or extent of tumor invasion.

6.2 Long-term Surgical Outcomes

The overall 3- and 5-year survival rates after surgical resection of distal bile duct cancer ranged from 33 to 63 % and 16 to 52 %, respectively [2, 4, 9, 12, 22, 28, 31, 33, 42,

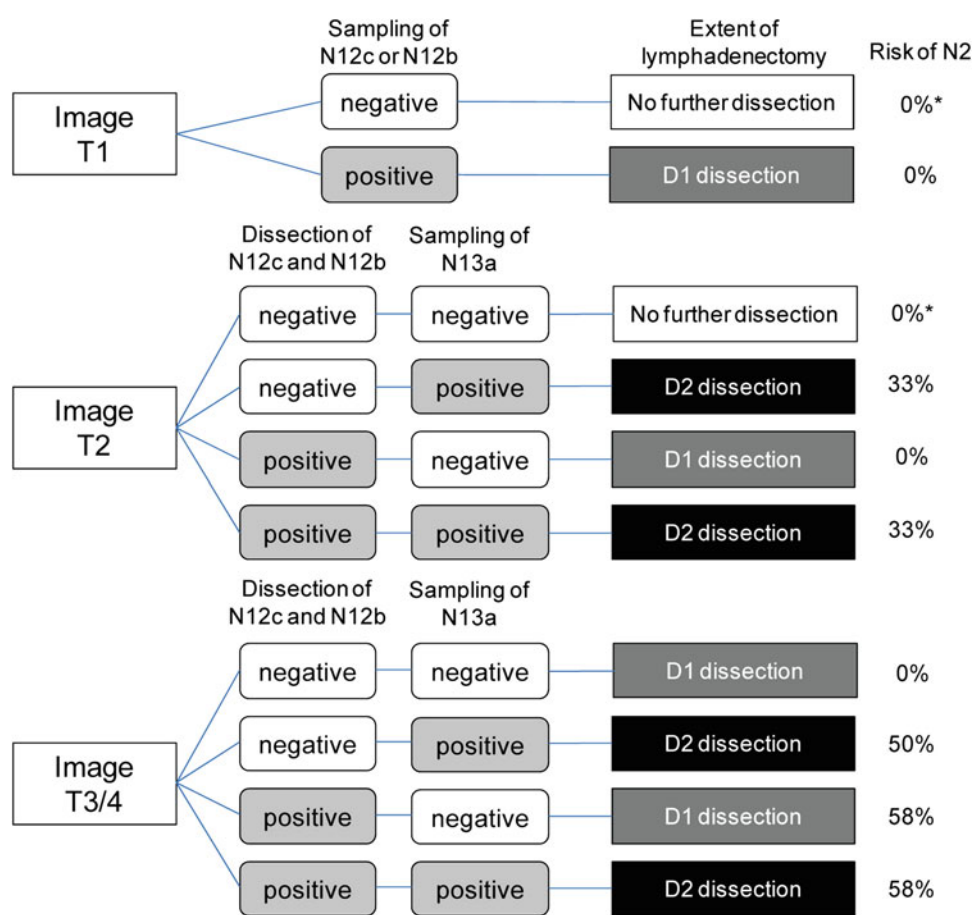


Fig. 9 Algorithm for the extent of lymph node dissection based on preoperative diagnosed T factor (Image T) and intraoperative frozen section of key lymph nodes. (Adapted from [39] with permission). D1 dissection, lymphadenectomy of first-echelon lymph nodes (N12c and N12b); D2 dissection, lymphadenectomy of second-echelon lymph nodes (N12p, N12a, N13a, N8a, and N8p). Number of lymph node is

based on the classification of Japanese Society of Biliary Surgery [29]. N12c, cystic nodes; N12b, pericholedochal nodes, N12p, portal nodes; N12a, nodes along the proper hepatic artery; N13a, superior retropancreatic nodes; N8a, anterior nodes along the common hepatic artery; N8p, posterior nodes along the common hepatic artery. * Risk of N1, 0 %

50, 60, 66, 69, 75, 84, 85]. The latest report from Japanese Biliary Tract Cancer Statistics Registry revealed that the overall 3- and 5-year survival rates after PD or PPPD for distal bile duct cancer ($n = 779$) were 58 and 44 %, respectively [46].

based on the previous clinical outcomes. In surgical treatment of gallbladder cancer, R0 resection is needed to achieve extended survival [73]. To achieve this surgical outcome, various surgical approaches have been attempted.

7 Surgical Principles for Gallbladder Cancer

7.1 Selection of Surgical Procedure Based on the Clinical Outcomes

Various surgical procedures have been indicated for gallbladder cancer according to the extent of the disease. However, precisely preoperative staging remains difficult. Therefore, surgical approach should be adopted systematically

7.1.1 Lymphadenectomy

Effectiveness of lymphadenectomy has been well documented [39, 73, 79]. Also, assessment of lymph node status is recommended for risk stratification after surgery [27]. Although there still remain controversies in optimal extent of lymphadenectomy, incidence of lymph node metastases is well correlated with depth of tumor invasion. Kokudo et al. [39] investigated the accuracy of preoperative image studies in predicting T stage of gallbladder cancer and proposed algorithms for stepwise lymphadenectomy based on the risk and incidence of lymph node involvement (Fig. 9).

7.1.2 Hepatic Resection

When direct invasion of gallbladder cancer to the liver or the bile duct is evidenced or suspected, gallbladder bed or further extensive hepatic resection, or combined bile duct resection is performed. However, optimal extent of resection remains controversial.

7.1.3 Pancreaticoduodenectomy and Combined Resection of Surrounding Organs

En bloc resection is required to achieve R0 resection according to the patterns of tumor invasion in advanced gallbladder cancer. In selected patients with advanced tumor, prognostic advantage of pancreaticoduodenectomy-combined major hepatectomy has been reported if R0 resection is feasible [83].

7.2 Additional Resection and Lymphadenectomy for Incidentally Diagnosed Gallbladder Cancer after Cholecystectomy

When gallbladder cancer is incidentally diagnosed in resected specimen by cholecystectomy, additional resection is required in some patients. For patients with T1 tumor, additional resection or lymphadenectomy is unnecessary because of the very low incidence of lymph node metastasis. However, when the depth of tumor is reaching subserosal layer (i.e., T2 or higher), additional resection with lymphadenectomy is required due to higher chance of lymph node metastasis as indicated in Fig. 9.

7.3 Long-term Surgical Outcomes

When R0 resection is feasible, long-term survival can be expected especially in early-stage gallbladder cancer. A five-year overall survival rate is of 41.6 % after curative resection in a large cohort of patients with gallbladder cancer ($n = 1,094$) [46]. Because the reported 5-year survival of Stage III gallbladder cancer (41.8 %) was higher than Stage III hilar or distal cholangiocarcinoma (30.5 %), systematic approach for R0 resection is important to achieve long survival.

8 Effectiveness of Adjuvant Therapy for Extrahepatic Biliary Tract Cancer

Extrahepatic biliary tract cancer has a propensity to recur locally or regionally [30], and it is the leading cause of morbidity and tumor-related mortality. For this reason, various adjuvant therapies have been attempted especially

in advanced biliary cancers. However, there have been only two prospective studies [63, 76] on the effectiveness of adjuvant chemotherapy or radiotherapy for extrahepatic biliary cancer, and no prognostic advantages have been shown. In addition, our group previously investigated the efficacy of adjuvant chemoradiotherapy for extrahepatic biliary tract cancer. Although time to local recurrence tended to be longer in patient treated with postoperative chemoradiotherapy, total relapse-free survival and overall survival rates were not improved by adding additional chemoradiotherapy after surgery [7]. Therefore, there has been no established adjuvant therapy for extrahepatic biliary cancer, and accordingly, surgery plays a central role in treatment for extrahepatic biliary cancer.

9 Conclusion

Because the extrahepatic biliary tract is in a critical anatomic location surrounded by complex vascular structures and multiple organs, surgical resection of a biliary malignancy often requires resection of adjacent organs. In addition, because of jaundice caused by biliary obstruction and very small future liver remnant in extended hepatic resection for biliary cancer, careful preparations including biliary drainage and portal vein embolization are needed prior to extensive resections. However, surgical resection is the only therapeutic option offering a chance of cure for patients with extrahepatic biliary tract cancers. Thus, optimization of the condition of the patient and selection of appropriate surgical procedure are needed to achieve favorable surgical outcomes.

References

1. Aljiffry M, Walsh MJ et al (2009) Advances in diagnosis, treatment and palliation of cholangiocarcinoma: 1990–2009. *World J Gastroenterol* 15(34):4240–4262
2. Allen PJ, Reiner AS et al (2008) Extrahepatic cholangiocarcinoma: a comparison of patients with resected proximal and distal lesions. *HPB (Oxford)* 10(5):341–346
3. Aloia TA, Charnsangavej C et al (2007) High-resolution computed tomography accurately predicts resectability in hilar cholangiocarcinoma. *Am J Surg* 193(6):702–706
4. Bahra M, Jacob D et al (2008) Carcinoma of the distal and middle bile duct: surgical results, prognostic factors, and long-term follow-up. *J Hepatobiliary Pancreat Surg* 15(5):501–507
5. Baton O, Azoulay D et al (2007) Major hepatectomy for hilar cholangiocarcinoma type 3 and 4: prognostic factors and longterm outcomes. *J Am Coll Surg* 204(2):250–260
6. Bismuth H, Corlette MB (1975) Intrahepatic cholangioenteric anastomosis in carcinoma of the hilus of the liver. *Surg Gynecol Obstet* 140(2):170–178
7. Borghero Y, Crane CH et al (2008) Extrahepatic bile duct adenocarcinoma: patients at high-risk for local recurrence treated

- with surgery and adjuvant chemoradiation have an equivalent overall survival to patients with standard-risk treated with surgery alone. *Ann Surg Oncol* 15(11):3147–3156
8. Capussotti L, Muratore A et al (2002) Liver resection for hilar cholangiocarcinoma: in-hospital mortality and longterm survival. *J Am Coll Surg* 195(5):641–647
 9. Cheng Q, Luo X et al (2007) Distal bile duct carcinoma: prognostic factors after curative surgery. A series of 112 cases. *Ann Surg Oncol* 14(3):1212–1219
 10. Couinaud C (1957) *Le Foie. Etudes anatomiques et chirurgicales*, Paris
 11. Couinaud C (1989) *Surgical anatomy of the liver*. Couinaud, Paris
 12. DeOliveira ML, Cunningham SC et al (2007) Cholangiocarcinoma: thirty-one-year experience with 564 patients at a single institution. *Ann Surg* 245(5):755–762
 13. Dinant S, Gerhards MF et al (2006) Improved outcome of resection of hilar cholangiocarcinoma (Klatskin tumor). *Ann Surg Oncol* 13(6):872–880
 14. Ebata T, Nagino M et al (2003) Hepatectomy with portal vein resection for hilar cholangiocarcinoma: audit of 52 consecutive cases. *Ann Surg* 238(5):720–727
 15. Fong Y, Blumgart LH et al (1996) Outcome of treatment for distal bile duct cancer. *Br J Surg* 83(12):1712–1715
 16. Grandadam S, Compagnon P et al (2010) Role of preoperative optimization of the liver for resection in patients with hilar cholangiocarcinoma type III. *Ann Surg Oncol* 17(12):3155–3161
 17. Hasegawa S, Ikai I et al (2007) Surgical resection of hilar cholangiocarcinoma: analysis of survival and postoperative complications. *World J Surg* 31(6):1256–1263
 18. Hemming AW, Mekeel K et al (2011) Portal vein resection in management of hilar cholangiocarcinoma. *J Am Coll Surg* 212(4):604–613
 19. Hemming AW, Reed AI et al (2005) Surgical management of hilar cholangiocarcinoma. *Ann Surg* 241(5):693–699
 20. Hirano S, Kondo S et al (2009) No-touch resection of hilar malignancies with right hepatectomy and routine portal reconstruction. *J Hepatobiliary Pancreat Surg* 16(4):502–507
 21. Hirano S, Kondo S et al (2010) Outcome of surgical treatment of hilar cholangiocarcinoma: a special reference to postoperative morbidity and mortality. *J Hepatobiliary Pancreat Sci* 17(4):455–462
 22. Hong SM, Pawlik TM et al (2009) Depth of tumor invasion better predicts prognosis than the current American Joint Committee on Cancer T classification for distal bile duct carcinoma. *Surgery* 146(2):250–257
 23. Igami T, Nishio H et al (2010) Surgical treatment of hilar cholangiocarcinoma in the “new era”: the Nagoya University experience. *J Hepatobiliary Pancreat Sci* 17(4):449–454
 24. Ikeyama T, Nagino M et al (2007) Surgical approach to bismuth Type I and II hilar cholangiocarcinomas: audit of 54 consecutive cases. *Ann Surg* 246(6):1052–1057
 25. Ishizawa T, Hasegawa K et al (2007) Selective versus total biliary drainage for obstructive jaundice caused by a hepatobiliary malignancy. *Am J Surg* 193(2):149–154
 26. Ito F, Agni R et al (2008) Resection of hilar cholangiocarcinoma: concomitant liver resection decreases hepatic recurrence. *Ann Surg* 248(2):273–279
 27. Ito K, Ito H et al (2010) Adequate lymph node assessment for extrahepatic bile duct adenocarcinoma. *Ann Surg* 251(4):675–681
 28. Jang JY, Kim SW et al (2005) Actual long-term outcome of extrahepatic bile duct cancer after surgical resection. *Ann Surg* 241(1):77–84
 29. Japanese society of Biliary Surgery (2004) *Classification of biliary tract carcinoma*. Kanehara, Tokyo
 30. Jarnagin WR, Ruo L et al (2003) Patterns of initial disease recurrence after resection of gallbladder carcinoma and hilar cholangiocarcinoma: implications for adjuvant therapeutic strategies. *Cancer* 98(8):1689–1700
 31. Kawai M, Tani M et al (2010) The ratio between metastatic and examined lymph nodes is an independent prognostic factor for patients with resectable middle and distal bile duct carcinoma. *Am J Surg* 199(4):447–452
 32. Kawasaki S, Imamura H et al (2003) Results of surgical resection for patients with hilar bile duct cancer: application of extended hepatectomy after biliary drainage and hemihepatic portal vein embolization. *Ann Surg* 238(1):84–92
 33. Kayahara M, Nagakawa T et al (1999) Role of nodal involvement and the periductal soft-tissue margin in middle and distal bile duct cancer. *Ann Surg* 229(1):76–83
 34. Kayahara M, Nagakawa T et al (1993) Lymphatic flow in carcinoma of the distal bile duct based on a clinicopathologic study. *Cancer* 72(7):2112–2117
 35. Kennedy TJ, Yopp A et al (2009) Role of preoperative biliary drainage of liver remnant prior to extended liver resection for hilar cholangiocarcinoma. *HPB (Oxford)* 11(5):445–451
 36. Kishi Y, Abdalla EK et al (2009) Three hundred and one consecutive extended right hepatectomies: evaluation of outcome based on systematic liver volumetry. *Ann Surg* 250(4):540–548
 37. Kishi Y, Madoff DC et al (2008) Is embolization of segment 4 portal veins before extended right hepatectomy justified? *Surgery* 144(5):744–751
 38. Klempnauer J, Ridder GJ et al (1997) Resectional surgery of hilar cholangiocarcinoma: a multivariate analysis of prognostic factors. *J Clin Oncol* 15(3):947–954
 39. Kokudo N, Makuuchi M et al (2003) Strategies for surgical treatment of gallbladder carcinoma based on information available before resection. *Arch Surg* 138(7):741–750
 40. Kraybill WG, Lee H et al (1994) Multidisciplinary treatment of biliary tract cancers. *J Surg Oncol* 55(4):239–245
 41. Kubota K, Makuuchi M et al (1997) Measurement of liver volume and hepatic functional reserve as a guide to decision-making in resectional surgery for hepatic tumors. *Hepatology* 26(5):1176–1181
 42. Lee HG, Lee SH et al (2009) Carcinoma of the middle bile duct: is bile duct segmental resection appropriate? *World J Gastroenterol* 15(47):5966–5971
 43. Lee SG, Song GW et al (2010) Surgical treatment of hilar cholangiocarcinoma in the new era: the Asan experience. *J Hepatobiliary Pancreat Sci* 17(4):476–489
 44. Madoff DC, Abdalla EK et al (2005) Transhepatic ipsilateral right portal vein embolization extended to segment IV: improving hypertrophy and resection outcomes with spherical particles and coils. *J Vasc Interv Radiol* 16(2 Pt 1):215–225
 45. Mise Y, Hasegawa K et al (2011) Venous reconstruction based on virtual liver resection to avoid congestion in the liver remnant. *Br J Surg* 98(12):1742–1751
 46. Miyakawa S, Ishihara S et al (2009) Biliary tract cancer treatment: 5,584 results from the Biliary Tract Cancer Statistics Registry from 1998 to 2004 in Japan. *J Hepatobiliary Pancreat Surg* 16(1):1–7
 47. Miyazaki M, Kimura F et al (2007) Recent advance in the treatment of hilar cholangiocarcinoma: hepatectomy with vascular resection. *J Hepatobiliary Pancreat Surg* 14(5):463–468
 48. Miyazaki M, Kimura F et al (2010) One hundred seven consecutive surgical resections for hilar cholangiocarcinoma of Bismuth types II, III, IV between 2001 and 2008. *J Hepatobiliary Pancreat Sci* 17(4):470–475
 49. Mizumoto R, Kawarada Y et al (1986) Surgical treatment of hilar carcinoma of the bile duct. *Surg Gynecol Obstet* 162(2):153–158
 50. Murakami Y, Uemura K et al (2007) Pancreatoduodenectomy for distal cholangiocarcinoma: prognostic impact of lymph node metastasis. *World J Surg* 31(2):337–342 (discussion 343–334)

51. Murakami Y, Uemura K et al (2009) Gemcitabine-based adjuvant chemotherapy improves survival after aggressive surgery for hilar cholangiocarcinoma. *J Gastrointest Surg* 13(8):1470–1479
52. Nagino M, Kamiya J et al (2000) Right trisegment portal vein embolization for biliary tract carcinoma: technique and clinical utility. *Surgery* 127(2):155–160
53. Nagino M, Kamiya J et al (2006) Two hundred forty consecutive portal vein embolizations before extended hepatectomy for biliary cancer: surgical outcome and long-term follow-up. *Ann Surg* 243(3):364–372
54. Nagino M, Nimura Y et al (2010) Hepatectomy with simultaneous resection of the portal vein and hepatic artery for advanced perihilar cholangiocarcinoma: an audit of 50 consecutive cases. *Ann Surg* 252(1):115–123
55. Neuhaus P, Jonas S (2000) Surgery for hilar cholangiocarcinoma—the German experience. *J Hepatobiliary Pancreat Surg* 7(2):142–147
56. Neuhaus P, Jonas S et al (1999) Extended resections for hilar cholangiocarcinoma. *Ann Surg* 230(6):808–818
57. Nimura Y, Hayakawa N et al (1991) Combined portal vein and liver resection for carcinoma of the biliary tract. *Br J Surg* 78(6):727–731
58. Nimura Y, Kamiya J et al (2000) Aggressive preoperative management and extended surgery for hilar cholangiocarcinoma: Nagoya experience. *J Hepatobiliary Pancreat Surg* 7(2):155–162
59. Nishio H, Nagino M et al (2005) Is caudate lobectomy necessary for hilar cholangiocarcinoma? *Tan-to-sui* 26(3):309–314
60. Nomura T, Tsuchiya Y et al (2009) Prognostic factors for radical resection of middle and distal bile duct cancer. *Hepatogastroenterology* 56(90):294–298
61. Palavecino M, Abdalla EK et al (2009) Portal vein embolization in hilar cholangiocarcinoma. *Surg Oncol Clin N Am* 18(2):257–267, viii
62. Parikh AA, Abdalla EK et al (2005) Operative considerations in resection of hilar cholangiocarcinoma. *HPB* 7(4):254–258
63. Pitt HA, Nakeeb A et al (1995) Perihilar cholangiocarcinoma. Postoperative radiotherapy does not improve survival. *Ann Surg* 221(6):788–797
64. Radtke A, Sotiropoulos GC et al (2010) Computer-assisted surgery planning for complex liver resections: when is it helpful? A single-center experience over an 8-year period. *Ann Surg* 252(5):876–883
65. Saito S, Yamanaka J et al (2005) A novel 3D hepatectomy simulation based on liver circulation: application to liver resection and transplantation. *Hepatology* 41(6):1297–1304
66. Sakamoto Y, Kosuge T et al (2005) Prognostic factors of surgical resection in middle and distal bile duct cancer: an analysis of 55 patients concerning the significance of ductal and radial margins. *Surgery* 137(4):396–402
67. Sano T, Shimada K et al (2007) Changing trends in surgical outcomes after major hepatobiliary resection for hilar cholangiocarcinoma: a single-center experience over 25 years. *J Hepatobiliary Pancreat Surg* 14(5):455–462
68. Sasaki R, Takahashi M et al (2001) Prognostic significance of lymph node involvement in middle and distal bile duct cancer. *Surgery* 129(6):677–683
69. Sasaki R, Takeda Y et al (2007) Significance of ductal margin status in patients undergoing surgical resection for extrahepatic cholangiocarcinoma. *World J Surg* 31(9):1788–1796
70. Schnitzbauer AA, Lang SA et al (2012) Right portal vein ligation combined with in situ splitting induces rapid left lateral liver lobe hypertrophy enabling 2-staged extended right hepatic resection in small-for-size settings. *Ann Surg* 255(3):405–414
71. Seyama Y, Kubota K et al (2003) Long-term outcome of extended hemihepatectomy for hilar bile duct cancer with no mortality and high survival rate. *Ann Surg* 238(1):73–83
72. Shindoh J, Mise Y et al (2010) The intersegmental plane of the liver is not always flat—tricks for anatomical liver resection. *Ann Surg* 251(5):917–922
73. Shirai Y, Wakai T et al (2012) Regional lymphadenectomy for gallbladder cancer: rational extent, technical details, and patient outcomes. *World J Gastroenterol* 18(22):2775–2783
74. Sugiura Y, Nakamura S et al (1994) Extensive resection of the bile ducts combined with liver resection for cancer of the main hepatic duct junction: a cooperative study of the Keio Bile Duct Cancer Study Group. *Surgery* 115(4):445–451
75. Suzuki M, Unno M et al (2000) Surgical treatment and postoperative outcomes for middle and lower bile duct carcinoma in Japan—experience of a single institute. *Hepatogastroenterology* 47(33):650–657
76. Takada T, Amano H et al (2002) Is postoperative adjuvant chemotherapy useful for gallbladder carcinoma? A phase III multicenter prospective randomized controlled trial in patients with resected pancreaticobiliary carcinoma. *Cancer* 95(8):1685–1695
77. Todoroki T, Kawamoto T et al (2000) Radical resection of hilar bile duct carcinoma and predictors of survival. *Br J Surg* 87(3):306–313
78. Toyoda T, Yoshida M et al (1986) Studies on hepatobiliary lymph flow with radioactive colloid—for lymph node metastasis resection of carcinoma of the bifurcation of hepatic duct. *Gastroenterol Jpn* 21(1):35–43
79. Tsukada K, Kurosaki I et al (1997) Lymph node spread from carcinoma of the gallbladder. *Cancer* 80(4):661–667
80. Unno M, Katayose Y et al (2010) Major hepatectomy for perihilar cholangiocarcinoma. *J Hepatobiliary Pancreat Sci* 17(4):463–469
81. Vauthey JN, Abdalla EK et al (2002) Body surface area and body weight predict total liver volume in Western adults. *Liver Transpl* 8(3):233–240
82. Wade TP, Prasad CN et al (1997) Experience with distal bile duct cancers in U.S. Veterans affairs hospitals: 1987–1991. *J Surg Oncol* 64(3):242–245
83. Wakai T, Shirai Y et al (2008) Combined major hepatectomy and pancreaticoduodenectomy for locally advanced biliary carcinoma: long-term results. *World J Surg* 32(6):1067–1074
84. Yeo CJ, Cameron JL et al (2002) Pancreaticoduodenectomy with or without distal gastrectomy and extended retroperitoneal lymphadenectomy for perihilar adenocarcinoma, part 2: randomized controlled trial evaluating survival, morbidity, and mortality. *Ann Surg* 236(3):355–366 (discussion 366–358)
85. Yoshida T, Matsumoto T et al (2002) Prognostic factors after pancreaticoduodenectomy with extended lymphadenectomy for distal bile duct cancer. *Arch Surg* 137(1):69–73
86. Young AL, Prasad KR et al (2010) Surgical treatment of hilar cholangiocarcinoma in a new era: comparison among leading Eastern and Western centers Leeds. *J Hepatobiliary Pancreat Sci* 17(4):497–504

Surgical Management of Gallbladder Cancer

Amudhan Pugalenthil and Yuman Fong

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Abstract

Gallbladder (GB) cancer carries a dismal prognosis with a 5-year survival rate of less than 5 % for advanced disease. Only 20 % of GB cancers are detected at an early stage when the disease is confined to the gallbladder. Improvements in surgical techniques have resulted in improved outcomes. We clearly understand that surgery is the only hope for survival and can have an impact on the natural history of the disease. In this article, we will summarize the most recent data with regards to the surgical options. Accurate diagnosis based on imaging and pathological staging will help us achieve better results. We will conclude by providing an algorithm to manage GB cancer based on T-staging.

1 Introduction

Gallbladder (GB) cancer is an aggressive disease. Because of its insidious onset, it is usually detected at an advanced stage. The five-year survival rate for advanced disease is less than 5 %. Surgery is the most effective and only curative form of treatment for GB cancer. Improvements in surgical techniques have resulted in improved outcomes. In this chapter, we will review data for surgical treatment and provide an algorithm of how to treat patients based on current evidence.

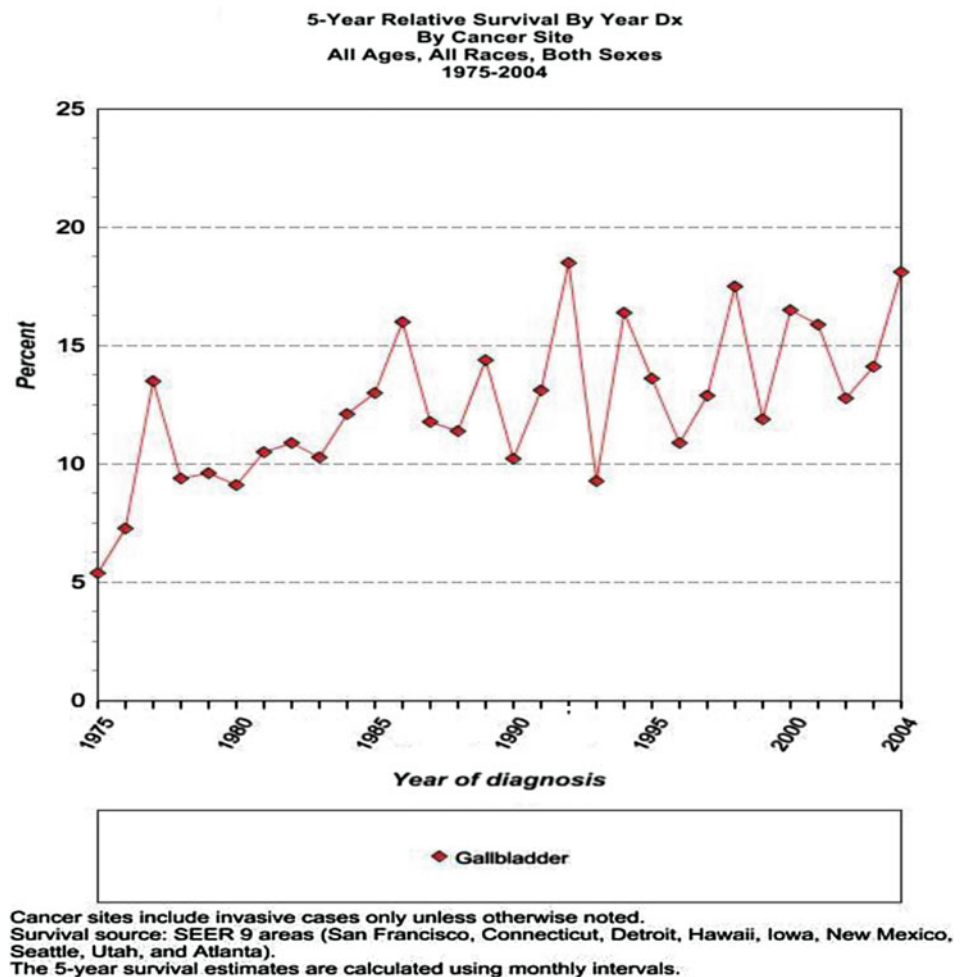
2 Epidemiology

Cancer of the GB is rare, but it is the commonest site of occurrence in the biliary tract. It is also the fifth most common tumor of the gastrointestinal tract. The estimated number of new cases from gallbladder (and other biliary) cancer in the United States (US) in 2012 was 9,810 new cases, including 4,480 males and 5,330 females. The number of deaths was 3,200 including 1,240 males and

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Fig. 1 Five-year survival for gallbladder cancer generated from surveillance epidemiology and end results (SEER) database [4]



1,960 females. Of these, more than half (about 60 %, which is almost 6,000 cases) were GB cancers [1]. Worldwide it is more common in Asia, East Europe and South America than the United States. The age-adjusted invasive cancer incidence rate in the US between the years 2005 and 2009 in males was 0.82 per 100,000 population and among females was 1.38 per 100,000 population [2]. Thus, it is more common in women than in men.

By the time GB cancer is detected, it is usually well advanced. Only 20 % of the cancers are detected in early stages—where the cancer has not spread beyond the gallbladder. The five-year survival rate for GB cancer in the United States based on more than 10,000 patients diagnosed between the years 1989 and 1996 are 80, 50, 28, 8 % and less than 4 % for Stages 0, 1, 2, 3 and 4, respectively [3].

Because of its silent onset, propensity for local invasion and rapid disease progression, treatment results have been dismal. The disease is usually diagnosed either incidentally after cholecystectomy or at an advanced stage, when it presents with jaundice, as a mass, peritoneal disease or ascites. With a clearer understanding of surgical anatomy, disease biology, advancement in imaging techniques and

improvement in surgical procedures, the outcomes are better now. Appropriate workup of the extent of disease and radical resection can result in cure. There is no doubt that results for treatment for gallbladder cancer have improved over the 25 years [4] (Fig. 1). It is also clear from this figure that the majority of patients still die from this terrible cancer.

Gallstones and chronic gallbladder inflammation are two important risk factors for development of GB cancer. Cholelithiasis is an associated finding in the majority of cases, but less than 1 % of patients with cholelithiasis develop cancer. Stones larger than 3 cm are associated with a tenfold increased risk of cancer [5]. Porcelain GB was once suspected to be a risk factor for development of GB cancer, but recently this relationship has been questioned. Towfigh et al. [6] evaluated the pathology of 10,741 GB specimens between the years 1955 and 1998 and identified fifteen porcelain GB, and none had cancer. Similarly, Khan et al. analyzed 1,200 cholecystectomies and identified 13 patients with porcelain GB, and all were benign. They concluded that prophylactic cholecystectomy is not indicated for porcelain GB for risk of development of later cancer.

The most common symptoms caused by GB cancer are jaundice, pain, and fever. These symptoms are common for both benign and malignant diseases and that is a key reason for delay in diagnosis. Any GB mass or polyp found on imaging should lead to a high degree of suspicion for early cancer. Ninety percentage of primary GB cancers are adenocarcinomas, and they originate from the fundus (60 %), body (30 %) and neck (10 %). Among the morphological types, the papillary growth pattern seems to have better prognosis as this subtype tends to have little invasion. In contrast, the infiltrative pattern seems to invade early and grow along the subserosal plane (plane of dissection during simple cholecystectomy). The third morphological pattern is the nodular type, which shows early invasion, but the margins seem to be well defined; hence, they tend to have a better prognosis.

2.1 History of Biliary Surgery

Early part of the eighteenth century was the beginning of surgery for biliary diseases. In 1743, Jean Louis Petit, Paris, coined the term “biliary colic” (“colique hepaticque”). He presented his seminal paper at the Paris Surgical Academy entitled “Considerations Concerning Tumors Produced by Retained Bile in the Gallbladder....” [7]. In the United States, John Bobbs [8] is credited with the first cholecystostomy in Indianapolis in 1867. A decade later in 1878, Theodore Kocher in Berne, Switzerland, who was trained under Billroth and Langenbuch did his cholecystostomy in two stages [9]. The Kocher maneuver named was first used for gastric surgery, and only later utilized for biliary surgery by Vautrin. Kocher also pioneered internal choledochoduodenostomy to remove common bile duct calculi. Along with Dr. Matti, he wrote the book *Hundert Operationen an den Gallenwegen* (A hundred operations on the bile ducts). Carl Langenbuch of Germany is credited with performing the first cholecystectomy at the Lazarus Hospital in Berlin in July, 1882 [10]. George Pack in 1955 was the first to report 3 cases, where radical liver resection (right hepatic lobectomy) along with portal lymph node dissection was performed for the treatment of gallbladder cancer [11].

Toward the end of the twentieth century in 1985, Prof Dr Med Erich Muhe of Böblingen, Germany, did the first laparoscopic cholecystectomy [12]. Since then it has become the gold standard for removal of gallbladder for benign disease. At present, surgeons use other minimally invasive techniques to remove the gallbladder [13, 14]. Biliary surgery continues to evolve with the help of advancements in imaging with ultrasound in liver and biliary surgery [15], computerized tomography (CT) scan of

the liver [16] and magnetic resonance (MR) imaging [17]. After popularization of laparoscopic cholecystectomy, there is a new presentation of gallbladder cancer, namely after minimally invasive cholecystectomy. The number of incidental cancers detected in the pathology specimen is approximately one for every hundred gallbladders that are removed for a presumed benign etiology [7].

2.2 Surgical Anatomy

Anatomically, the gallbladder lies in the subhepatic area close to Couinaud’s liver segments 4b and 5. The GB wall is composed of an innermost layer of mucosa which is lined by simple columnar epithelium; beneath this is a layer of lamina propria. The GB wall lacks submucosa. A layer of muscular tissue (smooth muscle) is present beneath the lamina propria. The outermost layer is made up of thick connective tissue called adventitia which is present in the area where GB is attached to the liver tissue (GB bed). In the unattached area, facing the free peritoneal cavity, there is an outer layer of mesothelium and loose connective tissue called serosa. The subserosal plane is the least bloody plane of dissection during routine cholecystectomy. The cystic plate is the gallbladder serosa on the liver which is usually left behind after dissection in the subserosal plane. According to American Joint Committee on Cancer staging, the lymph nodal station N1 is defined as regional (hepatic hilus) which corresponds to nodes around cystic duct, common hepatic duct, portal vein and hepatic artery. N2 station refers to metastases nodes around celiac artery, superior mesenteric artery, periduodenal and peripancreatic nodes.

GB cancers spread by direct extension to contiguous structures or via veins draining segments IV and V to the liver. GB cancer can also spread to regional lymph nodes and to the peritoneal cavity by direct extension or after bile spillage. In addition, GB cancer has a propensity to seed and grow along needle track sites, including port sites. GB cancers very rarely metastasize via blood stream.

3 Presentation

There are three common presentations of GB cancer. The most common is a presentation as **incidental gallbladder cancer**. As such, it may be (a) detected during surgery (cholecystectomy) or (b) detected on postoperative pathology review of the resected gallbladder specimen. Gallbladder cancer can also be detected as a **large invasive mass in GB fossa detected on imaging**. Finally, it may be

detected as a **small mass or polyp in the GB found on preoperative imaging**. These presentations will be discussed separately.

1. Management of incidental gallbladder cancer (IGBC)

IGBC refers to GB cancer discovered incidentally at the time of surgery (cholecystectomy) or detected postoperatively in the GB specimen removed for a presumed benign pathology.

a. Intraoperative detection

Since the liberal use of laparoscopic technology for cholecystectomies in the early 1990s, there is increased detection of GB cancers. In a study by [18], almost 50 % of the cancers were discovered incidentally. If suspicion of gallbladder cancer arises during initial laparoscopic surgery for presumed gallstone disease or cholecystitis, intraoperative staging should be done. Metastatic disease should be ruled out by sending frozen section on any suspected lesion or lymph node. A thorough laparoscopic examination of the abdominal cavity should be done. The exploration should include inspection of the liver, including intraoperative ultrasound, gastrohepatic ligament, porta hepatis, pelvis and peritoneal cavity. Additionally, frozen section of the gallbladder should be sent when the diagnosis is in doubt, after resection, and to confirm negative margins. In one study, frozen section biopsy had an accuracy of 88 % [19] and sensitivity and specificity of over 90 and 100 %, respectively [20]. However, the accuracy of T stage which is critical for management though is less than 100 % [20].

If frozen section confirms GB malignancy and the lesion is resectable, then an extended cholecystectomy should be performed after conversion to an open procedure [21]. If expertise is not available, then surgery should be deferred. The patient should then be transferred to an experienced center. Such approach is reasonable and does not affect the prognosis [22, 23].

b. Cancer discovered incidentally in postoperative pathology specimen

The most common presentation of early GB cancer is incidental discovery after unsuspected cholecystectomy in the pathology specimen. In a study by Duffy et al., 47 % of the GB cancers were detected after laparoscopic cholecystectomy [24, 25]. In those patients, a careful review of the GB specimen for the level of invasion (T staging), assessment of resected margins and search for malignancy in the nodes if any retrieved is important. Also, these patients need to be worked up carefully to rule out any metastatic disease. Documentation of spillage of bile or gall stones during the initial surgery must be done, as GB cancer is notorious to spread and recur at port sites and peritoneal surfaces. Further management after cholecystectomy is based on staging and surgical margins.

3.1 Management Based on T Staging

For **carcinoma in situ (Cis)** and **T1a lesions** (i.e., lesion confined to the mucosa), the treatment recommendation is simple cholecystectomy. Most often, these lesions are identified after cholecystectomy as incidental cancers found in the specimens. A high degree of suspicion is required to identify GB cancer by preoperative imaging. In a study of 27 patients with T1a lesions, eight of whom underwent lymph nodal dissection; none had lymph nodal metastasis [26]. A careful review of the pathology for the level of invasion and negative margins is important. There is no need for additional surgical procedures or re-exploration in these patients if the staging workup is negative. The five-year survival rate for Cis and T1a lesions ranges from 85 to 100 % [27–29].

3.2 Tumors Confined to Muscularis Propria (T1b)

According to the AJCC staging system, 7th edition, T1b lesion is one which invades the muscularis propria but does not involve the perimuscular connective tissue. Many studies show T1b lesion treated by laparoscopic cholecystectomy alone had a 5-year survival rate of around 90 % and in some others as high as 100 % [19, 27–29]. However, whether an operation beyond a simple cholecystectomy is needed remains controversial.

Otero et al. [30] concluded that for T1b lesions, additional procedures are needed after cholecystectomy, which was a recommendation shared by Principe et al. [31] and in the review by Miller et al. [23]. Most data, however, argue against radical resection. The occurrence of lymph nodal metastasis was only 3.8 % in the study by You et al. [26]. Similarly, in a study in 1996 by Tsukada et al. [32], all 15 patients with T1 lesions had no lymph nodal metastasis. De Aretxabala et al. concluded after their study in 46 patients that lesions with invasion of muscle layer do not need additional procedures following cholecystectomy.

It must be stated that the 2009 NCCN guidelines mention additional hepatic resection and lymphadenectomy for T1b lesion [33]. We do not routinely perform such radical resection except in cases with clinically positive lymph node metastases. Our reasoning is since lymphatics are only present in the subserosal layer; those tumors that have not yet fully penetrated the muscularis layer have a minimal risk of lymph nodal involvement. Hence, nodal dissection is not required for T1b lesions. However, an open discussion with the patient is worthwhile. There is convincing enough data to demonstrate that early-stage cancers that have not

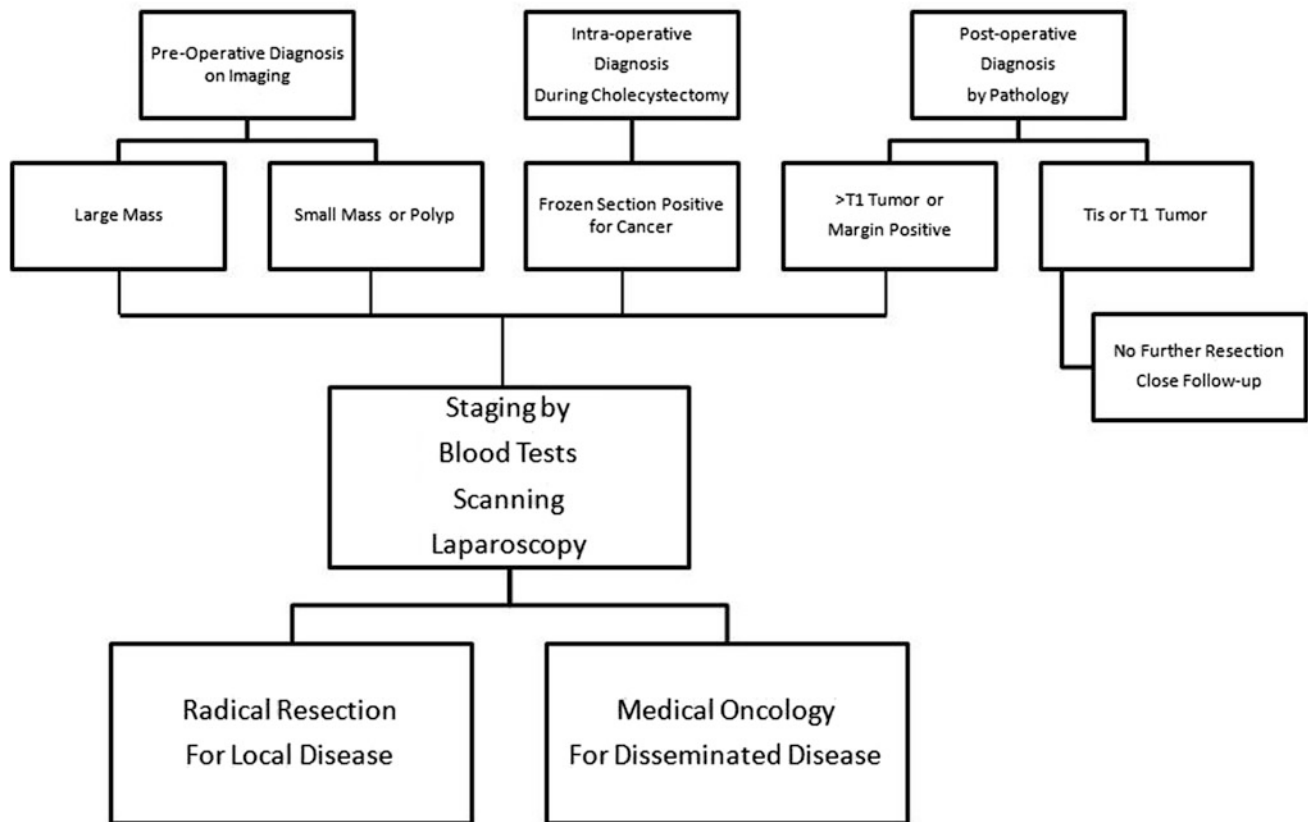


Fig. 2 Algorithm of management of gallbladder cancer

penetrated through the entire muscularis layer can be adequately treated by cholecystectomy alone [34, 35].

In those patients where diagnosis of T1 GB cancer is made after initial cholecystectomy, a careful review of the specimen for level of invasion and margins must be done. Patients will need an additional procedure only if the margins or lymph nodes are positive or the depth of invasion is higher than T1. Postoperatively if CT and CEA levels are normal, they will need routine follow-up as shown in algorithm (Fig. 2).

3.3 Tumors Penetrating Full Thickness of the Muscularis Propria into Subserosa: T2 (stage 2)

Tumors that penetrate through the entire thickness of the muscularis layer but do not involve the serosa are defined as T2 lesions and have been grouped as stage 2 per the AJCC staging (Table 1). The subserosa (avascular) is the space between the muscle layer and the outer serosal layer. Lymphatics are present immediately outside of the muscle layer in the subserosa. Hence, T2 lesions have a high incidence of lymph nodal metastasis ranging from 20 to 40 % (Table 2). While performing a simple cholecystectomy, this

is the plane where the gallbladder is dissected off the GB fossa from the liver. The serosa on the GB fossa that is present on the liver surface and which is left behind after dissecting the GB along the avascular subserosal plane in a simple cholecystectomy is called the cystic plate. Therefore, a conventional cholecystectomy in a T2 lesion will result in high likelihood of positive margins as the tumor plane may be violated. Hence, these patients have to be subjected to additional exploration if R0 resection needs to be achieved.

Hence, for a T2 lesion, the recommendation is a cholecystectomy, along with resection of sufficient liver to achieve a negative margin, and a regional lymph node dissection. Such an operation is known as an extended or radical cholecystectomy. The incidence of nodal disease in T2 lesion has been reported from 23 % in a series by Konstantinidis [36] to as high as 61 % by Duffy et al. [24]. This group of patients benefit from re-exploration and additional radical or extended resection to achieve negative margins. Similarly, Ogura et al. [35], Japan, reported after a nationwide survey that 5-year survival rate for 499 patients with T2 carcinoma was 37 %. Among them, 44 % of patients had lymph node metastases. In a study by Fong et al., those with T2 lesions after prior cholecystectomy who never underwent a subsequent re-exploration and additional procedure had a 5-year survival rate of 19 %. By comparison, those who

Table 1 Seventh edition of AJCC staging

AJCC staging 7th edition			
<i>Primary Tumor (T)</i>			
TX—primary tumor cannot be assessed			
T0—no evidence of primary tumor			
Tis—carcinoma in situ			
T1—tumor invades lamina propria or muscular layer			
T1a—tumor invades lamina propria			
T1b—tumor invades muscular layer			
T2—tumor invades perimuscular connective tissue; no extension beyond serosa or into liver			
T3—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			
T4—tumor invades main portal vein or hepatic artery or invades two or more extrahepatic organs or structures			
<i>Regional Lymph Nodes (N)</i>			
N0—no regional lymph node metastases			
N1—metastases to nodes along the cystic duct, common bile duct, hepatic artery and/or portal vein			
N2—metastases to periaortic, pericaval, superior mesenteric artery and/or celiac artery lymph nodes			
<i>Distant Metastasis (M)</i>			
M0—no distant metastasis			
M1—distant metastasis			
<i>Anatomic stage/prognostic groups</i>			
Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage IIIA	T3	N0	M0
Stage IIIB	T1-3	N1	M0
Stage IVA	T4	N0-1	M0
Stage IVB	Any T	N2	M0
	Any T	Any N	M1

Table 2 Results of cholecystectomy alone versus additional re-exploration and resection for T2 lesions

Author, year, country	N	Only cholecystectomy 5-year survival (%)	Re-exploration and additional radical resection			
			N	Nodal disease (%)	Positive margins (%)	5-year survival (%)
Shirai, 1992 [28], Japan	35	40	10	30	20	90
Fong, 2000 [22], USA	16	19	37	33	NR	61
Chijiwa, 2001 [55], Japan	NR	17	28	39	21	59
Wakai, 2002 [56], Japan	6	50	7	28	0	100
Toyonaga, 2003 [57], Japan	25	65	18	20	63	38
Foster, 2007 [58], USA	10	38	19	33	NR	78

N number of patients; NR not reported

underwent further radical procedure for T2 lesion had a survival rate of 61 % (Table 2) [22]. Similarly, De Aretxabaleta reported a 5-year survival of 70 % for patients treated by radical re-resection versus 20 % 5-year survival for simple cholecystectomy alone [37]. Most data over the past 15 years have consistently shown that T2-stage patients benefit the most from radical cholecystectomy that included a segment 4, 5 liver resection and regional lymphadenectomy. Radical re-resection is not only safe but also rational therapy for T2 cancers.

Two factors need to be addressed in treatment of T2 tumors are the extent of lymph node removal and extent of liver resection along with the GB removal. We know that GB cancer has a high propensity to involve lymph nodes. The presence of lymph nodal disease in T2 lesions ranges from 20 to 39 % in different studies (Table 2). In a study by Shirai et al. [38], lymphatic drainage mapping using a dye was done to follow the drainage pattern from gallbladder. They found that the first echelon group of lymph nodes were cystic or pericholedochal nodes and the second echelon

Table 3 Results of surgical treatment for advanced (T3/T4) lesions

Author, year, Country	N	Stage	3-year OS (%)	5-year OS (%)	Comment
Gall, 1991 [59], Germany	9	III	11	NR	
	11	IV	9	NR	
Onoyama, 1995 [42], Japan	12	III	44*	44*	
	14	IV	8*	8*	
Nakamura, 1999 [60], Japan	23	IV	17	11	Morbidity—60 % Mortality—nil
Fong, 2000 [22], USA	58	III/IV	28	28	
Kondo, 2002 [40], Japan	9	III	44	33	
	29	IVa(M0)	24	17	
		IVb(M1)	7	3	
D'Angelica, 2009 [61], USA	63	III/IV	NA	25	

* Incidentally discovered GB cancers after laparoscopic cholecystectomy; OS—overall survival

group of nodes were located around the portal vein, hepatic arteries, and postero-superior to the head of pancreas, which correspond to the N1 nodes as per the AJCC TNM staging, 7th edition [39]. Although it is not uniform throughout the world, the extent of lymphadenectomy for T2 disease ranges from cystic node removal alone to *en bloc* portal lymphadenectomy and in some series combined with pancreatoduodenectomy. It must be noted that combined liver resection and pancreatic resections, done usually to improve nodal clearance, have a mortality rate of nearly 18 % [40].

For curative surgery, an adequate portal lymphadenectomy is required with resection of CBD as the periportal lymph nodes are closely related to CBD, and its removal facilitates nodal clearance. A full Kocher maneuver should be performed; the CBD should be transected close to the pancreas posterior to the duodenum to clear lymph nodal tissue behind the duodenum and pancreas; the portal vein and hepatic artery should be skeletonized, and all tissue should be swept superiorly along with the divided CBD. At the confluence of the right and left hepatic ducts, the CBD should be divided and a Roux—en—Y hepaticojejunostomy should be performed. This regional lymphadenectomy should include periportal, peripancreatic and celiac nodes, and any aorto-caval or superior mesenteric nodes should be included if possible. This entire procedure is justified for any cancer which is $T \geq 2$ as they have high incidence of lymph nodal disease (Table 2). Combined radical resection with pancreaticoduodenectomy should be reserved for very fit patients.

2. Large invasive mass detected on imaging (locally advanced)

Although resection is the treatment of choice for large GB cancer, only 25 % of the patients are candidates for a curative procedure due to the advanced presentation of the disease [41].

Treatment for advanced tumors (T3 and T4): For T3 and T4 lesions, there is an increased risk of nodal disease as well as peritoneal and systemic metastasis. A careful workup

that includes imaging studies and staging laparoscopy should be performed to rule out M1 or N2 disease. If these patients are referred after initial cholecystectomy, there will often be a residual mass in the imaging done postoperatively, as well as positive margins in the specimen.

Although radical surgeries, including hepatic lobectomies and extended lobectomies, for advanced disease have been performed since 1990s, there was controversy whether such procedures are justifiable when associated mortality and morbidity was high. There is no doubt that such extensive liver resections are necessary to get a clear margin that is the first step toward long-term survival. However, morbidity and mortality for such operations in the 1990s were quite high. As safety of liver resection has improved, more studies are reporting substantial survival benefit of such procedures. Onoyama et al. [42] reported from Japan, a 5-year survival of 44 and 8 % for stage III and IV GB cancers that underwent radical resection. These patients should be evaluated for major liver resection, which includes assessment of liver residual volumes to maintain adequate hepatic function after surgery.

If the lesion is deemed resectable and patient is medically fit, the patient should be explored for radical resection. This usually includes a liver resection and lymphadenectomy, with or without bile duct resection. In some cases, resection may also include contiguous organs like colon or part of the stomach for tumor clearance. Doty et al. [43] reported in a small series of five patients, the safety of combined pancreatoduodenectomy for nodal clearance along with liver resection for GB cancer. But, combined pancreas and liver resections have resulted in a high mortality of up to 21 % in a study by Nimura et al. [44].

As in Table 3, more studies show better outcomes after radical operations for T3 and T4 lesions. These data indicate that radical surgery for advanced disease may be potentially curative. In selected group of patients, extended radical resection is the only hope for long-term survival.

3. Small mass or polyp found on imaging

Any polyp more than 1 cm, solitary, sessile, growing or vascular in nature should raise suspicion for cancer [45]. Similarly, any irregular wall thickening and evidence of ultrasonographic invasion at the liver interface in an older patient should arouse suspicion [46]. US is a good modality to evaluate the direct extension of GB cancer [47], but it is not very useful to evaluate lymph nodes. If a suspicious mass is found in the gallbladder on preoperative imaging, patients need further workup to evaluate the extent of disease. Metastasis needs to be ruled out with CT, MRI, PET (positron emission tomography) and staging laparoscopy. When metastasis is ruled out, these patients need exploration and radical resection.

3.4 Complications

GB cancer is a disease of elderly population. Most of the patients are in their seventh or eighth decade of life with age related co-morbidities. The curative procedures described for treatment of advanced disease are extensive procedures posing significant risks. The mortality associated with such radical procedures ranges from 1 to 7 % in many series. The mortality increases up to 18 % in one series when liver resection was combined with pancreas resection for adequate nodal clearance [44]. Hence, risks of resection should be weighed against the benefits before undertaking such radical procedures for GB cancers [35].

3.5 Role of Palliation

The median survival for unresectable GB carcinoma is 2–4 months. Palliation is required to relieve pain, jaundice and bowel obstruction. For unresectable cancer, radiologic and endoscopic approaches for biliary drainage have replaced surgery. If a surgical bypass is indeed necessary, then segment III bypass should be done to relieve jaundice because of the advanced disease at porta hepatis. Systemic chemotherapy and radiation therapy have very little effect on these tumors. If patients are willing, they should be enrolled in investigational trials as a last option. For pain relief, celiac ganglion block can be offered. For bowel obstruction, patients need gastrointestinal bypass procedures.

4 Special Considerations

4.1 Port-site Resection

GB cancer is notorious for seeding and growing along needle biopsy tracts and port sites. Review of operative

records after detection of IGBC in the pathology specimen is essential to identify GB perforation or bile spillage. In one study, the risk of port-site recurrence following laparoscopic cholecystectomy was 9 % when the initial operation on GB was without perforation. On the other hand, the incidence goes up to 40 % if GB perforation occurs during initial cholecystectomy [48]. Paolucci 2001 reported 174 cases of recurrence at port sites after laparoscopic cholecystectomy and 12 cases in the surgical scar in open cholecystectomy, with an incidence rate of 14 %. Recently, a study by Maker et al. [49] reported that port-site metastases was as high as 19 % and resection of the port sites did not improve survival or disease recurrence. They also state that resection should not be considered mandatory during definite surgical treatment [48–52]. At this time, we are not sure whether these port-site recurrences are just isolated areas of metastases or a marker of diffuse peritoneal disease.

4.2 Role of Staging Laparoscopy

Laparoscopy is helpful in prelaparotomy staging of GB cancer to identify occult metastases, especially peritoneal metastases. Any suspicious area needs to be biopsied because previous inflammation can make it difficult to differentiate scar tissue from tumor. Diagnostic laparoscopy complements high-quality imaging in detection of peritoneal disease which is common in advanced disease. In cases of previous cholecystectomy, biliary spillage and gallbladder perforation increase the chance of intra-abdominal spread [53].

Disseminated disease is relatively uncommon in patients with incidental GB cancer, and staging laparoscopy provides a very low yield. In one study by Vollmer et al. up to 50 % of patients were found to have unresectable disease at the time of laparoscopy [54]. However, patients with poorly differentiated T3/T4 or positive-margin gallbladder tumors are at high risk for disseminated disease, and targeting these patients may increase the yield of staging laparoscopy [53, 54].

5 Summary

Gallbladder cancer is an aggressive disease. Radical surgery offers the only form of cure. Improvements in surgery have resulted in improved outcomes. The rarity of gallbladder cancer prevents us from conducting randomized control studies regarding surgical options, and majority of the cases are unresectable at presentation. In an elderly patient, any mass or polyp >1 cm in the GB on imaging should raise suspicion for cancer. Because of its indolent nature, any long-term obstruction of mid common bile duct (CBD) should be considered GB cancer until proven otherwise.

Appropriate staging workup with the help of US, CT, MRI, chest imaging, PET and staging laparoscopy will help us to correctly stage these tumors. N2 and M1 disease have to be ruled out, because these findings preclude any surgical intervention. In advanced cases, MRCP, PTC or ERCP may be required to evaluate the extent of the disease. For early-stage GB cancer, (T1)-cholecystectomy alone seems to be an adequate procedure, if the margins are negative. For any lesion, $\geq$ T2 will require standard extended cholecystectomy which includes removal of lymph nodes around porta hepatis, peripancreatic, celiac axis and superior mesenteric artery. The common bile duct may have to be resected for adequate lymphadenectomy. Also, liver segments 4b and 5 needs to be removed to achieve R0 resection. In selected patients with T3 and T4 lesions, adequacy of liver functional reserve needs to be assessed before any major hepatic resection. Most of these patients are elderly; hence, their general medical condition should be evaluated before any major procedure. We now understand that surgery remains the only form of therapy that has had an impact on the natural history of the disease.

References

1. American Cancer Society (2012) Cancer facts and figures 2012. Atlanta, Ga. [cited 2013 Jan 11]. Available from: <http://www.cancer.org/acs/groups/content/@epidemiologysurveillance/documents/document/acspc-031941.pdf>
2. North American Association of Central Cancer Registries (2013) CINA + Online Cancer in North America
3. American Cancer Society (2012) Survival statistics for gallbladder cancer, by stage. [cited 2013 Jan 14 2013]. Available from: <http://www.cancer.org/gallbladder/gallbladder-cancer/detailedguide/gallbladder-survival-rates>
4. SEER (2013) Statistics stratified by cancer site. [cited 2013 Feb 5 2013]. Available from: <http://seer.cancer.gov/faststats/selections.php?#Output>
5. Diehl AK (1983) Gallstone size and the risk of gallbladder cancer. *JAMA* 250(17):2323–2326
6. Towfigh S et al (2001) Porcelain gallbladder is not associated with gallbladder carcinoma. *Am Surg* 67(1):7–10
7. Jarnagin WR, Belghiti J, Blumgart LH (2012) Blumgart's surgery of the liver, biliary tract, and pancreas. Available from: <http://www.sciencedirect.com/science/book/9781437714548>
8. Bobbs J (1868) Case of lithotomy of the gallbladder. *Trans Med Soc Indiana* 18:68
9. Glenn F (1971) Biliary tract disease since antiquity. *Bull N Y Acad Med* 47(4):329–350
10. Hardy KJ (1993) Carl Langenbuch and the Lazarus Hospital: events and circumstances surrounding the first cholecystectomy. *Aust N Z J Surg* 63(1):56–64
11. Pack GT, Miller TR, Brasfield RD (1955) Total right hepatic lobectomy for cancer of the gallbladder; report of three cases. *Ann Surg* 142(1):6–16
12. Reynolds W Jr (2001) The first laparoscopic cholecystectomy. *JSL* 5(1):89–94
13. Marescaux J et al (1998) Virtual reality applied to hepatic surgery simulation: the next revolution. *Ann Surg* 228(5):627–634
14. Satava RM (1999) Emerging technologies for surgery in the 21st century. *Arch Surg* 134(11):1197–1202
15. Bryan PJ et al (1977) Correlation of computed tomography, gray scale ultrasonography, and radionuclide imaging of the liver in detecting space-occupying processes. *Radiology* 124(2):387–393
16. Grossman ZD et al (1977) Radionuclide imaging, computed tomography, and gray-scale ultrasonography of the liver: a comparative study. *J Nucl Med* 18(4):327–332
17. Damadian R (1971) Tumor detection by nuclear magnetic resonance. *Science* 171(3976):1151–1153
18. Shih SP et al (2007) Gallbladder cancer: the role of laparoscopy and radical resection. *Ann Surg* 245(6):893–901
19. Sun CD et al (2005) Laparoscopic cholecystectomy for treatment of unexpected early-stage gallbladder cancer. *J Surg Oncol* 91(4):253–257
20. Kwon AH et al (2008) Unsuspected gallbladder cancer diagnosed during or after laparoscopic cholecystectomy. *J Surg Oncol* 97(3):241–245
21. Benson AB 3rd et al (2009) NCCN clinical practice guidelines in oncology: hepatobiliary cancers. *J Natl Compr Canc Netw* 7(4):350–391
22. Fong Y, Jarnagin W, Blumgart LH (2000) Gallbladder cancer: comparison of patients presenting initially for definitive operation with those presenting after prior noncurative intervention. *Ann Surg* 232(4):557–569
23. Miller G, Jarnagin WR (2008) Gallbladder carcinoma. *Eur J Surg Oncol (EJSO)* 34(3):306–312
24. Duffy A et al (2008) Gallbladder cancer (GBC): 10-year experience at Memorial Sloan-Kettering Cancer Centre (MSKCC). *J Surg Oncol* 98(7):485–489
25. de Aretxabala X et al (2006) Gallbladder cancer: an analysis of a series of 139 patients with invasion restricted to the subserosal layer. *J Gastrointest Surg* 10(2):186–192
26. You DD et al (2008) What is an adequate extent of resection for T1 gallbladder cancers? *Ann Surg* 247(5):835–838
27. Yamaguchi K, Tsuneyoshi M (1992) Subclinical gallbladder carcinoma. *Am J Surg* 163(4):382–386
28. Shirai Y et al (1992) Inapparent carcinoma of the gallbladder. An appraisal of a radical second operation after simple cholecystectomy. *Ann Surg* 215(4):326–331
29. Ouchi K, Mikuni J, Kakugawa Y (2002) Laparoscopic cholecystectomy for gallbladder carcinoma: results of a Japanese survey of 498 patients. *J Hepatobiliary Pancreat Surg* 9(2):256–260
30. Otero JC et al (2006) Gallbladder cancer: surgical results after cholecystectomy in 25 patients with lamina propria invasion and 26 patients with muscular layer invasion. *J Hepatobiliary Pancreat Surg* 13(6):562–566
31. Principe A et al (2006) Radical surgery for gallbladder carcinoma: possibilities of survival. *Hepatogastroenterology* 53(71):660–664
32. Tsukada K et al (1996) Outcome of radical surgery for carcinoma of the gallbladder according to the TNM stage. *Surgery* 120(5):816–821
33. Jensen EH et al (2009) A critical analysis of the surgical management of early-stage gallbladder cancer in the United States. *J Gastrointest Surg* 13(4):722–727
34. de Aretxabala X et al (2004) Management of gallbladder cancer with invasion of the muscular layer. *Rev Med Chil* 132(2):183–188
35. Ogura Y et al (1991) Radical operations for carcinoma of the gallbladder: present status in Japan. *World J Surg* 15(3):337–343

36. Konstantinidis IT et al. (2009) Trends in presentation and survival for gallbladder cancer during a period of more than 4 decades: a single-institution experience. *Arch Surg* 144(5): 441–447 (discussion 447)
37. de Aretxabala XA et al (1997) Curative resection in potentially resectable tumours of the gallbladder. *Eur J Surg* 163(6):419–426
38. Shirai Y et al (2012) Regional lymphadenectomy for gallbladder cancer: rational extent, technical details, and patient outcomes. *World J Gastroenterol* 18(22):2775–2783
39. Greene FL et al. (ed) (2010) *AJCC Cancer Staging Handbook*, 7th edn. American Joint Committee on Cancer, Chicago
40. Kondo S et al (2002) Extensive surgery for carcinoma of the gallbladder. *Br J Surg* 89(2):179–184
41. Piehler JM, Crichlow RW (1978) Primary carcinoma of the gallbladder. *Surg Gynecol Obstet* 147(6):929–942
42. Onoyama H et al (1995) Extended cholecystectomy for carcinoma of the gallbladder. *World J Surg* 19(5):758–763
43. Doty JR et al (2002) Cholecystectomy, liver resection, and pylorus-preserving pancreaticoduodenectomy for gallbladder cancer: report of five cases. *J Gastrointest Surg* 6(5):776–780
44. Nimura Y et al (1991) Hepatopancreatoduodenectomy for advanced carcinoma of the biliary tract. *Hepatogastroenterology* 38(2):170–175
45. Yang HL, Sun YG, Wang Z (1992) Polypoid lesions of the gallbladder: diagnosis and indications for surgery. *Br J Surg* 79(3):227–229
46. Konstantinidis IT et al (2012) Gallbladder lesions identified on ultrasound. Lessons from the last 10 years. *J Gastrointest Surg* 16(3):549–553
47. Pandey M et al (2000) Carcinoma of the gallbladder: role of sonography in diagnosis and staging. *J Clin Ultrasound* 28(5):227–232
48. Z'Graggen K et al (1998) Incidence of port site recurrence after laparoscopic cholecystectomy for preoperatively unsuspected gallbladder carcinoma. *Surgery* 124(5):831–838
49. Maker AV et al (2012) Is port site resection necessary in the surgical management of gallbladder cancer? *Ann Surg Oncol* 19(2):409–417
50. Suzuki K, Kimura T, Ogawa H (1998) Is laparoscopic cholecystectomy hazardous for gallbladder cancer? *Surgery* 123(3):311–314
51. Lundberg O, Kristoffersson A (2000) Wound recurrence from gallbladder cancer after open cholecystectomy. *Surgery* 127(3):296–300
52. Yamaguchi K et al (1996) Gallbladder carcinoma in the era of laparoscopic cholecystectomy. *Arch Surg* 131(9):981–984 discussion 985
53. Corvera CU, Weber SM, Jarnagin WR (2002) Role of laparoscopy in the evaluation of biliary tract cancer. *Surg Oncol Clin N Am* 11(4):877–891
54. Butte JM et al (2011) The role of laparoscopic staging in patients with incidental gallbladder cancer. *HPB (Oxford)* 13(7):463–472
55. Chijiwa K et al (2001) Surgical treatment of patients with T2 gallbladder carcinoma invading the subserosal layer. *J Am Coll Surg* 192(5):600–607
56. Wakai T, Shirai Y, Hatakeyama K (2002) Radical second resection provides survival benefit for patients with T2 gallbladder carcinoma first discovered after laparoscopic cholecystectomy. *World J Surg* 26(7):867–871
57. Toyonaga T et al (2003) Completion radical surgery after cholecystectomy for accidentally undiagnosed gallbladder carcinoma. *World J Surg* 27(3):266–271
58. Foster JM et al (2007) Gallbladder cancer: Defining the indications for primary radical resection and radical re-resection. *Ann Surg Oncol* 14(2):833–840
59. Gall FP et al (1991) Radical operations for carcinoma of the gallbladder: present status in Germany. *World J Surg* 15(3):328–336
60. Nakamura S et al (1999) Outcome of extensive surgery for TNM stage IV carcinoma of the gallbladder. *Hepatogastroenterology* 46(28):2138–2143
61. D'Angelica M et al (2009) Analysis of the extent of resection for adenocarcinoma of the gallbladder. *Ann Surg Oncol* 16(4):806–816

Systemic Therapy: Current Strategies and New Directions

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Abstract

A majority of patients diagnosed with malignancy of the intrahepatic bile ducts, (cholangiocarcinoma) or the extrahepatic bile ducts, either present initially with extensive unresectable disease, or will either develop recurrent disease after surgical resection. These patients will need systemic treatment with chemotherapy to palliate symptoms and prolong survival. Many challenges are faced with the development of effective chemotherapy regimens for these patients.

1 Introduction

There have been many advances in the management of patients with early-stage cancer of the biliary tract, including improved radiographic imaging to optimize tumor staging and identification of resectable tumors, pathologic assessment of prognostic features of resected tumors, and improvements in biliary hygiene, all of which contribute to improved patient survival [1–8]. Unfortunately, most patients with bile duct cancer will eventually develop advanced disease that is not readily curable with surgery, radiation therapy, and/or other liver-directed treatment options. These patients will seek systemic therapy hoping for prolonged survival and palliation of tumor-related symptoms such as pain, anorexia, ascites, and fatigue. A large number of clinical trials have been conducted over the previous decades that evaluated a variety of cytotoxic chemotherapy agents. However, few chemotherapy regimens have been widely accepted as providing clinical benefit to patients based on prospective controlled trials. Many of the published studies of systemic therapy for cancer of the biliary tract have significant limitations including:

- They are retrospective case series.
- Many of the prospective studies are single-arm trials, with a small sample size and no control arm.

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- Because cancers of the bile ducts and gallbladder are orphan tumors, it is very challenging to conduct trials specifically for these malignancies. Many trials have included a variety of tumor types, including pancreatic adenocarcinoma, intrahepatic cholangiocarcinoma (ICC), extrahepatic bile duct cancer (EHCC), gallbladder cancer, and ampullary cancer. This group of tumors is very heterogeneous and is distinct entities with different etiologies, risk factors, molecular characteristics, patterns of dissemination, prognoses, and response to treatment [9, 10].

A few prospective chemotherapy clinical trials have included subgroup analyses to ascertain whether there are differences in clinical outcome based on ICC, EHCC, or gallbladder cancer. However, due to many challenges with conducting prospective clinical trials in these patients, there have not yet been separate trials in ICC and EHCC, or trials that are stratified by tumor location, that are adequately powered to identify true differences in chemotherapy benefit.

2 The Challenges of Developing Effective Systemic Therapy for Advanced Cholangiocarcinoma

When evaluating systemic therapy for solid tumors, there are a number of factors that affect the design of clinical trials, selection of study endpoints, and which agents are to be studied. With respect to biliary tract cancer, in summary, these include

- *Clinical behavior:* As is the case in many solid tumors, but particularly notable in cholangiocarcinoma, a wide range of tumor behavior is observed. Many of these tumors display relatively indolent clinical behavior, may remain stable for many months, appear to grow slowly, and progress over years rather than months. These indolent ICC often do not cause tumor-related symptoms until the tumor burden is very extensive. Other cholangiocarcinomas exhibit a more aggressive phenotype, progress rapidly, and result in more tumor-related morbidity [9].
- *Imaging characteristics:* Tumors of the bile ducts often appear radiographically in two general forms: The mass-forming ICC appear as low-attenuation masses with irregular peripheral enhancement and may be accompanied by liver capsule retraction, satellite nodules and peripheral intrahepatic ductal dilatation. The periductal infiltrating cholangiocarcinomas are characterized by growth along dilated or narrowed bile ducts without mass formation [10]. Both ICC and ECC are highly desmoplastic tumors and tend to spread along bile duct walls and periductal tissue, thus making them challenging to

image adequately with conventional imaging techniques, in order to establish a baseline and assess radiographic tumor response [11–16].

- *Tumor biology:* The putative cells of origin in ICC and EHCC, the cholangiocytes, are multifunctional pro-proliferative cells. Cholangiocytes produce stimulatory cytokines (including TGF, IL-6, PDGF, TNF) as part of both autocrine and paracrine modulatory pathways. They mediate inflammation in the liver, which is known to play a key role in the initiation and maintenance of carcinogenesis [17, 18]. The liver–cholangiocyte microenvironment is thought to be pro-carcinogenic. Cholangiocytes are also able to “detoxify” foreign substances as a normal cellular function and thus are inherently chemotherapy resistant [19–21].

3 Systemic Therapy for Biliary Tract Cancers: State of the Clinical Science

A large number of clinical trials of chemotherapy, using single agents, doublets, and multidrug combinations, have been published in recent years. Most trials have been conducted to ascertain whether chemotherapy can provide clinical benefit to patients with advanced cancer of the biliary tract, in terms of palliation of tumor-related symptoms, or increased survival. Most trials have been small, single-arm studies that commonly evaluate tumor response rate at the primary endpoint. For trials that follow the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 guidelines, tumor response is defined as complete response (CR disappearance of all target lesions) or partial response (PR at least a 30 % decrease in the sum of diameters of target lesion taking as reference the baseline sum diameters) [22]. Tumor response is an indication of antitumor activity, but may not correlate with clinical benefit to patients with advanced disease. Selected studies are summarized in Table 1.

In April 2010, the ABC-02 trial was published, which was the first phase III randomized, controlled trial in subjects with advanced biliary tract cancer. The ABC-02 trial compared doublet therapy consisting of gemcitabine and cisplatin, to single-agent gemcitabine in 410 patients with locally advanced or metastatic biliary tract cancer. The combination of gemcitabine + cisplatin demonstrated improved progression-free survival (PFS) and overall survival (OS) compared to gemcitabine alone. In this trial, 59 % (241 patients) had bile duct tumors, but the particular site of disease within the bile duct was not specified. After a median follow-up of 8.2 months, the combination group had a significantly improved OS (11.7 vs. 8.1 months) [23].

Table 1 Selected cytotoxic chemotherapy regimens for biliary tract cancer

Regimen	Study phase	Sample size	Response rate (%)	PFS ^b	Overall survival (months)
				TTP ^c (months)	
Gemcitabine + cisplatin versus gemcitabine [23]	III	410	81 ^a	8.0	11.7
			71	5.0	8.1
Gemcitabine [24]	II	24	13		7.2
Gemcitabine + 5-fluorouracil [25]	II	42	1		9.7
Gemcitabine + S-1 [26]	II	35	34.3	TTP 5.9	11.6
Gemcitabine + cisplatin [27]	II	43	28		8.4
Gemcitabine + capecitabine [28]	II	45	31		14
Gemcitabine + oxaliplatin (GEMOX) [29]	II	53	18.9	PFS 4.8	8.3
GEMOX + cetuximab [30]	II	30	63		11.6
Oxaliplatin + capecitabine (CAPEOX) [31]	II	65	37 (GB, ECC) ^d		12.8
			0 (ICC)		16.8
Capecitabine [32]	II	26	19		8.1
5-fluorouracil + leucovorin + irinotecan (FOLFIRI) [33]	II	30	10	3	5.9
		17 ICC		5.9	9.75
		13 GB			

^a Tumor control rate = SD + PR + CR (stable disease + partial response + complete response)

^b PFS progression-free survival

^c TTP time to progression

^d GB gallbladder, ECC extrahepatic cholangiocarcinoma, ICC intrahepatic cholangiocarcinoma

Table 2 Clinical trials of “targeted” therapies for biliary tract cancer

Regimen	Study phase	Sample size	Response rate (%)	PFS	Overall survival (months)
				TTP (months)	
Bevacizumab + erlotinib [36]	II	49	18.4	TTP 4.4	9.9
Sorafenib [37]	II	31		3	9
Sorafenib [38]	II	46	0	PFS 3	9
Lapatinib [39]	II	17		PFS 1.8	5.2
Selumetinib [40]	II	28	12	3.7	9.8
Erlotinib [41]	II	42	8		7.5
GEMOX + panitumumab [42]	II	46	33	PFS 8.3	10.0
GEMOX versus GEMOX + erlotinib [43]	RIII, first line	133	29	PFS 4.2	9.5
		135	15.7	PFS 5.8	9.5
Sunitinib [44]	II, second line	56	8.9	1.7	4.8
GEMOX + bevacizumab [45]	II, first line	35	40	7	12.7

This regimen is currently considered the standard-of-care first-line therapy for patients with advanced cancer of the bile ducts and gallbladder. The results of the ABC-02 trial have stimulated enthusiasm by many investigators to conduct trials in the second-line setting, as well as of novel targeted anticancer agents based on existing understanding of the molecular carcinogenesis of biliary tract cancers.

4 Advancing the Field: Incorporating “Targeted” Therapies into Clinical Trials

Clearly, there remains a significant unmet medical need to develop effective and safe systemic therapy regimens for patients with advanced cholangiocarcinoma. The two main areas of unmet clinical need are to extend the benefit of

Table 3 Novel “targeted” agents with preclinical rationale in the treatment for cholangiocarcinoma

Pathway/Target	Rationale	Outcome
EGFR + VEGF inhibition	Both EGFR and VEGF overexpressions common in cholangiocarcinoma	Vandetanib, dual VEGF2/EGFR inhibitor significantly decreased in cell lines and xenografts [52, 53]
PDGF (platelet-derived growth factor)	Myofibroblasts are abundant in cholangiocarcinoma microenvironment and display pro-carcinogenic cross talk with cancer cells, mediated partly by PDGF-B [48, 54, 55]	Cytotoxic agent navitoclax induced apoptosis in cancer-associated fibroblasts (CAF) in a cholangiocarcinoma rat model [47]
Epidermal growth factor receptor (EGFR)	ERB1 and ERB2 overexpressions are prominent in biliary tract [56–60]. Activating mutations rate very low [61]	Several preclinical studies suggest benefit of therapeutic efficacy with EGFR inhibitors [62]
Cyclooxygenase	Cyclooxygenase plays important role in biliary cancer cell signaling [63–66]	COX-2 inhibitor NS-398 showed dose-dependent growth inhibition in rat model of cholangiocarcinoma [67]
Vascular endothelial growth factor (VEGF) expression	VEGF expression linked to poor prognostic features and decreased survival [37, 38, 52, 68–70]	Some suggestion of clinical benefit shown in single-arm clinical trials [37, 38, 45, 70, 71]
MEK (mitogen-activated) ERK (extracellular signal-regulated kinase)	MEK is critical element of Ras/Raf/MEK/ERK signal transduction pathway [72, 73]	Evidence of gallbladder cancer cell line growth inhibition by MEK inhibitor UO126 [74, 75]
c-MET (hepatocyte growth factor)	Several studies show overexpression of c-MET in preclinical cholangiocarcinoma models [76–78]	NK4, which acts as an HGF antagonist and angiogenesis inhibitor, when transfected cholangiocarcinoma cell line clones, showed cell growth inhibition by arresting cell cycle progression [79]
Molecular subclasses including “inflammatory” and “proliferative” subclasses	This important work may provide biomarkers of therapeutic efficacy to design biomarker-driven clinical trials [49, 51]	
BRAF activating mutations	Present in 7 % of cholangiocarcinoma specimens [49, 51, 80]	
Hedgehog signaling	Sonic hedgehog ligand highly expressed by human cholangiocarcinoma tissue specimens and cell lines [55, 81, 82]	In vitro inhibition of sonic hedgehog signaling decreased epithelial–mesenchymal transition and cholangiocarcinoma cell viability [81]

gemcitabine and cisplatin, potentially by adding one or more “targeted” agents to the combination, and to develop effective regimens for patients who have failed first-line chemotherapy. Unfortunately, the numerous single-arm clinical trials in advanced biliary tract cancer conducted over the previous two decades have done little to advance the field [34, 35]. Some trials of single-agent-targeted therapies, and combinations with cytotoxic agents, have been reported, as listed in Table 2.

The foremost task in biliary tract cancer research is to improve our understanding of the key molecular carcinogenic mechanisms in this group of malignancies with a focus on identifying the oncogenic driver mechanisms or mutations. Given the high cost, the time required to complete, and very low yield of empiric clinical trials, it is essential that a better understanding of the rationale for new agents and combinations be established in the preclinical setting. In order to identify potential “relevant” molecular target(s) or combinations of targets in ICC and or EHCC, some important concepts include the following:

- The results from measuring “overexpression” of a potential molecular target are highly variable, depending on the quality of tumor specimen analyzed and the analytic method used.
- Consistent overexpression of a cell receptor or a protein does not guarantee that it is a “driver mechanism” in the particular cancer or will be an “actionable” target for drug development.
- Screening of potential new agents and combinations in cholangiocarcinoma cell lines, of which there are very few, is one step in assessing new therapeutics. However, cell lines have lost many characteristics of the original tumor and thus have significant limitations in predicting behavior of human tumors.
- Development of new anticancer drugs in cholangiocarcinoma must utilize appropriate preclinical models. The “ideal” models include the role of the tumor microenvironment [46–48].
- Genetic and genomic profiling utilizing any of the many new cancer gene panels, or broader whole-genome

sequencing methods, holds the promise of identifying validated molecular targets in both populations and individuals. These are powerful tools that provide rationale determining “druggable” targets in ICC and EHCC.

- Future clinical trials in biliary tract cancer ideally will stratify by molecular expression profile if potential targets differ by site and enrich the trial patient population by the target of interest. Table 3 summarizes current understanding of several important “actionable” molecular targets in cholangiocarcinoma.

Several recent studies that employed advanced molecular analytical approaches to ICC have provided exciting insight into molecular classifications of these tumors that are providing much-needed basis for rational development of future clinical trials in this malignancy. Sia et al. [49] identified two “classes” of cholangiocarcinoma through Genomic Identification of Significant Targets in Cancer (GISTIC) analysis of 149 subject specimens. The categories, including a “proliferative” class and “inflammatory” class, displayed different gene signatures, activated oncogenic pathways, and clinical outcomes. This work represents important progress in developing therapeutic biomarkers in cholangiocarcinoma.

Thorgeirsson and colleagues have carried out extensive genetic profiling of ICC in the Laboratory of Experimental Carcinogenesis at the National Cancer Institute of the NIH, Bethesda, MD [50, 51]. This important work performed on 109 patient specimens has also identified several subclasses of cholangiocarcinomas based on clinical outcome, presence or absence of KRAS gene mutations, growth factor expression, and other markers of malignant transformation.

Developing more effective systemic therapy options that improve outcome for patients with advanced cancer of the biliary tract is possible but depends on continued, large-scale molecular profiling of tumor specimens to guide drug selection and novel clinical trial designs that assign patients to therapies based on their tumor profile.

References

1. Ishizawa T, Mise Y, Aoki T et al (2010) Surgical technique: new advances for expanding indications and increasing safety in liver resection for HCC: the Eastern perspective. *J Hepatobiliary Pancreat Sci* 17:389–393
2. LaFemina J, Jarnagin WR (2012) Surgical management of proximal bile duct cancers. *Langenbecks Arch Surg* 397:869–879
3. Kawakubo K, Isayama H, Mizuno S et al (2013) One-step, simultaneous triple endoscopic nasobiliary drainage for hilar biliary stricture. *Dig Endosc* 25:76–79
4. Valle JW (2010) Advances in the treatment of metastatic or unresectable biliary tract cancer. *Ann Oncol* 21(Suppl 7):vii345–vii348
5. de Jong MC, Nathan H, Sotiropoulos GC et al (2011) Intrahepatic cholangiocarcinoma: an international multi-institutional analysis of prognostic factors and lymph node assessment. *J Clin Oncol* 29:3140–3145
6. Gerhardt T, Rings D, Hoblinger A et al (2010) Combination of bilateral metal stenting and trans-stent photodynamic therapy for palliative treatment of hilar cholangiocarcinoma. *Z Gastroenterol* 48:28–32
7. Nathan H, Aloia TA, Vauthey JN et al (2009) A proposed staging system for intrahepatic cholangiocarcinoma. *Ann Surg Oncol* 16:14–22
8. Blehacz B, Komuta M, Roskams T et al (2011) Clinical diagnosis and staging of cholangiocarcinoma. *Nat Rev Gastroenterol Hepatol* 8:512–522
9. Aishima S, Fujita N, Mano Y et al (2009) Different roles of S100P overexpression in intrahepatic cholangiocarcinoma: carcinogenesis of perihilar type and aggressive behavior of peripheral type. *Am J Surg Pathol* 35:590–598
10. Chung YE, Kim MJ, Park YN et al (2009) Varying appearances of cholangiocarcinoma: radiologic-pathologic correlation. *Radiographics* 29:683–700
11. Inui K, Yoshino J, Miyoshi H (2009) Differential diagnosis and treatment of biliary strictures. *Clin Gastroenterol Hepatol* 7:S79–S83
12. Motosugi U, Ichikawa T, Nakajima H et al (2009) Cholangiolocellular carcinoma of the liver: imaging findings. *J Comput Assist Tomogr* 33:682–688
13. Kim SH, Lee WJ, Lim HK et al (2007) Sclerosing hepatic carcinoma: helical CT features. *Abdom Imaging* 32:725–729
14. Gu XJ, Wang BF, Liu R (2012) Application of (1)(8)F-fluorodeoxyglucose positron emission tomography/computed tomography in preoperative assessment of hilar cholangiocarcinoma. *Zhonghua Yi Xue Za Zhi* 92:1409–1412
15. Alvaro D, Bragazzi MC, Benedetti A et al (2011) Cholangiocarcinoma in Italy: a national survey on clinical characteristics, diagnostic modalities and treatment. Results from the “Cholangiocarcinoma” committee of the Italian Association for the Study of Liver disease. *Dig Liver Dis* 43:60–65
16. Lee SW, Kim HJ, Park JH et al (2010) Clinical usefulness of 18F-FDG PET-CT for patients with gallbladder cancer and cholangiocarcinoma. *J Gastroenterol* 45:560–566
17. Wehbe H, Henson R, Meng F et al (2006) Interleukin-6 contributes to growth in cholangiocarcinoma cells by aberrant promoter methylation and gene expression. *Cancer Res* 66:10517–10524
18. Fava G, Alpini G, Rychlicki C et al (2008) Leptin enhances cholangiocarcinoma cell growth. *Cancer Res* 68:6752–6761
19. Marin JJ, Romero MR, Briz O (2010) Molecular bases of liver cancer refractoriness to pharmacological treatment. *Curr Med Chem* 17:709–740
20. Namwat N, Amimanan P, Loilome W et al (2008) Characterization of 5-fluorouracil-resistant cholangiocarcinoma cell lines. *Chemotherapy* 54:343–351
21. Kongpetch S, Kukongviriyapan V, Prawan A et al (2012) Crucial role of heme oxygenase-1 on the sensitivity of cholangiocarcinoma cells to chemotherapeutic agents. *PLoS ONE* 7:e34994
22. Eisenhauer EA, Therasse P, Bogaerts J et al (2009) New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* 45:228–247
23. Valle J, Wasan H, Palmer DH et al (2010) Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. *N Engl J Med* 362:1273–1281
24. Lin MH, Chen JS, Chen HH et al (2003) A phase II trial of gemcitabine in the treatment of advanced bile duct and perihampullary carcinomas. *Chemotherapy* 49:154–158
25. Alberts SR, Al-Khatib H, Mahoney MR et al (2005) Gemcitabine, 5-fluorouracil, and leucovorin in advanced biliary tract and

- gallbladder carcinoma: a North Central Cancer Treatment Group phase II trial. *Cancer* 103:111–118
26. Sasaki T, Isayama H, Nakai Y et al (2012) Multicenter phase II study of S-1 monotherapy as second-line chemotherapy for advanced biliary tract cancer refractory to gemcitabine. *Invest New Drugs* 30:708–713
 27. Thongprasert S, Napapan S, Charoentum C et al (2005) Phase II study of gemcitabine and cisplatin as first-line chemotherapy in inoperable biliary tract carcinoma. *Ann Oncol* 16:279–281
 28. Knox JJ, Hedley D, Oza A et al (2005) Combining gemcitabine and capecitabine in patients with advanced biliary cancer: a phase II trial. *J Clin Oncol* 23:2332–2338
 29. Jang JS, Lim HY, Hwang IG et al (2010) Gemcitabine and oxaliplatin in patients with unresectable biliary cancer including gall bladder cancer: a Korean Cancer Study Group phase II trial. *Cancer Chemother Pharmacol* 65:641–647
 30. Gruenberger B, Schueller J, Heubrandtner U et al (2010) Cetuximab, gemcitabine, and oxaliplatin in patients with unresectable advanced or metastatic biliary tract cancer: a phase 2 study. *Lancet Oncol* 11:1142–1148
 31. Nehls O, Oettle H, Hartmann JT et al (2008) Capecitabine plus oxaliplatin as first-line treatment in patients with advanced biliary system adenocarcinoma: a prospective multicentre phase II trial. *Br J Cancer* 98:309–315
 32. Patt YZ, Hassan MM, Aguayo A et al (2004) Oral capecitabine for the treatment of hepatocellular carcinoma, cholangiocarcinoma, and gallbladder carcinoma. *Cancer* 101:578–586
 33. Feisthammel J, Schoppmeyer K, Mossner J et al (2007) Irinotecan with 5-FU/FA in advanced biliary tract adenocarcinomas: a multicenter phase II trial. *Am J Clin Oncol* 30:319–324
 34. Furuse J, Kasuga A, Takasu A et al (2012) Role of chemotherapy in treatments for biliary tract cancer. *J Hepatobiliary Pancreat Sci* 19:337–341
 35. Eckmann KR, Patel DK, Landgraf A et al (2011) Chemotherapy outcomes for the treatment of unresectable intrahepatic and hilar cholangiocarcinoma: a retrospective analysis. *Gastrointest Cancer Res* 4:155–160
 36. Lubner SJ, Mahoney MR, Kolesar JL et al (2010) Report of a multicenter phase II trial testing a combination of biweekly bevacizumab and daily erlotinib in patients with unresectable biliary cancer: a phase II Consortium study. *J Clin Oncol* 28:3491–3497
 37. Bengala C, Bertolini F, Malavasi N et al (2010) Sorafenib in patients with advanced biliary tract carcinoma: a phase II trial. *Br J Cancer* 102:68–72
 38. El-Khoueiry AB, Rankin CJ, Ben-Josef E et al (2012) SWOG 0514: a phase II study of sorafenib in patients with unresectable or metastatic gallbladder carcinoma and cholangiocarcinoma. *Invest New Drugs* 30:1646–1651
 39. Ramanathan RK, Belani CP, Singh DA et al (2009) A phase II study of lapatinib in patients with advanced biliary tree and hepatocellular cancer. *Cancer Chemother Pharmacol* 64:777–783
 40. Bekaii-Saab T, Phelps MA, Li X et al (2011) Multi-institutional phase II study of selumetinib in patients with metastatic biliary cancers. *J Clin Oncol* 29:2357–2363
 41. Philip PA, Mahoney MR, Allmer C et al (2006) Phase II study of erlotinib in patients with advanced biliary cancer. *J Clin Oncol* 24:3069–3074
 42. Jensen LH, Lindebjerg J, Ploen J et al (2012) Phase II marker-driven trial of panitumumab and chemotherapy in KRAS wild-type biliary tract cancer. *Ann Oncol* 23:2341–2346
 43. Lee J, Park SH, Chang HM et al (2012) Gemcitabine and oxaliplatin with or without erlotinib in advanced biliary-tract cancer: a multicentre, open-label, randomised, phase 3 study. *Lancet Oncol* 13:181–188
 44. Yi JH, Thongprasert S, Lee J et al (2012) A phase II study of sunitinib as a second-line treatment in advanced biliary tract carcinoma: a multicentre, multinational study. *Eur J Cancer* 48:196–201
 45. Zhu AX, Meyerhardt JA, Blaszkowsky LS et al (2010) Efficacy and safety of gemcitabine, oxaliplatin, and bevacizumab in advanced biliary-tract cancers and correlation of changes in 18-fluorodeoxyglucose PET with clinical outcome: a phase 2 study. *Lancet Oncol* 11:48–54
 46. Utispan K, Sonongbua J, Thuwajit P et al (2012) Periostin activates integrin $\alpha 5 \beta 1$ through a PI3 K/AKT-dependent pathway in invasion of cholangiocarcinoma. *Int J Oncol* 41:1110–1118
 47. Mertens JC, Fingas CD, Christensen JD et al (2013) Therapeutic effects of deleting cancer-associated fibroblasts in cholangiocarcinoma. *Cancer Res* 73:897–907
 48. Fingas CD, Mertens JC, Razumilava N et al (2012) Targeting PDGFR- β in cholangiocarcinoma. *Liver Int* 32:400–409
 49. Sia D, Hoshida Y, Villanueva A et al (2013) Integrative molecular analysis of intrahepatic cholangiocarcinoma reveals 2 classes that have different outcomes. *Gastroenterology* 144:829–840
 50. Andersen JB, Thorgeirsson SS (2012) Genetic profiling of intrahepatic cholangiocarcinoma. *Curr Opin Gastroenterol* 28:266–272
 51. Andersen JB, Spee B, Blechacz BR et al (2012) Genomic and genetic characterization of cholangiocarcinoma identifies therapeutic targets for tyrosine kinase inhibitors. *Gastroenterology* 142:1021–1031 e15
 52. Yoshikawa D, Ojima H, Iwasaki M et al (2008) Clinicopathological and prognostic significance of EGFR, VEGF, and HER2 expression in cholangiocarcinoma. *Br J Cancer* 98:418–425
 53. Yoshikawa D, Ojima H, Kokubu A et al (2009) Vandetanib (ZD6474), an inhibitor of VEGFR and EGFR signalling, as a novel molecular-targeted therapy against cholangiocarcinoma. *Br J Cancer* 100:1257–1266
 54. Boonjaraspinyo S, Boonmars T, Wu Z et al (2012) Platelet-derived growth factor may be a potential diagnostic and prognostic marker for cholangiocarcinoma. *Tumour Biol* 33:1785–1802
 55. Fingas CD, Bronk SF, Werneburg NW et al (2011) Myofibroblast-derived PDGF-BB promotes Hedgehog survival signaling in cholangiocarcinoma cells. *Hepatology* 54:2076–2088
 56. Yoon JH, Werneburg NW, Higuchi H et al (2002) Bile acids inhibit Mcl-1 protein turnover via an epidermal growth factor receptor/Raf-1-dependent mechanism. *Cancer Res* 62:6500–6505
 57. Jan YY, Yeh TS, Yeh JN et al (2004) Expression of epidermal growth factor receptor, apomucins, matrix metalloproteinases, and p53 in rat and human cholangiocarcinoma: appraisal of an animal model of cholangiocarcinoma. *Ann Surg* 240:89–94
 58. Terada T, Ashida K, Endo K et al (1998) c-erbB-2 protein is expressed in hepatolithiasis and cholangiocarcinoma. *Histopathology* 33:325–331
 59. Lai GH, Zhang Z, Shen XN et al (2005) erbB-2/neu transformed rat cholangiocytes recapitulate key cellular and molecular features of human bile duct cancer. *Gastroenterology* 129:2047–2057
 60. Kiguchi K, Carbajal S, Chan K et al (2001) Constitutive expression of ErbB-2 in gallbladder epithelium results in development of adenocarcinoma. *Cancer Res* 61:6971–6976
 61. Leone F, Cavalloni G, Pignochino Y et al (2006) Somatic mutations of epidermal growth factor receptor in bile duct and gallbladder carcinoma. *Clin Cancer Res* 12:1680–1685
 62. Zhang Z, Oyesanya RA, Campbell DJ et al (2010) Preclinical assessment of simultaneous targeting of epidermal growth factor receptor (ErbB1) and ErbB2 as a strategy for cholangiocarcinoma therapy. *Hepatology* 52:975–986

63. Sirica AE, Lai GH, Endo K et al (2002) Cyclooxygenase-2 and ERBB-2 in cholangiocarcinoma: potential therapeutic targets. *Semin Liver Dis* 22:303–313
64. Sirica AE, Lai GH, Zhang Z (2001) Biliary cancer growth factor pathways, cyclo-oxygenase-2 and potential therapeutic strategies. *J Gastroenterol Hepatol* 16:363–372
65. Han C, Demetris AJ, Stolz DB et al (2006) Modulation of Stat3 activation by the cytosolic phospholipase A2alpha and cyclooxygenase-2-controlled prostaglandin E2 signaling pathway. *J Biol Chem* 281:24831–24846
66. Han C, Wu T (2005) Cyclooxygenase-2-derived prostaglandin E2 promotes human cholangiocarcinoma cell growth and invasion through EP1 receptor-mediated activation of the epidermal growth factor receptor and Akt. *J Biol Chem* 280:24053–24063
67. Zhang Z, Lai GH, Sirica AE (2004) Celecoxib-induced apoptosis in rat cholangiocarcinoma cells mediated by Akt inactivation and Bax translocation. *Hepatology* 39:1028–1037
68. Dobashi A, Imazu H, Tatsumi N et al (2013) Quantitative analysis of VEGF-C mRNA of extrahepatic cholangiocarcinoma with real-time PCR using samples obtained during endoscopic retrograde cholangiopancreatography. *Scand J Gastroenterol* 48:848–855
69. Benckert C, Jonas S, Cramer T et al (2003) Transforming growth factor beta 1 stimulates vascular endothelial growth factor gene transcription in human cholangiocellular carcinoma cells. *Cancer Res* 63:1083–1092
70. Sugiyama H, Onuki K, Ishige K et al (2011) Potent in vitro and in vivo antitumor activity of sorafenib against human intrahepatic cholangiocarcinoma cells. *J Gastroenterol* 46:779–789
71. Wiedmann MW, Mossner J (2010) Molecular targeted therapy of biliary tract cancer—results of the first clinical studies. *Curr Drug Targets* 11:834–850
72. Meng F, Yamagiwa Y, Taffetani S et al (2005) IL-6 activates serum and glucocorticoid kinase via p38alpha mitogen-activated protein kinase pathway. *Am J Physiol Cell Physiol* 289:C971–C981
73. Tan FL, Ooi A, Huang D et al (2010) p38delta/MAPK13 as a diagnostic marker for cholangiocarcinoma and its involvement in cell motility and invasion. *Int J Cancer* 126:2353–2361
74. Horiuchi H, Kawamata H, Furihata T et al (2004) A MEK inhibitor (U0126) markedly inhibits direct liver invasion of orthotopically inoculated human gallbladder cancer cells in nude mice. *J Exp Clin Cancer Res* 23:599–606
75. Horiuchi H, Kawamata H, Fujimori T et al (2003) A MEK inhibitor (U0126) prolongs survival in nude mice bearing human gallbladder cancer cells with K-ras mutation: analysis in a novel orthotopic inoculation model. *Int J Oncol* 23:957–963
76. Radaeva S, Ferreira-Gonzalez A, Sirica AE (1999) Overexpression of C-NEU and C-MET during rat liver cholangiocarcinogenesis: a link between biliary intestinal metaplasia and mucin-producing cholangiocarcinoma. *Hepatology* 29:1453–1462
77. Terada T, Nakanuma Y, Sirica AE (1998) Immunohistochemical demonstration of MET overexpression in human intrahepatic cholangiocarcinoma and in hepatolithiasis. *Hum Pathol* 29:175–180
78. Socoteanu MP, Mott F, Alpini G et al (2008) c-Met targeted therapy of cholangiocarcinoma. *World J Gastroenterol* 14:2990–2994
79. Ge X, Wang Y, Wang Y et al (2013) NK4 gene therapy inhibits HGF/Met-induced growth of human cholangiocarcinoma cells. *Dig Dis Sci* 58:1636–1643
80. Tannapfel A, Sommerer F, Benicke M et al (2003) Mutations of the BRAF gene in cholangiocarcinoma but not in hepatocellular carcinoma. *Gut* 52:706–712
81. El Khatib M, Kalnytska A, Palagani V et al (2013) Inhibition of hedgehog signaling attenuates carcinogenesis in vitro and increases necrosis of cholangiocellular carcinoma. *Hepatology* 57:1035–1045
82. Omenetti A, Diehl AM (2011) Hedgehog signaling in cholangiocytes. *Curr Opin Gastroenterol* 27:268–275

External Beam Radiation Therapy: 3D-Conformal, Intensity-Modulated, and Proton Beam

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Abstract

Radiation plays an important role in the treatment for gallbladder and biliary tract cancers. In the adjuvant setting, the goal of radiation is to provide local disease control and, by doing so, slow overall disease progression and prolong survival. Furthermore, local control is critical because of the morbidity of local progression in the biliary tract. Thus, radiation may help prevent or palliate symptomatic or uncontrolled local disease in both the adjuvant and unresectable settings. Historically, radiation has had a limited role in these malignancies. This was primarily related to the concern about radiation injury to organs at risk (OARs). With a better understanding of dose tolerances of OARs and improved conformality of treatment modalities, radiation has become more widely used.

1 Rationale for Radiotherapy

Several reports have examined the impact of radiation therapy on either the adjuvant or unresectable setting. Both external beam radiation therapy (EBRT) and, to a lesser degree, brachytherapy and intraoperative radiation therapy (IORT) were used in these series. The majority of the studies are retrospective, but several have comparison cohorts of patients who did not receive radiation. In the adjuvant series, the extent of resection (complete vs. partial) was variable. Neoadjuvant radiotherapy has been limited to unresectable disease prior to liver transplantation.

1.1 Adjuvant Radiotherapy

In 2012, a systemic review and meta-analysis of adjuvant therapy in biliary tract cancer presented data on twenty studies between 1960 and 2010 involving 6,712 patients with gallbladder and biliary tract tumors [1]. The vast majority of studies (19 of 20) in this meta-analysis did not

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Table 1 Adjuvant radiotherapy or chemoradiotherapy for extrahepatic cholangiocarcinoma

Study	No. of patients	R0 (%)	Radiotherapy	Chemotherapy	Locoregional failure (%)	Median survival (months)	P value
Kim et al. [29]	72	65	EBRT 40 Gy (split course, in 6 weeks)	Bolus 5-FU	47	25	–
Todoroki et al. [2]	29	4	IORT 21 Gy, EBRT 43 Gy, or the combination	None	21	32	0.01
	20		No radiation	None	69	10	
Schoenthaler et al. [28]	6	0	EBRT 54 Gy, 1.8 Gy/fraction	None	–	21.5	0.01
	15	60	No radiation	None		16	
Sagawa et al. [5]	39	49	EBRT 37 Gy + ILBT 37 Gy or EBRT 38 Gy	None	–	23	NS
	30		No radiation	None		20	
Gerhards et al. [3]	71	14	EBRT 46 Gy or EBRT 42 Gy + ILBT 10 Gy	None	–	24	<0.01
	20		No radiation	None		8	
Pitt et al. [49]	14	68	EBRT ± Ir-192 13 Gy	None	–	20	NS
	17		No radiation	None		20	
Nakeeb et al. [50]	42	75	EBRT (no details)	Bolus and CI 5-FU; gemcitabine	–	16.4	–
Ben-David et al. [12]	28	43	EBRT 54 Gy (median)	54 % of patients; 5-FU, gemcitabine, floxuridine, bromodeoxyuridine	39	24.1 (R0); 15 (R1)	–
Kim et al. [4]	115	78	EBRT 45 Gy (median), 1.8 Gy/fraction	Concurrent 5-FU-based chemo	41.5	36.4	0.007 (LRF); 0.049 (OS)
	53	38	No radiation	None	55.6	27.9	–
Nelson et al. [31]	45	80	EBRT 50.4 Gy (median); ILBT (4 patients)	5-FU-based	22	34	–
Hughes et al. [30]	34	24	EBRT 50.4 Gy (median), 1.8 Gy/fraction	5-FU-based	30	36.9	–

EBRT external beam radiation therapy; IORT intraoperative radiation therapy; ILBT intraluminal brachytherapy; 5-FU 5-fluorouracil; OS overall survival; LRF locoregional failure; NS not significant; CI continuous infusion

include intrahepatic cholangiocarcinoma. Adjuvant therapy, which included chemotherapy, radiation, and chemoradiation, was associated with a borderline significant improvement in survival ($p = 0.06$). In patients who had undergone R1 resections, adjuvant radiation had a survival benefit ($p = 0.01$). The authors concluded that radiation therapy should be administered in margin-positive disease, but that the benefit after R0 resection was unclear.

Extrahepatic Cholangiocarcinoma The majority of the literature on radiation in cancers of the gallbladder and biliary tract is focused on adjuvant therapy for extrahepatic cholangiocarcinoma. In these studies, median survival is approximately 2 years (Table 1). Radiation was administered to the tumor bed and draining lymph nodes (see Target Definition) at a dose of 37–54 Gy in 1.8 Gy per fraction, sometimes in combination with intraoperative radiation or brachytherapy to total doses approaching 60 Gy.

Todoroki et al. [2] published a retrospective analysis of 63 patients who underwent resection of Klatskin tumors between 1976 and 1999. Forty-nine patients had R0 or R1 resections, of which 29 were treated adjuvantly with IORT, EBRT, or a combination. The 5-year survival was 33.9 % in the cohort that was treated with adjuvant radiation and 13.5 % in those who were observed ($p < 0.01$). Patients who had a combination of EBRT and IORT had better survival than those treated with either modality alone. Locoregional failure was diminished in the group that received adjuvant radiation: 20 % compared to 69 %. Initially, high toxicity rates were seen in the IORT group thought to be related to large single electron doses. These toxicities diminished after dose adjustment.

Likewise, a 2003 study by Gerhards et al. [3] suggested a survival benefit with adjuvant radiation. Ninety-one patients underwent mostly margin-positive surgical resection (86 %)

for hilar cholangiocarcinoma, of which 71 received EBRT, intraluminal radiation, or a combination. The median survival for those that received radiation was 24 months, compared to 8 months in those observed ($p < 0.01$).

Most recently, Kim et al. [4] reported on 168 patients with extrahepatic biliary tract cancer who underwent resection between 2001 and 2009, of which approximately 70 % were margin negative. Postoperative chemoradiation with EBRT and concurrent 5-fluorouracil-based chemotherapy was administered to 115 of 168 patients. After a median follow-up of 33.8 months, the median survival was 36.4 months in the adjuvant treatment group, versus 27.9 months in the observation group, which was statistically significant on univariate analysis ($p = 0.049$) and multivariate analysis ($p = 0.005$). Likewise, locoregional failure was lower in the adjuvant treatment group on univariate analysis (41.5 vs. 55.6 %, $p = 0.007$) and multivariate analysis ($p = 0.001$). Other significant poor prognostic indicators on multivariate analysis included perineural invasion, vascular invasion, poor differentiation on histology, and positive resection margin.

Other series, however, were more equivocal in regard to benefit of adjuvant radiation therapy. Sagawa et al. [5], who reported on patients with hilar cholangiocarcinoma who underwent surgical resection, did not reveal an overall survival benefit in a subset that received adjuvant radiation. Of the 69 patients reported, approximately 50 % had R0 resections. Thirty-nine patients received EBRT with or without brachytherapy, and the others were observed. After a median follow-up of 32 months, 3-year survival was 40.9 % in the adjuvant therapy group compared to 33.3 % with surgery alone ($p = 0.554$).

Population studies have not demonstrated a clear benefit from adjuvant radiotherapy. In a Surveillance, Epidemiology and End Results (SEER) analysis by Shinohara et al. [6], 4,758 patients with extrahepatic cholangiocarcinomas treated with surgery or radiation between 1998 and 2003 were assessed for overall survival. Of these patients, 28.8 % underwent surgery alone, and 14.7 % underwent surgery and radiation therapy. Although the median survival was 16 months in the surgery and radiation group compared to 9 months with surgery alone ($p < 0.0001$), this did not hold after adjusting for potential confounders. A similar SEER analysis of patients with resected extrahepatic cholangiocarcinoma, which excluded patients with less than 3 months of follow-up, demonstrated no benefit from adjuvant radiation in local or locally advanced disease [7].

Gallbladder Cancer In the case of gallbladder cancer, there are fewer studies of adjuvant radiotherapy (Table 2). Like studies in extrahepatic cholangiocarcinoma, median survival in the majority of studies was approximately 2 years. Balachandran et al. [8] published a report on 117 patients with gallbladder cancer, of which only 37 underwent

extended resections. Of the 117 patients, 73 received adjuvant chemoradiotherapy. Although no details were given regarding adjuvant chemoradiotherapy, the median survival for the adjuvant treatment group was 24 months compared to 11 months in the surgery-alone group ($p = 0.001$). Those patients who did not have extended surgical resections or had node-positive or T3 disease appeared to benefit more from adjuvant chemoradiotherapy.

A more recent study by Gold et al. [9] of 73 patients with stage I and II gallbladder cancer who underwent R0 resection reported a median survival approaching 5 years. In the 25 patients that received adjuvant chemoradiotherapy, which involved 50.4 Gy in 1.8 Gy per fraction with concurrent bolus 5-FU, the median survival was 4.8 years (vs. 4.2 years for surgery alone). Although not significant on univariate analysis ($p = 0.56$), overall survival was statistically improved with adjuvant chemoradiation on multivariate analysis, adjusting for T and N stages as well as pathologic diagnosis.

In 2008, Wang et al. [10] described a prediction model for gallbladder cancer using SEER data of 4,180 patients with resected disease, of whom 18 % received adjuvant radiation. In addition to factors such as age, histology, and stage of disease, adjuvant radiation was associated with a significant survival benefit on multivariate analysis. The median survival of those who received radiation therapy was 15 months, versus 8 months in those who did not. In the prediction model, the greatest benefit from adjuvant radiation therapy occurs in patients with T2 or node-positive disease.

1.2 Definitive Radiotherapy

In the series of definitive radiotherapy for unresectable disease, which included patients with gallbladder cancer as well as intrahepatic and extrahepatic cholangiocarcinoma (Table 3), the median survival was approximately 1 year. Although there were no direct comparison cohorts in most series, there was an improvement compared to historical data where the median survival for untreated patients with unresectable cancers of the gallbladder and biliary tract had been only 6–9 months.

Alden and Mohiuddin [11] described 48 patients with extrahepatic cholangiocarcinoma in one of the earliest reports of radiation in the unresectable setting. Of these patients, 24 were treated with radiation therapy (EBRT, brachytherapy, or combination) or chemoradiotherapy, 6 underwent resection, 7 were treated with chemotherapy alone, and 11 were untreated. The median survival of the untreated group and chemotherapy-alone group was 4 and 9 months, respectively. The median survival of the group receiving radiation was 12 months, compared to 5.5 months for the 24 patients that did not receive radiation ($p = 0.01$).

Table 2 Adjuvant radiotherapy or chemoradiotherapy for gallbladder cancer

Study	No. of patients	Radiotherapy	Chemotherapy	Median survival (months)	P value
Kresl et al. [32]	21	54 Gy EBRT	5-FU bolus	31.2	–
Czito et al. [51]	22	45 Gy EBRT $\pm$ 5.4 to 50 Gy boost (5 patients)	5-FU bolus or CI (82 % of patients)	22.8	–
Balachandran et al. [8]	44	None	None	11	0.001
	73	Yes; no details	Yes; no details	24	
Ben-David et al. [12]	14	54 Gy EBRT	Mostly 5-FU-based (54 % of patients)	23	–
Duffy et al. [52]	16	No details	Mostly 5-FU-based during radiotherapy; 8 received additional systemic therapy	23.4	0.4
	99	None	None	30.3	
Gold et al. [9]	25	50.4 Gy EBRT	5-FU bolus	4.8 years	0.56
	48	None	None	4.2 years	

Table 3 Definitive radiotherapy or chemoradiotherapy for unresectable cholangiocarcinoma

Study	No. of patients	Radiotherapy	Chemotherapy	Median survival (months)
Hayes et al. [53]	14	63.5–108.2 Gy; EBRT + ILBT	None	12.8
Alden and Mohiuddin [11]	24	46 Gy EBRT + 25 ILBT	5-FU $\pm$ adriamycin; 5-FU $\pm$ mitomycin	12
Morganti et al. [15]	20	39.6–50.4 Gy EBRT $\pm$ 30–50 Gy ILBT (12 patients)	5-FU CI days 1–4 in 19 patients	21.2
Shin et al. [54]	31	50.4 Gy EBRT $\pm$ 15 Gy ILBT (14 patients)	None	7
Crane et al. [34]	52	30–85 Gy; EBRT $\pm$ ILBT	5-FU CI in 38 patients	10
Buskirk et al. [55]	34	45–55 Gy EBRT $\pm$ ILBT (20–25 Gy; 10 pts) or IORT (15–20 Gy; 7 patients)	5-FU in 7 patients	12
Urego et al. [37]	34	49.5 Gy (median) EBRT $\pm$ ILBT (4 patients)	5-FU + INFa (27 patients)	14
Ben-David et al. [12]	52	23–86.3 Gy (median 60.2 Gy) EBRT	Mostly 5-FU-based	13.1
Habermehl et al. [13]	15	EBRT 45 Gy (median; 25.2–69 Gy); brachytherapy boost in 3 patients	Gemcitabine or FU-based chemotherapy	12.0
Polistina et al. [16]	10	SBRT 30 Gy to 80 % isodose in 3 fractions (CyberKnife)	Gemcitabine	35.5
Kopek et al. [18]	27	SBRT 45 Gy to isocenter in 3 fractions over 5–8 days	–	10.6
Momm et al. [17]	13	SFRT 32–56 Gy, in 4 Gy/fraction given 3 times a week	Gemcitabine or FU-based in 6/13 pts	33.5
Leong et al. [14]	20	EBRT 46 Gy (median) in 1.8–2.0 Gy/fraction	Cisplatin/5-FU and gemcitabine	20.4

In a retrospective study by Ben-David et al. [12], a subset of 52 patients with extrahepatic cholangiocarcinoma and gallbladder cancer had unresectable or gross residual disease and underwent radiation therapy. The median overall survival in this group was 13.1 months, similar to the study by Alden and Mohiuddin [11]. More recent studies have reported similar median survival times with chemoradiation in the unresectable setting [13, 14].

Long-term survival has also been reported with definitive radiotherapy. In a cohort of 20 patients who received EBRT for extrahepatic cholangiocarcinoma or gallbladder cancer reported by Morganti et al. [15], 2 patients survived beyond 5 years. The majority of patients also received concurrent chemotherapy (5-fluorouracil) and intraluminal brachytherapy.

Studies using hypofractionated or stereotactic body radiotherapy reported promising results, achieving median

survival exceeding 30 months in the unresectable setting [16–18] (Table 3). However, these studies report only a limited number of patients and toxicity, at least in some, has been high. This approach requires further investigation. This will be discussed in more detail in the next chapter entitled [Emerging Techniques in Image-Guided Radiation Therapy and Stereotactic Body Radiation Therapy](#).

2 Radiation Technique

The vast majority of the studies supporting the use of radiation therapy in cancers of the gallbladder and biliary tract used 3D-conformal technique. In this chapter, we will discuss this technique in detail including target definition, organs at risk (OARs), and dose selection. We will then introduce the use of intensity-modulated RT (IMRT) and proton beam radiotherapy in the treatment for gallbladder and biliary tract cancers.

2.1 3D-Conformal Radiotherapy

External beam radiotherapy using 3D-conformal technique relies on cross-sectional imaging and three-dimensional reconstruction to define target structures and OARs. Current radiation planning systems use CT-based imaging. Simulation CT scans are obtained with intravenous and oral contrast to (1) delineate vasculature structures around which nodal basins are defined, (2) identify gross tumor volume (GTV), and (3) delineate liver, stomach, duodenum, and small bowel. Preferably, 4D-CT scans are obtained to assess tumor and organ motion. Magnetic resonance imaging (MRI) may also be obtained at time of simulation to assist in target delineation. Hepatobiliary structures are better visualized on MR-based imaging. In particular, the extent of tumor and nodal involvement, proximity to biliary and vascular structures, and proximity to small bowel are better defined on MRI.

Target structures include the GTV, which is defined by gross disease on imaging, and clinical target volume (CTV), which includes the GTV as well as any potential microscopic disease. Finally, dose is prescribed to a planning target volume (PTV), which is the CTV plus an additional margin (typically 1 to 1.5 cm) to account for motion and setup uncertainty. When an internal tumor volume (ITV) is generated incorporating motion as determined by 4D-CT planning, the PTV will only consist of setup uncertainty (0.5 mm in all directions, when daily image guidance is used).

In the adjuvant setting, appropriate postoperative healing should occur prior to starting radiation therapy. A rule of thumb is to begin radiation planning approximately 4 weeks

after surgery. In the adjuvant or definitive setting, radiation may be administered concurrently with chemotherapy, or sequentially. One common approach is to begin with systemic chemotherapy and follow with chemoradiotherapy. The initial preradiation chemotherapy allows for the selection of patients who do not have early distant failure, akin to approaches used in pancreatic cancer [19].

Target Definition Target definition depends primarily on two factors: (1) goals of treatment and (2) disease failure patterns. In the definitive setting, the aim is to eradicate all gross and microscopic locoregional disease. Disease failure patterns dictate the extent of regional nodal irradiation.

In the adjuvant setting, gross disease is not present unless the resection was grossly incomplete. Thus, the CTV is defined by the surgical bed and the lymphatic drainage basin. The surgical bed is best defined by careful examination of the operative report and may be highlighted on imaging by radio-opaque clips or staples left by the surgeon. It is important to note that normal anatomic relationships may be disrupted postoperatively.

For hilar cholangiocarcinoma, surgery requires resection of the involved extrahepatic biliary structures and adjacent hepatic parenchyma. Thus, the surgical bed follows along the medial aspect of the remaining liver, within a reasonable radius around the surgical clips. Reconstruction of the tumor and surgical bed on the simulation scan is difficult because of the major change in anatomy and the considerable deformation typically encountered. Careful study of the preoperative scans and detailed discussion with the surgeon are critical. Any gross residual disease on imaging is the GTV and should be delineated separately and included within the CTV.

For distal extrahepatic cholangiocarcinoma, resection consists of the involved extrahepatic biliary ducts, with or without a pancreaticoduodenectomy. The postoperative bed is centered on the new anastomosis between the biliary tree and small bowel, and guided by surgical clips and an appropriate margin. For gallbladder cancer, surgery requires radical or extended cholecystectomy, which involves resection of a margin of hepatic tissue around the gallbladder. This relates to the tendency of gallbladder cancers to infiltrate through Rokitansky–Aschoff sinuses and the gallbladder wall into adjacent hepatic tissue. Thus, the tumor bed includes a rim of hepatic tissue in the space previously occupied by the gallbladder (as indicated by surgical clips). In each case, the CTV encompasses the tumor bed.

In the adjuvant setting, the regional lymph nodes are included in the CTV. Compared to intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinomas have higher rates of lymph node metastasis [20, 21]. This pattern of lymphatic drainage for extrahepatic and hilar cholangiocarcinomas has been described in a study using blue dye technique [22]. The first site of drainage is the pericholedochal lymph node station. The lymphatic drainage then

descends along the portal vein into the surrounding nodes, along the common hepatic artery into the surrounding nodes, or along the biliary tree to the pancreaticoduodenal node station. Notably, lymph flow does not ascend toward the hepatic hilum. The tertiary nodal stations include the nodes surrounding the celiac axis and superior mesenteric artery as well as the aortocaval nodes.

This flow pattern is supported by clinical studies. In a study by Kitagawa et al. [23], 110 patients underwent lymph node dissection in addition to surgical resection, of which 52 % of patients had nodal disease. The pericholedochal lymph node group was the most frequent site of lymph node metastasis (42 %), followed by the nodes along the portal vein (31 %), nodes along the common hepatic artery (27 %), and the pancreaticoduodenal nodes (15 %). Another study examined failure patterns based on imaging in 76 patients with hilar cholangiocarcinoma who had undergone resection [20]. Of the 52 patients who had disease recurrence, 59 % failed with isolated locoregional disease. Sites of local recurrence included the hepatic resection margin (12/59), porta hepatis (7/59), and bilioenteric anastomosis (5/59), while others recurred regionally in retroperitoneal lymph nodes (14/59).

The CTV for extrahepatic and hilar cholangiocarcinoma therefore includes the pericholedochal lymph nodes. For hilar cholangiocarcinomas, these nodes are within the hepatic hilum and porta hepatis. The CTV also extends to a 1-cm margin around the portal vein from the hepatic hilum to its junction with superior mesenteric and splenic veins to include the surrounding nodes. To encompass the pancreaticoduodenal nodes, the CTV will also include the area surrounding the groove between the pancreatic head and duodenum and, in particular, its posterior aspect. The celiac trunk and the proximal superior mesenteric artery, typically with a 1-cm margin, are also within the CTV to include corresponding lymph nodes.

For intrahepatic cholangiocarcinoma, the lymphatic drainage is similar with a few exceptions. Because the tumor originates intrahepatically, the first echelon pericholedochal lymph nodes lie within hepatic tissue. These nodes lie within the tumor bed volume and corresponding CTV. Subsequent lymph drainage occurs in a pattern similar to extrahepatic cholangiocarcinoma: to nodes in the hepatic hilum, along the common hepatic artery, to retropancreaticoduodenal region, the celiac axis, and the root of the superior mesenteric artery. These findings were confirmed in study of 39 patients who had undergone surgical resection as well as radical lymph node dissection for intrahepatic cholangiocarcinoma [24]. The study also found that intrahepatic cholangiocarcinoma in the left peripheral biliary tract also spreads to the left gastric nodes along the lesser curvature of the stomach. However, for intrahepatic cholangiocarcinoma, prophylactic irradiation of the regional

lymph node basin is more limited and typically does not include second echelon lymphatics.

Gallbladder carcinoma likely has a lower rate of isolated locoregional recurrence compared to hilar cholangiocarcinoma. In a study of 80 patients with gallbladder carcinoma after surgical resection, only 8 patients had isolated locoregional recurrence at a median follow-up of 24 months [20]. However, there are no confirmatory reports of this finding and other series suggested a local control and possibly a survival benefit with adjuvant chemoradiotherapy. When such therapy is administered, the CTV typically includes regional nodes. The nodal areas are those described above.

For unresectable disease, the benefit of radiotherapy is unknown. Radiation therapy, though not curative, is administered to decrease tumor size, slow progression, prevent local complications, and perhaps prolong survival. For intrahepatic tumors, the CTV includes gross disease with a margin for microscopic extension. For extrahepatic tumors, the regional nodes have typically also been included in the CTV. However, given the pattern of failure (primarily within the PTV of the primary), the rationale for this practice is questionable.

Organs at Risk Ultimately, target dose is limited by the dose to OARs. The primary OARs in the hepatobiliary region include the liver, small bowel, and ipsilateral kidney. The acute toxicities arising from radiation include nausea and vomiting, abdominal pain, and fatigue. Subacute and late toxicities can occur in the liver and gut. Radiation-induced liver disease, which pathophysiologically resembles venoocclusive disease, can occur between 4–6 weeks and 3–4 months postradiation. The incidence of RILD is related to the mean dose of radiation to the liver and volume of normal liver spared from radiation [25, 26]. In the absence of underlying cirrhosis, mean liver dose is limited to 30 Gy.

Patients with preexisting liver conditions (e.g., cirrhosis) are also at risk for liver failure. Unlike hepatocellular carcinoma, the majority of patients with cancers of the gallbladder and biliary tree do not have underlying cirrhosis. Cirrhotic livers are more sensitive to radiation injury and require more stringent constraints [27].

Stomach and small bowel are also at risk for radiation injury, including ulceration, bleeding and perforation and obstruction. Difficulty meeting the dose–volume constraints of these organs (Table 4) is naturally more common when treating hilar and distal extrahepatic cholangiocarcinomas.

Dose There are limited data to guide dose selection in radiation therapy of cancers of the gallbladder and bile ducts. Most series support radiation doses consistent with other tumors of the gastrointestinal tract because of shared OARs. The use of concurrent chemotherapy should also be considered in dose determination.

For extrahepatic and hilar cholangiocarcinoma in the adjuvant setting, most studies using external beam

Table 4 Dose constraints for organs at risk

Organ at risk	Maximum tolerated dose	Volume constraint	Mean dose constraint
Liver		700 cc less than 15 Gy	<30 Gy
Stomach		95 % less than 50 Gy	
		75 % less than 45 Gy	
Small bowel	60 Gy	98 % less than 55 Gy	
		75 % less than 45 Gy	
Duodenum	60 Gy	95 % less than 50 Gy	
		66 % less than 45 Gy	
Large bowel	60 Gy	95 % less than 55 Gy	
Kidney, right		50 % less than 18 Gy	
		25 % less than 20 Gy	
Kidney, left		50 % less than 10 Gy	
		25 % less than 15 Gy	
Spinal cord	45 Gy		
Gallbladder	60 Gy	75 % less than 55 Gy	

radiotherapy alone report median doses ranging from 45 to 54 Gy in 1.8–2.0 Gy per fraction [4, 12, 28–31]. One acceptable approach is to treat the entire CTV, including the tumor bed and lymph node basin, to one dose (e.g., 45 Gy) and administer an additional dose (e.g., 9–14 Gy) to the tumor bed. This is based on the higher risk for residual disease at the site of the primary lesion and allows for compliance with normal tissue constraints (e.g., bowel). Similar doses were utilized in studies of adjuvant radiation in gallbladder cancers [9, 12, 32].

In the unresectable or definitive setting, for typical fractionated radiotherapy, most recent studies have reported similar doses—45–60 Gy in standard 1.8–2.0 Gy per fraction [12–14, 33]. Crane et al. [34] reported that the first site of local failure in a cohort of 52 patients with unresectable cholangiocarcinoma treated with definitive radiotherapy (with or without chemotherapy) was local in 72 % of cases. Some studies of conventionally fractionated radiotherapy have shown a correlation between dose delivered and survival outcomes for hepatic malignancies [34–36]. In a study conducted by Alden and Mohiuddin [11], radiation doses greater than 55 Gy in the definitive setting were associated with improved survival in patients with extrahepatic cholangiocarcinoma. Others did not find an association between dose and survival [34, 37]. Given the pattern of failure and the stated goal of delaying progression for as long as possible, a reasonable approach would be to deliver as high a dose as safely possible given the OAR constraints discussed above.

A number of groups have investigated the use of stereotactic fractionated (SFRT) or stereotactic body radiotherapy (SBRT)—a highly conformal technique that allows delivery of high biologically effective dose to the tumor by delivering increased dose per fraction.

Momm et al. [17] reported their experience of 13 patients with unresectable Klatskin tumors treated with SFRT—32–56 Gy in 4 Gy per fraction three times a week. They reported a median progression-free survival of 32.5 months. Adverse events included one grade 3 toxicity (nausea) as well as infectious cholangitis in 5 of 13 patients. A few groups have reported variable experience using SBRT for unresectable cholangiocarcinoma. One study used a dose of 30 Gy in 3 fractions and reported median time to progression of 30 months [16]. Another study used 45 Gy in 3 fractions, reported only 7-month median progression-free survival, and reported a significant rate of late duodenal toxicity in the 27 patients treated (6 with ulceration, 3 with stenosis) [18]. Given this toxicity, we believe that the use of SFRT and SBRT remains investigational in this setting.

2.2 Intensity-Modulated Radiation Therapy

IMRT typically refers to inverse radiation planning based on dose-volume goals and constraints to target and normal structures. After target goals and OAR constraints are defined, multiple radiation fields are placed. Planning software then optimizes the radiation dose distribution by varying the intensity of multiple beamlets within each beam. The technique results in dose distributions that tightly conform to the shape of the target.

Compared to 3D-Conformal radiation, IMRT allows sparing of high dose to nearby critical structures (Fig. 1). For hepatobiliary radiation, this is advantageous because it limits high doses to the spinal cord, kidneys, bowel, and liver. Because of the steep dose gradient, higher radiation doses may be delivered to the target [38]. However, inherently, this technique delivers a higher low-to-intermediate dose to

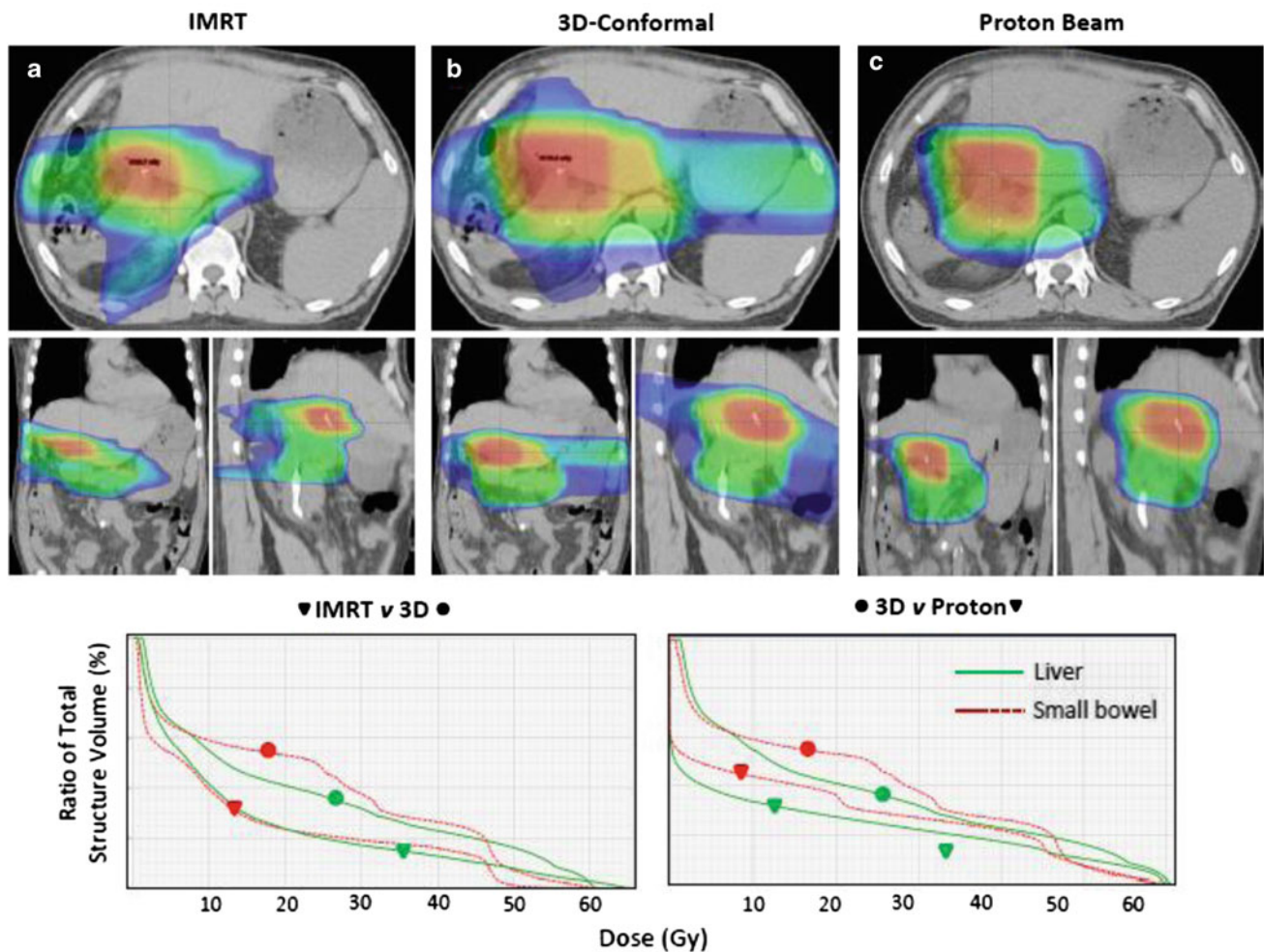


Fig. 1 Dose distribution for postoperative treatment for extrahepatic cholangiocarcinoma using **a** IMRT, **b** 3D-conformal therapy with four fields, and **c** proton radiotherapy with two fields. Axial (*top panel*), coronal, and sagittal (*middle panel*) views are shown. A dose gradient

of 25 Gy (*blue*) to 64 Gy (*red*) is shown. In the *bottom panel*, dose-volume histograms comparing 3D with IMRT (*left*) and proton therapy (*right*) show sparing of liver and small bowel with IMRT or proton radiotherapy

normal tissues around the target (but not immediately adjacent to it).

IMRT is widely used in pancreatic tumors [39], which have similar anatomic considerations to hepatobiliary malignancies, and consensus guidelines in pancreatic cancer recommend either 3D-conformal technique or IMRT [40]. A number of studies have demonstrated the dosimetric benefits of IMRT compared to 3D-conformal technique in pancreatic and bile duct malignancies. In pancreatic cancer, there is emerging prospective data, suggesting that high-dose radiotherapy delivered with IMRT may improve survival and local control [41]. Although no such data exist for cholangiocarcinoma, existing evidence does suggest that the use of IMRT in bile duct malignancies improves the rate of acute and/or late toxicity [42, 43].

Fuller et al. [44] reported on the use of IMRT (with ultrasound image guidance) for both gallbladder carcinoma and biliary adenocarcinoma. For 10 patients with gallbladder

carcinoma, adjuvant or definitive IMRT achieved a median dose of 59 Gy, while limiting mean liver dose to 28.8 Gy, mean right kidney dose to 14.3, and mean spinal cord dose to 10.6 (all median values). Another series of 24 patients treated with IMRT for biliary tract cancers (extrahepatic cholangiocarcinoma and gallbladder cancer) were compared to a similar cohort of 24 patients treated with 3D-conformal technique [45]. In the IMRT group, median target dose was 59 Gy, compared to 48 Gy in the 3D-conformal group. No significant differences were noted in clinical toxicity between the two modalities.

2.3 Proton Beam Radiotherapy

Proton beam radiotherapy, a form of heavy-charged-particle therapy, does not have a clinically proven benefit for cancers of the gallbladder and biliary tract at this time. Because of its

unique physical properties, protons have a finite penetration within tissue and deliver the majority of their energy at narrow depth window within tissue (i.e., Bragg peak). Historically, proton beam radiotherapy was limited to malignancies adjacent to critical structures (e.g., spinal cord) and pediatric malignancies. However, as the ability to deliver proton beam radiotherapy has advanced with isocentric gantry systems, its potential use in other malignancies has broadened.

In hepatic tumors, where restricting integral liver dose is critical, proton beam radiotherapy is of particular interest. Compared to a high-energy photon beam (e.g., 15 MV), each modulated proton beam delivers less radiation to normal surrounding liver in its path to the target and past the target. Dosimetric studies of proton beam radiotherapy in hepatobiliary malignancies have suggested an advantage in achieving maximal target coverage while limiting dose to nearby OARs (Fig. 1).

In a dosimetric analysis of four pancreatic or biliary large-volume treatment plans, proton beam radiotherapy (using “spot-scanning” technique) achieved target coverage while meeting dose constraints in all 4 patients [46]. IMRT plans with 9 fields were not able to simultaneously achieve large-volume target coverage and meet dose constraints in the same 4 patients. Likewise, in a dosimetric comparison between photon and proton plans in 9 patients with liver tumors, proton plans spared more liver of doses ≥ 30 Gy, decreased the mean liver dose, and reduced the volume of high-dose radiation to the stomach, duodenum, heart, and spinal cord [47]. And by limiting the number of beams from the contralateral side of the patient, the left kidney is better spared with proton radiotherapy [48].

The conformality of proton beam radiotherapy depends on the delivery technique. Less-conformal delivery techniques such as double scatter and uniform scanning do not achieve the same conformality as spot-scanning or pencil beam proton beam radiotherapy. Limitations of proton beam radiotherapy include range uncertainties related to tissue inhomogeneity and dose distribution at the distal edge of the Bragg peak.

Although clinical data have shown the feasibility of proton beam radiotherapy for hepatobiliary malignancies, there are no clinical data to suggest that proton beam radiotherapy is superior to photon-based treatments in terms of disease control or toxicity. There is currently an ongoing phase II trial to assess local control and safety of the use of proton beam radiotherapy in hepatocellular carcinoma and intrahepatic cholangiocarcinoma at the Massachusetts General Hospital (MGH), University of Pennsylvania, and MD Anderson Cancer Center.

3 Conclusion

Despite the lack of prospective data, the retrospective literature on the effects of radiation on cancers of the biliary tract suggests a benefit in the adjuvant and definitive setting in terms of local control and perhaps survival. Multiple external beam radiation technologies are now available to deliver target doses safely, including 3D-conformal, intensity-modulated, and proton beam radiotherapy. SBRT has been associated with a high rate of complications and needs further development in clinical trials. Although head-to-head comparisons of these modalities are unlikely, prospective clinical data on efficacy and toxicity will guide modality selection. In the meantime, assessment of each individual tumor and its relationship to surrounding structures, as well as the clinical context, is critical to the selection of target, dose, and technology.

References

1. Horgan AM, Amir E, Walter T, Knox JJ (2012) Adjuvant therapy in the treatment of biliary tract cancer: a systematic review and meta-analysis. *J Clinical Oncol* 30(16):1934–1940
2. Todoroki T, Ohara K, Kawamoto T, Koike N, Yoshida S, Kashiwagi H, Otsuka M, Fukao K (2000) Benefits of adjuvant radiotherapy after radical resection of locally advanced main hepatic duct carcinoma. *Int J Radiat Oncol Biol Phys* 46(3):581–587
3. Gerhards MF, Van Gulik TM, González González D, Rauws EaJ, Gouma DJ (2003) Results of postoperative radiotherapy for resectable hilar cholangiocarcinoma. *World J Surg* 27(2):173–179
4. Kim TH, Han S-S, Park S-J et al (2011) Role of adjuvant chemoradiotherapy for resected extrahepatic biliary tract cancer. *Int J Radiat Oncol Biol Phys* 81(5):e853–e859
5. Sagawa N, Kondo S, Morikawa T, Okushiba S, Katoh H (2005) Effectiveness of radiation therapy after surgery for hilar cholangiocarcinoma. *Surg Today* 35(7):548–552
6. Shinohara ET, Mitra N, Guo M, Metz JM (2009) Radiotherapy is associated with improved survival in adjuvant and palliative treatment of extrahepatic cholangiocarcinomas. *Int J Radiat Oncol Biol Phys* 74(4):1191–1198
7. Vern-Gross TZ, Shivanli AT, Chen K, Lee CM, Tward JD, MacDonald OK, Crane CH, Talamonti MS, Munoz LL, Small W (2011) Survival outcomes in resected extrahepatic cholangiocarcinoma: effect of adjuvant radiotherapy in a surveillance, epidemiology, and end results analysis. *Int J Radiat Oncol Biol Phys* 81(1):189–198
8. Balachandran P, Agarwal S, Krishnani N, Pandey CM, Kumar A, Sikora SS, Saxena R, Kapoor VK (2006) Predictors of long-term survival in patients with gallbladder cancer. *J Gastrointest Surg* 10(6):848–854
9. Gold DG, Miller RC, Haddock MG, Gunderson LL, Quevedo F, Donohue JH, Bhatia S, Nagorney DM (2009) Adjuvant therapy for

- gallbladder carcinoma: the Mayo Clinic experience. *Int J Radiat Oncol Biol Phys* 75(1):150–155
10. Wang SJ, Fuller CD, Kim J-S, Sittig DF, Thomas CR, Ravdin PM (2008) Prediction model for estimating the survival benefit of adjuvant radiotherapy for gallbladder cancer. *J Clin Oncol* 26(13):2112–2117
 11. Alden ME, Mohiuddin M (1994) The impact of radiation dose in combined external beam and intraluminal IR-192 brachytherapy for bile duct cancer. *Int J Radiat Oncol Biol Phys* 28(4):945–951
 12. Ben-David MA, Griffith KA, Abu-Isa E, Lawrence TS, Knol J, Zalupski M, Ben-Josef E (2006) External-beam radiotherapy for localized extrahepatic cholangiocarcinoma. *Int J Radiat Oncol Biol Phys* 66(3):772–779
 13. Habermehl D, Lindel K, Rieken S, Haase K, Goeppert B, Büchler M, Schirmacher P, Welzel T, Debus J, Combs S (2012) Chemoradiation in patients with unresectable extrahepatic and hilar cholangiocarcinoma or at high risk for disease recurrence after resection: analysis of treatment efficacy and failure in patients receiving postoperative or primary chemoradiation. *Strahlenther Onkol* 188(9):795–801
 14. Leong E, Chen WW, Ng E, Van Hazel G, Mitchell A, Spry N (2012) Outcomes from combined chemoradiotherapy in unresectable and locally advanced resected cholangiocarcinoma. *J Gastrointest Cancer* 43(1):50–55
 15. Morganti AG, Trodella L, Valentini V et al (2000) Combined modality treatment in unresectable extrahepatic biliary carcinoma. *Int J Radiat Oncol Biol Phys* 46(4):913–919
 16. Polistina FA, Guglielmi R, Baiocchi C, Francescon P, Scalchi P, Febbraro A, Costantin G, Ambrosino G (2011) Chemoradiation treatment with gemcitabine plus stereotactic body radiotherapy for unresectable, non-metastatic, locally advanced hilar cholangiocarcinoma. Results of a five year experience. *Radiother Oncol* 99(2):120–123
 17. Momm F, Schubert E, Henne K, Hodapp N, Frommhold H, Harder J, Grosu A-L, Becker G (2010) Stereotactic fractionated radiotherapy for Klatskin tumours. *Radiother Oncol* 95(1):99–102
 18. Kopeck N, Holt MI, Hansen AT, Høyer M (2010) Stereotactic body radiotherapy for unresectable cholangiocarcinoma. *Radiother Oncol* 94(1):47–52
 19. Huguet F, André T, Hammel P et al (2007) Impact of chemoradiotherapy after disease control with chemotherapy in locally advanced pancreatic adenocarcinoma in GERCOR phase II and III studies. *J Clin Oncol* 25(3):326–331
 20. Jarnagin WR, Luo L, Little SA, Klimstra D, D'Angelica M, DeMatteo RP, Wagman R, Blumgart LH, Fong Y (2003) Patterns of initial disease recurrence after resection of gallbladder carcinoma and hilar cholangiocarcinoma: implications for adjuvant therapeutic strategies. *Cancer* 98(8):1689–1700
 21. Jung SJ, Woo SM, Park HK et al (2012) Patterns of initial disease recurrence after resection of biliary tract cancer. *Oncology* 83(2):83–90
 22. Shirai Y, Yoshida K, Tsukada K, Ohtani T, Muto T (1992) Identification of the regional lymphatic system of the gallbladder by vital staining. *Brit J Surg* 79(7):659–662
 23. Kitagawa Y, Nagino M, Kamiya J, Uesaka K, Sano T (2001) Lymph node metastasis from hilar cholangiocarcinoma: audit of 110 patients who underwent regional and paraaortic node dissection. *Ann Surg* 233(3):385–392
 24. Tsuji T, Hiraoka T, Kanemitsu K, Takamori H, Tanabe D, Tashiro S (2001) Lymphatic spreading pattern of intrahepatic cholangiocarcinoma. *Surgery* 129(4):401–407
 25. Lawrence TS, Ten Haken RK, Kessler ML, Robertson JM, Lyman JT, Lavigne ML, Brown MB, DuRoss DJ, Andrews JC, Ensminger WD (1992) The use of 3-D dose volume analysis to predict radiation hepatitis. *Int J Radiat Oncol Biol Phys* 23(4):781–788
 26. Dawson LA, Normolle D, Balter JM, McGinn CJ, Lawrence TS, Ten Haken RK (2002) Analysis of radiation-induced liver disease using the Lyman NTCP model. *Int J Radiat Oncol Biol Phys* 53(4):810–821
 27. Cheng JC-H, Wu J-K, Lee PC-T, Liu H-S, Jian JJ-M, Lin Y-M, Sung J-L, Jan G-J (2004) Biologic susceptibility of hepatocellular carcinoma patients treated with radiotherapy to radiation-induced liver disease. *Int J Radiat Oncol Biol Phys* 60(5):1502–1509
 28. Schoenthaler R, Phillips TL, Castro J, Efrid JT, Better A, Way LW (1994) Carcinoma of the extrahepatic bile ducts. The University of California at San Francisco experience. *Ann Surg* 219(3):267–274
 29. Kim S, Kim SW, Bang YJ, Heo D-S, Ha SW (2002) Role of postoperative radiotherapy in the management of extrahepatic bile duct cancer. *Int J Radiat Oncol Biol Phys* 54(2):414–419
 30. Hughes MA, Frassica DA, Yeo CJ, Riall TS, Lillemoe KD, Cameron JL, Donehower RC, Laheru DA, Hruban RH, Abrams RA (2007) Adjuvant concurrent chemoradiation for adenocarcinoma of the distal common bile duct. *Int J Radiat Oncol Biol Phys* 68(1):178–182
 31. Nelson JW, Ghafoori AP, Willett CG et al (2009) Concurrent chemoradiotherapy in resected extrahepatic cholangiocarcinoma. *Int J Radiat Oncol Biol Phys* 73(1):148–153
 32. Kresl JJ, Schild SE, Henning GT, Gunderson LL, Donohue J, Pitot H, Haddock MG, Nagorney D (2002) Adjuvant external beam radiation therapy with concurrent chemotherapy in the management of gallbladder carcinoma. *Int J Radiat Oncol Biol Phys* 52(1):167–175
 33. Chen Y-X, Zeng Z-C, Tang Z-Y, Fan J, Zhou J, Jiang W, Zeng M-S, Tan Y-S (2010) Determining the role of external beam radiotherapy in unresectable intrahepatic cholangiocarcinoma: a retrospective analysis of 84 patients. *BMC Cancer* 10:492
 34. Crane CH, Macdonald KO, Vauthey JN, Yehuda P, Brown T, Curley S, Wong A, Delclos M, Charnsangavej C, Janjan NA (2002) Limitations of conventional doses of chemoradiation for unresectable biliary cancer. *Int J Radiat Oncol Biol Phys* 53(4):969–974
 35. Ben-Josef E, Normolle D, Ensminger WD, Walker S, Tatro D, Ten Haken RK, Knol J, Dawson LA, Pan C, Lawrence TS (2005) Phase II trial of high-dose conformal radiation therapy with concurrent hepatic artery floxuridine for unresectable intrahepatic malignancies. *J Clin Oncol* 23(34):8739–8747
 36. Dawson LA, McGinn CJ, Normolle D, Ten Haken RK, Walker S, Ensminger W, Lawrence TS (2000) Escalated focal liver radiation and concurrent hepatic artery fluorodeoxyuridine for unresectable intrahepatic malignancies. *J Clin Oncol* 18(11):2210–2218
 37. Urege M, Flickinger JC, Carr BI (1999) Radiotherapy and multimodality management of cholangiocarcinoma. *Int J Radiat Oncol Biol Phys* 44(1):121–126
 38. Eccles CL, Bissonnette J-P, Craig T, Taremi M, Wu X, Dawson LA (2008) Treatment planning study to determine potential benefit of intensity-modulated radiotherapy versus conformal radiotherapy for unresectable hepatic malignancies. *Int J Radiat Oncol Biol Phys* 72(2):582–588
 39. Ben-Josef E, Shields AF, Vaishampayan U, Vaitkevicius V, El-Rayes BF, McDermott P, Burmeister J, Bossenberger T, Philip PA (2004) Intensity-modulated radiotherapy (IMRT) and concurrent capecitabine for pancreatic cancer. *Int J Radiat Oncol Biol Phys* 59(2):454–459
 40. Huguet F, Goodman KA, Azria D, Racadot S, Abrams RA (2012) Radiotherapy technical considerations in the management of locally advanced pancreatic cancer: American–French consensus recommendations. *Int J Radiat Oncol Biol Phys* 83(5):1355–1364
 41. Ben-Josef E, Schipper M, Francis IR et al (2012) A phase I/II trial of intensity modulated radiation (IMRT) dose escalation with concurrent fixed-dose rate gemcitabine (FDR-G) in patients with

- unresectable pancreatic cancer. *Int J Radiat Oncol Biol Phys* 84(5):1166–1171
42. Milano MT, Chmura SJ, Garofalo MC, Rash C, Roeske JC, Connell PP, Kwon O-H, Jani AB, Heimann R (2004) Intensity-modulated radiotherapy in treatment of pancreatic and bile duct malignancies: toxicity and clinical outcome. *Int J Radiat Oncol Biol Phys* 59(2):445–453
43. Yovino S, Poppe M, Jabbour S, David V, Garofalo M, Pandya N, Alexander R, Hanna N, Regine WF (2011) Intensity-modulated radiation therapy significantly improves acute gastrointestinal toxicity in pancreatic and ampullary cancers. *Int J Radiat Oncol Biol Phys* 79(1):158–162
44. Fuller CD, Thomas CR, Wong A, Cavanaugh SX, Salter BJ, Herman TS, Fuss M (2006) Image-guided intensity-modulated radiation therapy for gallbladder carcinoma. *Radiother Oncol* 81(1):65–72
45. Fuller CD, Dang ND, Wang SJ, Desai P, Choi M, Thomas CR, Fuss M (2009) Image-guided intensity-modulated radiotherapy (IG-IMRT) for biliary adenocarcinomas: initial clinical results. *Radiother Oncol* 92(2):249–254
46. Zurlo A, Lomax A, Hoess A, Bortfeld T, Russo M, Goitein G, Valentini V, Marucci L, Capparella R, Loasses A (2000) The role of proton therapy in the treatment of large irradiation volumes: a comparative planning study of pancreatic and biliary tumors. *Int J Radiat Oncol Biol Phys* 48(1):277–288
47. Wang X, Krishnan S, Zhang X, Dong L, Briere T, Crane CH, Martel M, Gillin M, Mohan R, Beddar S (2008) Proton radiotherapy for liver tumors: dosimetric advantages over photon plans. *Med Dosim* 33(4):259–267
48. Skinner HD, Hong TS, Krishnan S (2011) Charged-particle therapy for hepatocellular carcinoma. *Semin Radiat Oncol* 21(4):278–286
49. Pitt HA, Nakeeb A, Abrams RA, Coleman J, Piantadosi S, Yeo CJ, Lillemoe KD, Cameron JL (1995) Perihilar cholangiocarcinoma: postoperative radiotherapy does not improve survival. *Ann Surg* 221(6):788–797
50. Nakeeb A, Tran KQ, Black MJ et al (2002) Improved survival in resected biliary malignancies. *Surgery* 132(4):555–563 (discussion 563–564)
51. Czito BG, Hurwitz HI, Clough RW, Tyler DS, Morse MA, Clary BM, Pappas TN, Fernando NH, Willett CG (2005) Adjuvant external-beam radiotherapy with concurrent chemotherapy after resection of primary gallbladder carcinoma: a 23-year experience. *Int J Radiat Oncol Biol Phys* 62(4):1030–1034
52. Duffy A, Capanu M, Abou-Alfa G, Huitzil D, Jarnagin W, Fong Y, D'Angelica M, Dematteo R, Blumgart L, O'Reilly E (2008) Gallbladder cancer (GBC): 10-year experience at Memorial Sloan-Kettering Cancer Centre (MSKCC). *J Surg Oncol* 98(7):485–489
53. Hayes J, Sapozink M, Miller F (1988) Definitive radiation therapy in bile duct carcinoma. *Int J Radiat Oncol Biol Phys* 15(3):735–744
54. Shin HS, Seong J, Kim WC, Lee HS, Moon SR, Lee IJ, Lee KK, Park KR, Suh CO, Kim GE (2003) Combination of external beam irradiation and high-dose-rate intraluminal brachytherapy for inoperable carcinoma of the extrahepatic bile ducts. *Int J Radiat Oncol Biol Phys* 57(1):105–112
55. Buskirk SJ, Gunderson LL, Schild SE, Bender CE, Williams HJ, McIlrath DC, Robinow JS, Tremaine WJ, Martin JK (1992) Analysis of failure after curative irradiation of extrahepatic bile duct carcinoma. *Ann Surg* 215(2):125–131

Brachytherapy in Hepatobiliary Malignancies

Subir Nag, L. Matthew Scala, and Andrew S. Kennedy

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Abstract

Brachytherapy (placement of radioactive material inside or close to tumors) is a very useful modality in the treatment of unresectable liver tumors. Solitary or a limited number of localized tumors can be treated with interstitial permanent ¹²⁵I seeds, HDR brachytherapy, or intraluminal ¹⁹²Ir to the unresected tumor or to the tumor bed after surgical resection. Diffuse or multiple primary or metastatic liver tumors can be palliated with ⁹⁰Y glass or resin microsphere radioembolization. These benefits are more when the radioembolization is done early in the treatment program or combined with appropriate chemotherapy. The role of these therapies must be investigated further in controlled clinical trials to integrate and quantify the benefit when combined with other therapies.

1 Introduction

It is estimated that over two-thirds of all cancer patients will receive radiation therapy at some point over the course of their disease. Many of these treatments will be delivered using brachytherapy, a highly conformal method of delivering high doses of radiation to tumor targets while sparing surrounding healthy tissue. While the primary treatment of hepatobiliary malignancy is surgical, brachytherapy has been used with success in both the primary treatment of unresectable patients and the palliation of those with recurrent disease. In fact, brachytherapy techniques can be safely used in medically inoperable patients as well as those found to be unresectable by virtue of the extent of their disease.

Brachytherapy differs from external beam radiotherapy (EBRT) in several fundamental ways. Typically, EBRT is delivered via a linear accelerator that generates high-energy X-rays. While some sparing of nontarget tissue is possible, there is inevitable deposition of radiation dose both proximal and distal to the target. Conversely, with brachytherapy,

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radioactive sources are implanted either directly in or next to target tumors. Due to the physical properties of brachytherapy sources, which are beyond the scope of this chapter, this results in highly conformal irradiation of the target tumor while allowing a high degree of normal tissue sparing.

Brachytherapy sources can be placed within the lumen of a visceral organ such as the bronchus or esophagus (intraluminal brachytherapy), within a preexisting body cavity such as the vagina (intracavitary brachytherapy), on the body surface as with skin cancer (surface brachytherapy), or directly within tissue or tumor such as with prostate, breast, and head and neck tumors (interstitial brachytherapy). Brachytherapy sources can also be placed during open surgical procedures with direct visualization of tumor (intraoperative brachytherapy) or, more commonly, through a percutaneous approach with image guidance such as fluoroscopy, ultrasound, computed tomography, or magnetic resonance imaging. In contradistinction to the methods mentioned above, there is also experience with unsealed source brachytherapy. Examples include the administration of bone-seeking isotopes such as samarium-153 or strontium-89 for the treatment of diffuse bony metastatic disease, radioimmunotherapy conjugates for relapsed and refractory lymphoma, and radioactive microparticles.

The decision to use brachytherapy must be a balance between the clinical status of the patient, disease characteristics, goals of care, and procedural risks. In general, brachytherapy is best suited for small tumors situated close to radiosensitive structures where it would be difficult to deliver high doses of EBRT.

Brachytherapy does have drawbacks compared to traditional EBRT. For example, procedural risks such as infection and bleeding are almost nonexistent when using EBRT. Additionally, brachytherapy techniques are not well suited for large treatment areas such as lymph node fields or very bulky tumors. Also, due to a high degree of dose inhomogeneity, care must be taken to avoid ultrahigh-dose regions within a target that may overlap with sensitive structures, such as the urethra as it runs centrally through the prostate gland.

The value of brachytherapy in treating hepatobiliary malignancies is evident as the liver is generally radiosensitive. Most authors accept that the overall tolerance of the whole liver to radiation is approximately 30 gray (Gy) [1]. Doses to the whole organ above this level can result in radiation-induced liver disease—a syndrome presenting 1–3 months after completing treatment consisting of hepatomegaly, alteration in liver enzymes, and ascites, which occurs secondary to central hepatic venous occlusion [2]. However, it is now recognized that, due to the liver's parallel cellular architecture, small volumes of the organ can receive very high radiation doses without compromising overall hepatic function [3]. This permits the use of brachytherapy in three general settings: as a method of dose

escalation following EBRT in non-operable patients, as an adjuvant treatment after surgery, and as a single modality for palliation.

1.1 Low-Dose-Rate (LDR) Brachytherapy

Historically, brachytherapy was delivered with low-dose-rate (LDR) techniques. With this approach, sources such as iridium wires were placed through tubes implanted near or into target tumors. These radioactive wires were left in place for several days to slowly deliver the therapeutic dose. Long exposure time to the target tumor theoretically allowed more repair of sublethal damage of normal tissues and allowed tumor cells to move into the more radiosensitive G_2 –M phase of the cell cycle [4]. While this radiobiological advantage is well accepted, there are numerous drawbacks to LDR brachytherapy. The long treatment time necessitates prolonged patient immobilization, increasing the risk of complications such as deep venous thrombosis (DVT), and decubitus ulceration. Additionally, radiation exposure to radiotherapy staff, clinical caregivers, and patient family members is not desirable.

1.2 High-Dose-Rate (HDR) Brachytherapy

Currently, brachytherapy is delivered using high-dose-rate (HDR) afterloading. With this technique, needles or catheters are placed within or near a tumor (Fig. 1). The spatial location of these applicators, along with the size and position of the tumor target and nearby critical normal tissue, is “mapped” in three dimensions with the use of imaging modalities such as ultrasound, computed tomography (CT), positron emission tomography (PET), or magnetic resonance imaging (MRI). Historically, a radiation treatment plan was developed through the use of plain film radiographs with radiation dose prescribed to a point near the applicators thought to represent tumor position. In the modern treatment era, the use of the above-mentioned imaging modalities has allowed a more sophisticated three-dimensional treatment planning methodology for target and normal tissue delineation in brachytherapy [5]. These targets are to be defined at the time of initial diagnosis and prior to each brachytherapy treatment, reinforcing the importance of recognizing tumor volume change during a course of therapy. Differential brachytherapy dose can be applied resulting in a more customized radiation treatment plan.

Next, a single high-activity source, typically iridium-192, is programmed to “dwell” at prespecified locations in each needle or catheter as per the radiation treatment plan. The high activity of the source results in treatment times of minutes in contrast to hours or days, typical of LDR

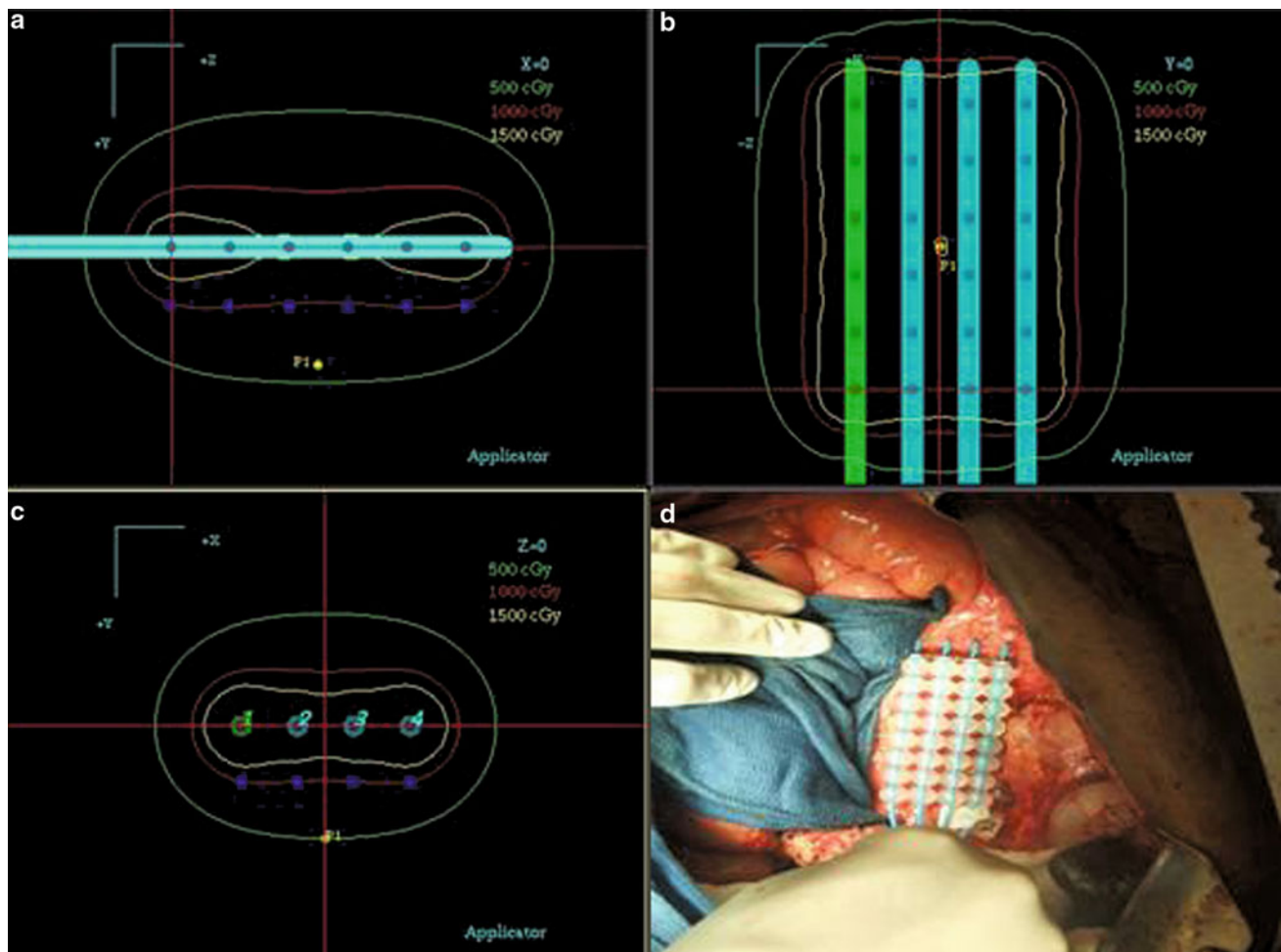


Fig. 1 A typical plan used for intraoperative radiation therapy using high-dose-rate brachytherapy (*HDR-IORT*). Dose distribution of an IORT plan prescribing 10 Gy to the surface in the (a) sagittal (b) coronal, and (c) axial planes. In (d), we see the catheters are placed in a silicone Freiburg flap that can be cut to conform to the tumor bed.

brachytherapy. Another advantage is the use of remote afterloading to guide the radioactive source from its shielded container through transfer tubes, to the treatment applicators implanted in the patient. This results in no radiation exposure to staff or caregivers as treatment can be given inside a shielded suite with the patient being remotely monitored with real-time video, audio, and even cardiac telemetry.

The following sections will detail specific applications of brachytherapy in the management of patients with hepatobiliary malignancy in terms of methods and treatment results.

2 Intraluminal Biliary Brachytherapy

The presence of malignant biliary obstruction, either from a primary hepatobiliary tumor or from secondary metastatic disease, often requires the placement of an intraluminal catheter to promote bile drainage and for symptom relief.

After the wires are secure, the flap is placed into the operative field with the appropriate shielding. A wire with a single high-activity source (typically iridium-192) at the end then passes through the catheters imbedded into the flap at evenly spaced 1 cm intervals to deliver the radiation

Once in place, these catheters can serve as a conduit to load brachytherapy sources for palliation of unresectable tumors, to boost the external beam dose, or as a preoperative approach prior to hepatic transplantation in potentially curative tumors [6–12].

2.1 Methods

Intraluminal brachytherapy for hepatobiliary tumors requires a multimodality approach including both interventional radiology and radiation oncology. The interventional radiologist first places a needle using a percutaneous approach into the liver, finding the dilated bile duct using real-time image guidance including CT or fluoroscopy. Next, the location and extent of the tumor is characterized by a transhepatic cholangiogram (THC). Following this, a thin guide wire is threaded through the percutaneous needle, passed the

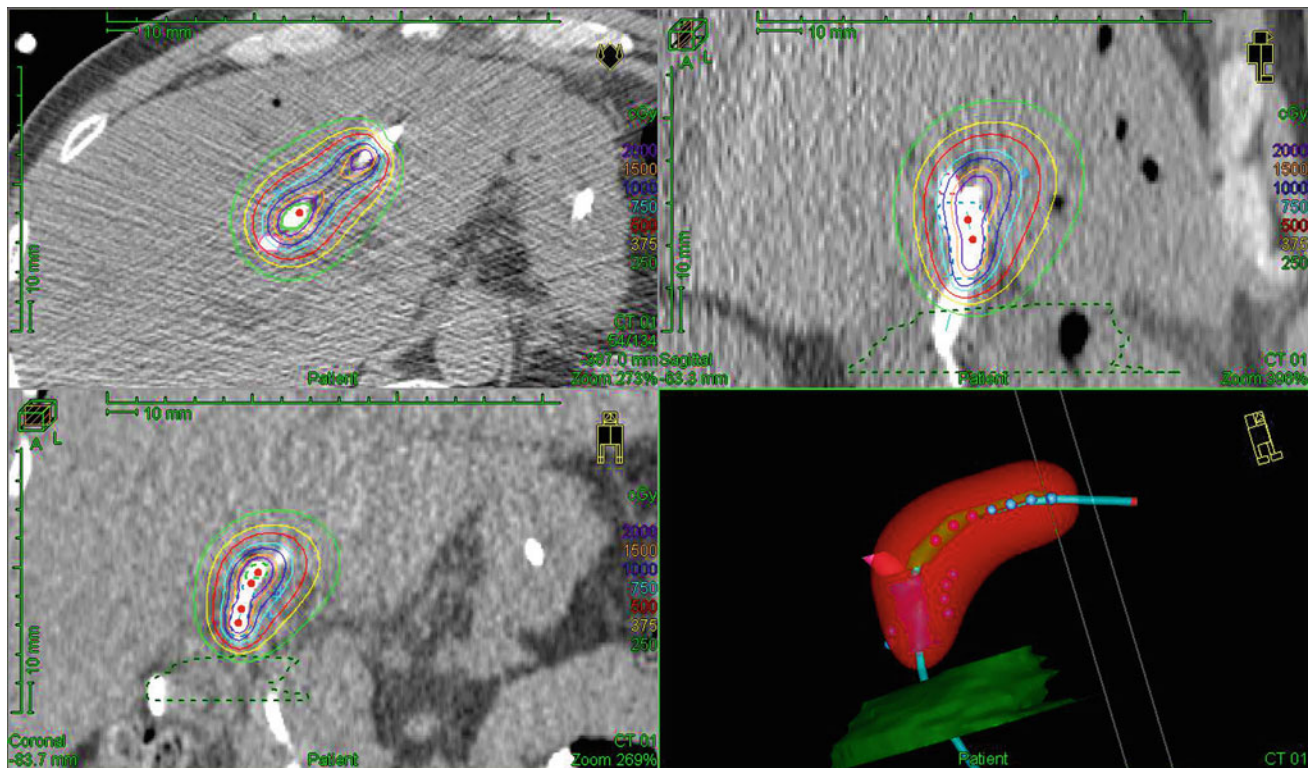


Fig. 2 Planning of intraluminal brachytherapy for biliary malignancy

obstructing tumor and into the duodenum. A flexible catheter is advanced over the guide wire and into the duodenum. If the catheter cannot be passed due to obstruction, it can be left in place to drain externally. After several days of drainage, it is often possible to advance the catheter distal to the tumor and into the duodenum due to decreased tissue edema. If the catheter can be passed by any portion of the tumor, brachytherapy may be useful to facilitate passage past the obstruction [12]. Once the catheter is in place, it can be used for both internal and external biliary drainage [13–15].

For brachytherapy treatment, a Tuohy-Borst sidearm adapter is fixed to the external portion of the catheter. This two-arm adapter allows both external biliary drainage and delivery of brachytherapy. Following this, a blind-ended brachytherapy nylon treatment catheter with stainless steel wire (to prevent kinking) is inserted through the Tuohy port into the larger diameter drainage catheter and is advanced into position under fluoroscopy.

It is important to account for the radioactive source diameter when selecting catheter size, as the catheter must have room to accommodate the radioactive source. Typically, LDR sources require an 8–10 French catheter; HDR sources require an 8–14 French catheter, depending on the type of HDR source and catheter used. The Varisource® afterloader has a narrow source; the Gamma-Med® and Nucletron® afterloaders have a wider diameter source. It is good practice to insert the afterloading catheter into the

drainage catheter and ensure easy passage before the performing the procedure.

The stainless steel wire is replaced with a dummy wire containing radiopaque markers at 1-cm intervals. An orthogonal radiograph or CT scan is obtained for brachytherapy planning purposes (Fig. 2). To deliver the brachytherapy treatment, the catheter is connected to an afterloader (for HDR brachytherapy) or an iridium or radium wire is inserted (for LDR brachytherapy). Once the prescription dose of radiotherapy has been delivered, the source is retracted and the drainage catheter flushed. It is recommended to keep a permanent biliary stent in place for 4–6 weeks to prevent biliary stenosis or stricture.

A transnasal approach using endoscopy at the time of ERCP has also been described [16–27]. This approach allows for internal biliary drainage after sphincterotomy with stent placement and avoids the hepatic puncture associated with the percutaneous technique described above. This approach may be difficult for the wire with the source to traverse the sharp curves in the treatment catheter introduced by this method.

Intraluminal LDR brachytherapy doses (prescribed at 1 cm from the source) are 20–30 Gy as a boost after EBRT or 40–50 Gy as monotherapy given over 1–3 days. HDR doses (again, at 1 cm from the source) are typically 15–20 Gy in 3–4 fractions as a boost or 30–40 Gy in 5–8 fractions, given BID as monotherapy.

Following the procedure, patients should receive broad-spectrum antibiotics to reduce the chance of infectious complications.

2.2 Results

Data supporting the use of intraluminal brachytherapy suggest that this treatment can prolong relief of biliary obstruction and possibly prolong survival.

Early data from Wheeler et al. [11] suggest that patients with malignant biliary obstruction undergoing biliary drainage benefit from intraluminal brachytherapy. In this retrospective report, combining tube drainage with brachytherapy caused a statistically significant “shift to the right” of the survival curve with a median survival of 4 versus 10 months in those treated without and with brachytherapy, respectively [11]. Work by Fletcher, Karani, and Nunnerly have also supported this finding [15, 28, 29].

More recently, Shinohara et al. [30] from the University of Pennsylvania reported a SEER analysis, demonstrating that patients with cholangiocarcinoma undergoing brachytherapy had an improved median survival of 11 versus 4 months. The result was statistically significant and persisted on multivariable analysis. To mitigate any potential bias inherent in an SEER analysis, they also performed a propensity score analysis. When patients were matched by propensity score, the overall survival (OS) benefit of brachytherapy held [30].

A small, randomized prospective trial of 21 patients with inoperable malignant biliary obstruction has been reported in Europe [31]. Patients were randomized to percutaneous insertion of a self-expanding metal stent or the same plus 30 Gy intraluminal brachytherapy delivered over 3 days. The mean stent patency was significantly longer in the brachytherapy group—378 versus 245 days. Additionally, overall survival was noted to be longer in the brachytherapy group—388 versus 298 days ($p < 0.05$).

When combined with surgical resection, postoperative intraluminal brachytherapy has been reported to increase survival in patients with cholangiocarcinoma [32, 33]. Median survival increased from 8.25 to 19 months with the addition of postoperative brachytherapy. The 1-, 2-, and 3-year OS rates were 36, 18, and 10 % for surgery alone versus 85, 42, and 31 % in patients treated with postoperative brachytherapy ($p = 0.0005$). Surgical resection with postoperative brachytherapy was also reported to be superior to biliary drainage with intraluminal brachytherapy, with a median survival of 12.3 months.

Montemaggi et al. [19, 20] noted improved locoregional control in patients treated with a combination of concurrent EBRT and intraluminal brachytherapy. Intraluminal brachytherapy combined with EBRT appeared to be superior to EBRT alone and encouraging evidence suggests that intraluminal brachytherapy may favorably impact survival

without significant associated toxicity [19]. In contrast, other reports have not supported the use of postoperative intraluminal brachytherapy. A study at Johns Hopkins reported similar survival in patients treated with radiotherapy (a combination of EBRT and intraluminal brachytherapy) and those who did not undergo postoperative treatment with median survival of 18 versus 20 months [34]. Cameron et al. [35] and Kraybill et al. [36] also reported no survival benefit with the use of postoperative brachytherapy.

Data on the role of neoadjuvant intraluminal brachytherapy in regard to liver transplantation in cholangiocarcinoma (CCA) patients is emerging. The LDR intraluminal brachytherapy that was administered at Mayo Clinic in 2005 was the first to utilize an endoscopic insertion of biliary brachytherapy catheters directly through internal biliary stents and without the use of nasobiliary tubes (NBTs) [37]. Survival was reported to be 92 % at 1 year and 82 % at 5 years after liver transplantation for the 38 patients that were treated with neoadjuvant chemoradiation, including biliary intraluminal brachytherapy, followed by orthotopic liver transplantation for cholangiocarcinoma [37, 38]. Furthermore, a complete hepatectomy was found to be curative due to the ability of neoadjuvant chemoradiation therapy (including external beam radiation therapy and intraluminal brachytherapy) to contain and treat the cancer in cholangiocarcinoma patients [37]. Because the patients were treated with multiple therapeutic modalities, it is difficult to establish a definitive causal relationship between intraluminal brachytherapy and improved outcome in patients with cholangiocarcinoma. However, long-term survival for a group of patients with unresectable CCA using neoadjuvant intraluminal brachytherapy alone with chemosensitization reports that 50 % of the patients were alive and disease-free 2.8–14.5 years after liver transplantation [39].

3 Interstitial Brachytherapy

Interstitial brachytherapy involves the placement of radioactive sources or afterloading catheters directly into target tumors or tissues. With hepatobiliary tumors, this is most often done in the intraoperative setting permitting direct visualization of the target. Typically, LDR iodine-125 sources are permanently implanted into the tumor or HDR iridium-192 can be afterloaded temporarily if an appropriately shielded operating suite is available.

3.1 Methods

Small unresectable tumors of the liver can be implanted with iodine-125 (I-125) seeds [40]. A nomograph is used to

determine the number and activity of the seeds based on tumor size [41]. After the target area is defined, hollow interstitial needles are placed into the target with 1 cm spacing. The use of intraoperative imaging is encouraged to avoid puncturing large blood vessels and most procedure-related bleeding can be controlled with simple pressure. After all needles are in place, a Mick applicator is used to deposit the I-125 seeds along the path of the needle track with one centimeter spacing between each seed. The Mick applicator allows the needle to be drawn back at predetermined “steps” and individual I-125 seeds deposited at the needle tip. Following seed deposition, the needles are removed. Post-implant CT scans are performed for dosimetric purposes. Doses of 140–160 Gy are typically prescribed with I-125 seeds.

There is also experience with intraoperative HDR [42]. With this technique, the liver is mobilized during laparotomy and several 14-gauge close-ended needles are placed within a non-resectable tumor. Doses in the range of 20 Gy are given in a single fraction, while the patient remains anesthetized and remotely monitored.

For resectable tumors for which margin are a concern (typically adjacent to large vessels), I-125 seeds can be fixed to a two-dimensional substrate and directly opposed to the area at risk [40]. The technique is as follows: I-125 seeds are placed 1 cm apart on a gelfoam sheet trimmed to the dimensions of the tumor bed and then covered with vicryl mesh. The mesh is then sutured directly to the area at risk and, if possible, should be covered by an omental pedicle flap to reduce radiation dose to nearby bowel. This functions as a permanent surface implant delivering a high radiation dose directly to the area at risk.

More recently, CT-guided percutaneous interstitial hepatic brachytherapy has been used [43]. The advantage of this technique is the less invasive nature of the procedure compared with laparotomy.

3.2 Results

Nag et al. [40] reported their results using permanent ^{125}I interstitial implants in a relatively large retrospective study of 64 patients with intrahepatic malignancies that were either unresectable or were incompletely resected. In this study, 58 patients had hepatic metastases from colorectal carcinoma, 4 patients had intrahepatic cholangiocarcinoma, and 2 patients had hepatic metastases from non-colorectal cancers. Plans were designed for a minimum peripheral dose of 160 Gy. The 1-, 3-, and 5-year actuarial control rates for liver disease in these patients were 44, 22, and 22 %, respectively, and the median time to liver recurrence was 9 months (95 % CI, 6–12 months). The overall liver recurrence rate was 75 %, and these were isolated

recurrences in 55 % of the patients. Overall, control rates of liver disease correlated with the number of liver metastases: Patients with solitary metastases had a 38 % 5-year control of liver disease; patients with 3 or fewer metastases had a 32 % 5-year control of liver disease; and patients with 4 or more metastases had an 8 % 5-year control of liver disease. Median times to liver recurrence in these subgroups were 17 months (95 % CI, 3–31), 12 months (95 % CI, 2–22), and 6 months (95 % CI, 5–7), respectively. Analysis of the number of implants, implant volume, and MPD failed to show any significant correlation with the control of liver disease. The 1-, 3-, and 5-year survival rates for all patients in this study were 73, 23, and 5 %, respectively, and the median survival time was 20 months (95 % CI, 16–24). The overall 5-year survival rate with no liver metastases was 3 %. Overall survival was found to correlate inversely with the size of the implant volume ($p = 0.049$); patients with implant volumes of ≤ 20 , 21–64, and ≥ 65 cc had median survival times of 25 months (95 % CI, 20–30), 14 months (95 % CI, 5–23), and 7 months (95 % CI, 1–13), respectively. Complications in this study were reported in only 6 patients (9 %). There were 2 deaths (3 %) within the 30-day postoperative period; one patient developed a small-bowel fistula distant from the implanted area and died of multi-organ failure, and the second patient died of aspiration pneumonia. Other complications included: a small-bowel obstruction, a small-bowel perforation, a liver abscess, and a wound abscess related to seed implantation.

Thomas et al. [42] from Georgetown University reported the results of a prospective phase I/II trial. In this study, 22 patients with 24 unresectable hepatic metastasis from colorectal cancer underwent interstitial HDR at the time of laparotomy. Treatment was given to the tumor periphery ranging from 20 to 30 Gy in a single fraction. No acute or chronic toxicity was noted at a median follow-up of 11 months. Actuarial local control was 26 % at 26 months with a median time to local failure of 8 months.

Pech et al. [44] reported a matched-pair analysis of 18 patients with 36 solitary metachronous liver metastasis from colorectal cancer that underwent either percutaneous image-guided HDR interstitial brachytherapy or interstitial laser ablation (ILT). The patients were matched upon tumor size, location, and use of adjuvant chemotherapy after the intervention. No major complications were observed with either technique. At a median follow-up of 14 months, the local control at 1 year was 72 % in the HDR brachytherapy group and 36 % in the patients undergoing ILT ($p = 0.004$).

A prospective phase II trial that enrolled patients with liver metastases from primary breast cancer was recently reported [45]. Forty-one patients with 115 unresectable tumors were treated with a single fraction of percutaneous image-guided brachytherapy. The median prescription dose

ranged from 12 to 25 Gy based upon dose to surrounding critical structures. Treatment planning parameters specified that no more than 33 % of the liver could receive more than 5 Gy. Only one “major” toxicity occurred—a post-procedure hemorrhage requiring a blood transfusion. No decline in liver function tests was noted at a median follow-up of 18 months. Local tumor control was 93.5 %, and overall survival was 60 % at 18 months.

A prospective phase II trial combining hepatic arterial chemotherapy infusion with interstitial HDR brachytherapy was reported by Wieners et al. [46]. Thirty-three patients with unresectable metastases from colorectal cancer were enrolled. A median dose of 15–25 Gy in a single fraction (based on tumor size and surrounding critical structures) was given along with hepatic infusional 5-fluorouracil. At a median follow-up of 28 months, local control was 69 % at 24 months. The median time to progression was 10.5 months. Overall survival was 30 % at 60 months. In terms of toxicity, grade I, II, III, and IV toxicity was noted in 6, 11, 7, and 1 patients, respectively.

A contemporary experience with CT-guided interstitial HDR brachytherapy has been recently reported. Tselis et al. [47] described the results of 36 tumors in 31 unresectable patients with a mix of primary and metastatic hepatic lesions. None of the patients underwent chemotherapy or external radiotherapy to the liver. The dose prescribed was variable but ranged from 30 Gy in 5 fractions, BID to a single fraction of between 7 and 14 Gy. The median prescribed DEQ2 (the total dose expressed as if delivered in standard 2 Gy fractions) was 30 Gy. The local control at 1 and 3 years was 82 and 57 % for all tumors, 79 and 59 % for metastatic tumors, and 88 and 50 % for primary hepatic malignancies. The median overall survival was 14.6 months. Two patients (16.5 %) developed “minor” toxicity (pain, nausea, vomiting) and 2 patients developed “major” toxicity noted to be hemorrhage that required transfusion.

Overall, these results indicate that interstitial brachytherapy is a safe and effective method of obtaining local tumor control for hepatobiliary tumors. These useful techniques are difficult to perform and not widely available. Patients thus need to be referred to specialized centers performing these techniques.

4 Radioembolization (^{90}Y Microspheres)

Radioembolization of liver cancers takes advantage of the unique vascular system of the liver. In normal liver tissue, approximately 70–80 % of the organ’s blood flow is supplied by the portal vein, and the hepatic artery accounts for the rest. This contrasts with both hepatocellular carcinoma (HCC) and metastatic tumors in the liver, in which the

hepatic artery supplies approximately 80–100 % of the blood flow [48–50]. This difference in perfusion is exploited by the technique known as radioembolization, whereby radioactive microspheres embedded with a beta-emitting isotope, Yttrium-90 (^{90}Y) are implanted into tumors of the liver by delivering the microspheres through the hepatic artery (Fig. 3). Although first performed in 1960 prior to other forms of embolization not using radiation, [51, 52] commercial agents were not available until the late 1980s [53]. An independent group of international experts from the fields of interventional radiology, radiation oncology, nuclear medicine, medical oncology, and surgical oncology involved with Y90 microsphere therapy, the Radioembolization Brachytherapy Oncology Consortium (REBOC), has recently issued clinical guidelines for Y90 microsphere brachytherapy [54].

The use of radioactive isotopes released into blood vessels in the treatment of cancer dates back to the 1940s. In the early years, unsealed sources, primarily ^{63}Zn , ^{198}Au , or radioactive carbon were used [55–57]. However, they could not be localized to a tumor and were, therefore, of limited use due to normal organ toxicity. An early version of resin microspheres containing ^{90}Y also had a problem with leaching of a portion of the radioactive moiety, but this was corrected quickly and is not an issue in modern microspheres. Currently, the use of ^{90}Y microspheres is favored; these are available in two forms: ^{90}Y bound resin microspheres (SIR-Spheres, Sirtex Medical, Australia) and ^{90}Y imbedded glass microspheres (TheraSphere, Nordion, Canada). ^{90}Y is an ideal isotope because it has a short half-life (64.2 h) and is a beta emitter (99.7 %) as it decays to stable Zirconium. Both of the commercially available microspheres contain ^{90}Y , which is produced either by bombarding ^{89}Y in the microspheres with neutrons in a nuclear reactor or using free ^{90}Y to bind irreversibly to resin microspheres. The “hot” radioactive microspheres are delivered to the facility where the treatment is to be performed either on the day of the procedure (resin) or days earlier (glass). Resin microspheres gained premarket approval from the FDA in 2002 for the treatment of hepatic metastases from colorectal adenocarcinoma concurrent with fluorodeoxyuridine (FUDR) hepatic artery infusion. Glass microspheres have use only under humanitarian device exemption (HDE) for the treatment of unresectable HCC. This requires all patients to be enrolled into an IRB-monitored clinical trial. Table 1 outlines the characteristics of each type of microsphere.

4.1 Methods

Guidelines regarding the use of ^{90}Y microspheres have recently been published by the REBOC [54] and are

Fig. 3 An injection of microspheres embedded with ^{90}Y into the hepatic artery causes cancer cells to die through the emission of beta particles (Yttrium-90 microsphere radioembolization—cancer treatment. Ria Endovascular. <http://www.riaendovascular.com/services-procedures/cancer-interventional-oncology/yttrium-90-radioembolization/>. Accessed November 08, 2013)

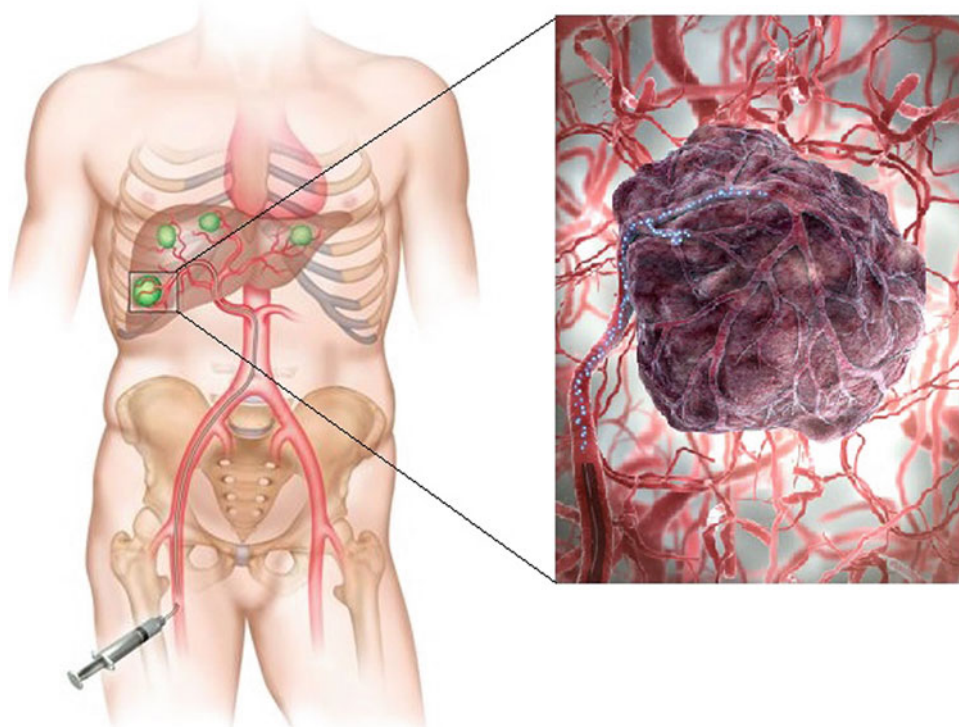


Table 1 Properties of resin and glass Y90 microspheres

Parameter	Resin	Glass
Trade name	SIR-spheres	TheraSpheres
Manufacturer	Sirtex Medical, Sydney, Australia	Nordion, Ottawa, Canada
Diameter	20–60 microns ^a	20–30 microns ^b
Specific gravity	1.6 g/dl	3.6 g/dl
Activity per particle	50 Bq	2,500 Bq
Number of microspheres average treatment	15 million	4 million
Material	Resin with bound yttrium	Glass with yttrium in matrix

^a SIR-Spheres®, Package Insert, Sirtex Medical, Inc., Sydney, Australia

^b TheraSphere®, Package Insert, Nordion, Ottawa, Canada

summarized here. Because this multidisciplinary technology has been developed by and involves the skill sets of the fields of Radiation Oncology, Interventional Radiology, and Nuclear Medicine, it is strongly recommended that a multidisciplinary team is established that includes individuals with the expertise needed to safely and successfully conduct radioembolization procedures. The team should be able to assume the overall medical management of a cancer patient, perform vascular catheterization, perform and interpret radiologic scans, assume responsibility for the delivery of the ^{90}Y microspheres and be an authorized user, and monitor radiation safety. Typically, institutions have achieved this by combining personnel from various disciplines, including interventional radiology, radiation oncology,

nuclear medicine, medical physics, hepatology, surgical oncology, medical oncology, and radiation safety.

Patients should always be evaluated for surgical resection before being considered for ^{90}Y microsphere radioembolization. In addition, the patient's hepatic disease should represent the bulk of their disease, and they should have a life expectancy of at least 3 months. Relative contraindications include limited hepatic reserve, irreversible hyperbilirubinemia (>2 mg/mL), compromised portal vein (unless selective radioembolization can be performed), and previous radiation to the liver. Goin et al. [58] have published a risk stratification analysis of ^{90}Y glass microspheres from data in 121 patients with unresectable HCC. Retrospectively, the group was divided into low-risk and

high-risk groups based on 3-month survival. Seven risk variables were identified as associated with 3-month mortality and were classified as either (1) liver reserve risk factors or (2) non-liver reserve risk factors. The five liver reserve risk factors included bulky disease (tumor volume greater-than-or-equal-to 70 %, or tumors too numerous to count), infiltrative disease (indistinct tumor/liver interface, exhibiting high degree of vascular infiltration on contrast CT), serum transaminase levels greater than five times the normal limit, bilirubin levels greater-than-or-equal-to 2 mg/dL, and tumor volume greater-than-or-equal-to 50 % with serum albumin levels less than 3 g/dL. The two non-liver reserve risk factors included lung dose greater than 30 Gy and a diagnosis of non-HCC disease. Patients grouped into the low-risk group according to this schema had improved survival compared with that of patients at high risk (median survival 466 vs. 108 days).

Prior to treatment with ^{90}Y microspheres, several important studies and procedures should be performed to establish patient eligibility and safety. To evaluate hepatic and renal function, the standard serum laboratory values should be obtained. A three-phase contrast CT and/or a gadolinium-enhanced MRI scan should be performed to evaluate portal vein patency and the hepatic and extrahepatic disease burden. An FDG-PET scan and SPECT imaging may also be useful in measuring hepatic and extrahepatic disease burden and provide information on dosimetry of ^{90}Y delivery (Fig. 4). Arteriograms of the aorta, superior mesenteric, celiac, and right and left hepatic arteries should be performed to evaluate the patient for any anatomic variations in vasculature and to document the perfusion characteristics of the areas of interest. For these studies, percutaneous catheterization is generally preferred over the use of indwelling arterial catheter devices. In order to reduce the risk of unwanted reflux of microspheres into the GI tract, it is also recommended that the gastroduodenal artery and right gastric artery be embolized. As revascularization can occur in a short period of time (days), repeat arteriograms should be performed immediately before the actual administration of ^{90}Y microspheres to make sure that revascularization of prophylactically embolized arteries has not occurred. A $^{99\text{m}}\text{Tc}$ macro-aggregated albumin (MAA) scan should be performed to evaluate the extent of any extrahepatic shunting; results suggesting radiation exposure to the lungs or gastrointestinal tract greater-than-or-equal-to 30 Gy represent a contraindication to radioembolization with microspheres. When the MAA scan is performed, catheter position and flow rates that are used should be representative of the anticipated catheter position and flow rates of the ^{90}Y infusion. Scintigraphy should be performed within 1 h of MAA administration to prevent false-positive extrahepatic activity due to free technetium. Once the results of these studies are reviewed and approved by the

treating team, and there is consensus regarding the planning tumor volume, proposed activity, and optimal catheter placement, treatment with ^{90}Y microspheres may safely proceed.

Whole liver or unilobar administrations are both acceptable approaches for ^{90}Y microspheres, and the decision between the two depends on multiple factors. Treating the entire liver in one session is called *whole liver delivery*, and treating a single lobe is called *lobar delivery*. Sometimes the entire liver will be treated one lobe at a time, which is referred to as *sequential delivery*. In sequential treatments, a 30–45-day interval between treatments is generally observed [59–61]. The activity, number of microspheres, and volumes to be treated will vary for an individual patient and differ depending on the type of microsphere being used (Table 1). Resin microspheres are received in bulk, and the individual medical center extracts the desired activity of ^{90}Y calculated for a specific patient from a 3-GBq source vial that arrives on the day prior to treatment. This process differs from that of glass microspheres, which arrive a few days prior to the procedure and all of which (i.e., the entire contents of the vial containing the spheres) are delivered to the tumor.

When choosing an activity, the significant physical differences between the two spheres must be considered: (1) *Activity per microsphere*: Glass microspheres contain up to 2,500 Bq/sphere and only 1–8 million spheres are delivered for the typical patient. This number of glass spheres is not sufficient to cause significant embolization in the main hepatic arteries. Resin microspheres contain approximately 50 Bq/sphere; thus, an average treatment contains 15–25 million spheres, a number that can cause embolic effects in the arteries. (2) *Embolic effect on dose delivery*: The total number of glass spheres in the vial is not usually sufficient to cause significant embolization in the hepatic arteries; hence, the entire prescribed dose of glass microsphere is completely infused. In contrast, because of reduced antegrade hepatic arterial flow, the prescribed activity of resin spheres cannot always be infused. When delivery of resin spheres is stopped earlier than planned, the residual activity in the delivery vial is measured and deducted from the activity present at the beginning of the procedure to obtain the amount implanted in the liver tumors.

Because the microspheres are designed to implant in the tumor end arteriole bed, it is important to monitor the rate of antegrade flow in the hepatic arteries. When forward flow of microspheres slows or stops, it is important to discontinue delivery of microspheres, thereby preventing reflux of the microspheres into unintended vessels. For this reason, termination of microsphere infusion before the prescribed activity has been delivered is acceptable when reduced antegrade blood flow is noticed during the procedure.

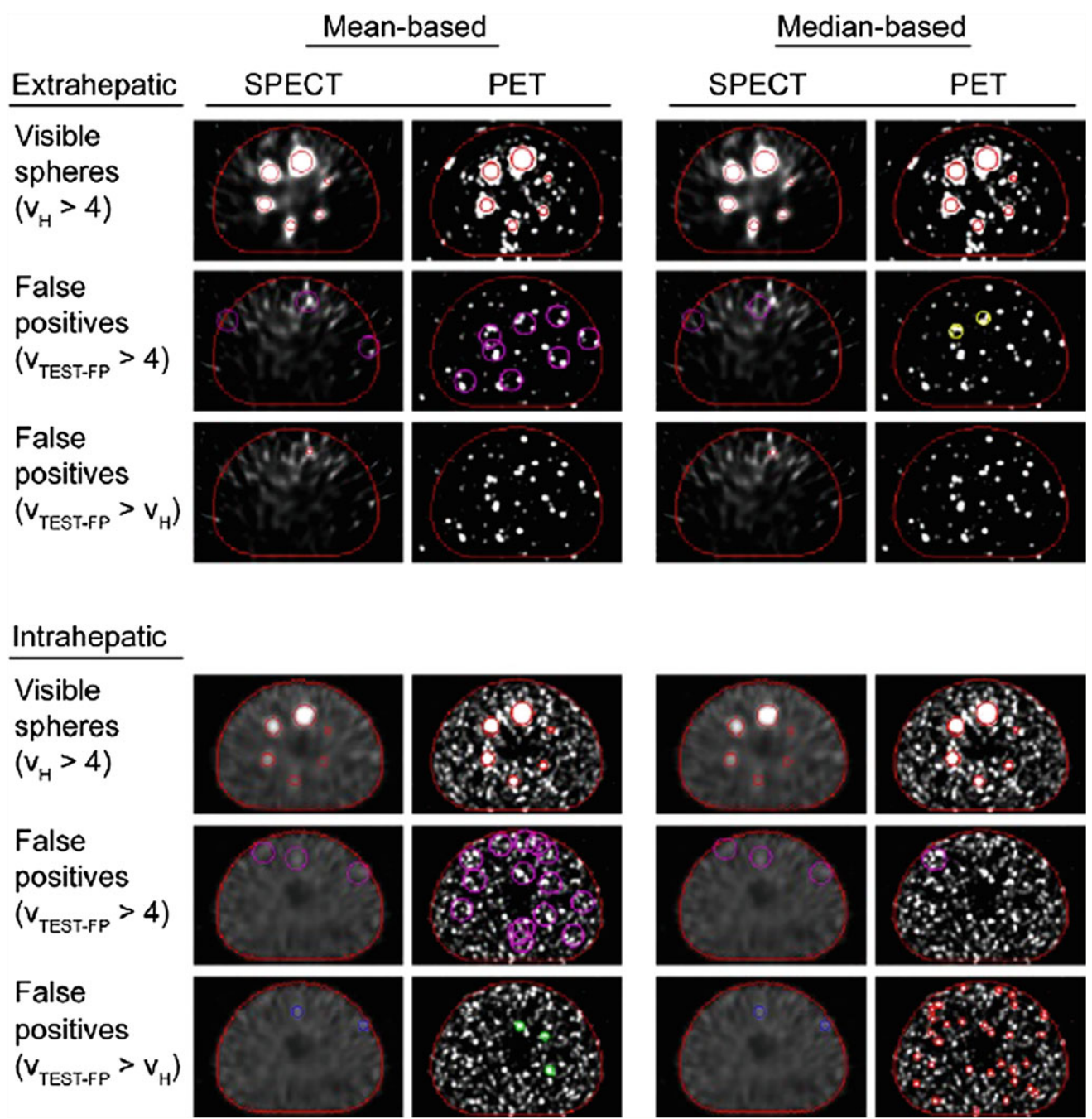


Fig. 4 A visual comparison of FDG-PET versus SPECT imaging for the assessment of intrahepatic and extrahepatic microsphere distribution after radioembolization. FDG-PET imaging often has superior

quality than that of SPECT although both methods can provide useful data regarding dosimetry and disease burden [88]

A Bremsstrahlung scan and other functional imaging studies should be obtained within 24 h after the delivery of either microsphere agent has concluded to confirm and evaluate the distribution of the ^{90}Y microspheres.

Radiologic studies performed after microsphere treatment to assess response must be interpreted with care, as liver edema, congestion, and microinfarctions will decrease attenuation on CT scan, or measure larger in diameter than

pretreatment scans, causing RECIST measurements to be erroneous and mistaken for tumor growth. PET scans may be able to demonstrate decreased metabolic activity suggesting tumor response, even though this may be discordant with findings by CT [60]. When the tumor markers such as CA 19-9, or carcinoembryonic antigen (CEA) has been used to track tumor response after treatment with microspheres, a nadir has been observed at about 12 weeks post-treatment;

this maximal response time has been noted by CT and MRI scans as well [59].

4.2 Results

It is estimated that from 2000 to 2013, more than 30,000 patients have been treated with ^{90}Y microspheres in over 220 medical centers worldwide. There is no published large prospective clinical study as yet, although a resin microsphere trial (SIRFLOX) with nearly 600 patients in metastatic colorectal cancer (mCRC) completed and closed accrual in 2013; the final results are awaiting. Nevertheless, substantial evidence has been published, demonstrating the safety and efficacy of ^{90}Y microspheres in the treatment of primary and metastatic cancers found in the liver. The majority of published clinical reports involve mCRC, HCC, and neuroendocrine liver metastases (mNET), although there are two small phase II studies specific to cholangiocarcinoma that will be presented. However, the most compelling support of radioembolization is found in mCRC and HCC studies. A few important experiences are presented to highlight safety, response and survival in these ill patients who have few or no other treatment options. Extensive data reporting can be found in a series of articles by international experts in radioembolization covering all aspects of this treatment approach [62–68].

4.2.1 Hepatocellular Carcinoma

Considerable experience using ^{90}Y microspheres for HCC has been published demonstrating their efficacy. One report by Carr studied the use of ^{90}Y glass microspheres in 65 patients with biopsy-proven unresectable HCC and made comparisons to historical controls [69]. In this report, 42 patients (64.6 %) had a substantial decrease in tumor vascularity in response to therapy, and 25 patients (38.4 %) had a partial response by CT scan. Median survival for Okuda stage I patients ($n = 42$) and Okuda stage II patients ($n = 23$) was 649 and 302 days, respectively. Historical controls for these two groups are estimated to be 244 and 64 days, respectively. Clinical toxicities included 9 episodes of abdominal pain and 2 episodes of acute cholecystitis requiring cholecystectomy. The main laboratory toxicity was elevated bilirubin, which increased by more than 200 % in 25 patients (30.5 %) during 6 months of therapy, but 18 of these patients had only transient elevation. A prominent finding was prolonged and profound (>70 %) lymphopenia in more than 75 % of the patients, but these were regarded to lack clinical significance.

In another study by Dancey et al. [70], 20 patients with HCC receiving ^{90}Y microspheres were evaluated for treatment efficacy. The median dose delivered was 104 Gy (range, 46–145 Gy), and response rate was 20 %. Nine

patients were Okuda stage I, and 11 were Okuda stage II. The median duration of response was 127 weeks, and the median survival was 54 weeks. Every patient in the study experienced at least 1 adverse event, and the most common were elevations in liver enzymes and bilirubin and upper GI ulceration. Multivariate analysis suggested that a dose of greater than 104 Gy ($p = 0.06$), tumor-to-liver activity uptake ratio of greater than 2 ($p = 0.06$), and Okuda stage I ($p = 0.07$) were associated with longer survival.

In a report by Geschwind et al. [71] of 80 patients with HCC receiving ^{90}Y microspheres delivering liver doses ranging from 47 to 270 Gy, 54 patients with Okuda stage I and 26 patients with Okuda stage II had median survival durations and 1-year survival rates of 628 days and 63 %, and 384 days and 51 %, respectively ($p = .02$). One patient died of liver failure judged as possibly related to the treatment.

Kim et al. [72] have published a case report describing use of ^{90}Y microsphere treatment as a bridge to transplantation in a patient with end-stage liver disease secondary to hepatitis C and HCC. This patient was not initially a candidate for transplantation because the size of his tumor exceeded the Milan criteria. After two treatments with ^{90}Y microspheres, the patient's tumor shrank; his AFP returned to the normal range, and he subsequently received a liver transplant. He was tumor free with normal AFP levels two years post-transplant. Kulik et al. [73] also published a case report in which a patient with an unresectable T3 HCC was downstaged to T2 disease after being treated with ^{90}Y microspheres. The patient received a liver transplant 42 days after treatment; pathology showed complete necrosis of the target tumor.

Kulik et al. [74] also reported using ^{90}Y microspheres in 35 patients with unresectable UNOS stage T3 HCC with the specific intent of downstaging to enable resection, radiofrequency ablation (RFA) or liver transplantation. Overall, 19 patients (56 %) were successfully downstaged from T3 to T2 following treatment, and 11 patients (32 %) were downstaged to target lesions measuring 3.0 cm or less. Also, 23 patients (66 %) were downstaged to either T2 status, lesion <3.0 cm (RFA candidate), or resection. A total of 17 patients (50 %) had an objective tumor response by WHO criteria, and 8 patients (23 %) were successfully downstaged and subsequently underwent liver transplant. One-, 2-, and 3-year survival rates were 84, 54, and 27 %, respectively; median survival for the entire cohort was 800 days.

The two largest reports of radioembolization in HCC confirm much of the data found in smaller studies to date (Table 2). Salem et al. [75] reported on a phase II, single arm, single institution trial using glass ^{90}Y microspheres of 291 patients with unresectable HCC. Multiple treatments with microspheres were allowed, with all patients treated in either segmental or lobar approach, but no whole liver

Table 2 Summary of the two largest reports of radioembolization in hepatocellular carcinoma

Parameter	Sangro et al. [76]	Salem et al. [75]
Patient number	325	291
⁹⁰ Y microsphere	Resin	Glass
Centers	8 EU centers	1 US center
Trial type	Retrospective	Phase II
Median followup	8.7 mo (95 % CI 0.4–46.8 mo)	30.9 mo (95 % CI 22.7–35.7 mo)
Overall survival	Child-Pugh A = 16.8 mo (CI 13.8–20.8 mo) Child-Pugh B = 10.3 mo (CI 7.4–12.6 mo)	Child-Pugh A = 17.2 mo (CI 14.9–24 mo) Child-Pugh B = 7.7 mo (CI 6.5–11.2 mo)
Time to progression	NS	7.9 mo (CI 6–10.3 mo)
⁹⁰ Y activity (median)	1.5 GBq	103 Gy (CI 99–108 Gy)

treatments were given. A total of 526 treatments (mean 1.8; range 1–5) were delivered in the 291 patients, with 30-day mortality rate from any cause 3 %. The radiographic response rate was 42 % by WHO criteria and 57 % by EASL criteria. Sangro et al. [76] reported a retrospective experience of 325 patients with unresectable HCC treated with resin ⁹⁰Y microspheres in 8 centers in Europe that had used the same eligibility criteria and treatment parameters. This report was significant for not only its high number of patients treated, but also for demonstrating safety and efficacy of resin microspheres in patients with portal vein thrombosis, prior TACE treatments, large tumor volumes, and ability to delivery microspheres more than once.

The acute and late side effects of using ⁹⁰Y microspheres in HCC have been well characterized in the literature [77–81]. Commonly, patients experience a mild post-embolization syndrome on the day of and up to 3 days post-treatment, and symptoms include fatigue, nausea, and abdominal pain. Damage to nontarget organs can also include gastrointestinal ulcers, pancreatitis, and radiation pneumonitis, but observing the recommended preventative pretreatment guidelines can minimize this risk. One potential serious late side effect is radiation-induced liver disease (RILD), also known as radiation hepatitis. Although this entity was also known from EBRT experiences with and without concurrent chemotherapy, it has been a rare finding in radioembolization. Kennedy et al., in a study at which time it was the largest collection of patients—515 total—who received 680 resin microsphere radioembolization fractions, found only a 0.8 % incidence in properly treated patients of RILD [82]. Fatal radiation pneumonitis is extremely rare (<0.1 %), and observing the radiation dose limit of <30 Gy to the lungs (total) can prevent this complication [83].

In addition to treating HCC, ⁹⁰Y microspheres have been used to treat metastatic disease in the liver. Kennedy et al. [59] published a retrospective study from 7 centers in the USA that examined the use of microspheres in patients with chemorefractory metastatic colorectal cancer with liver predominant disease. In this study, more than two-thirds of the patients responded to treatment despite a significant

history of previous chemotherapy treatments. In patients who responded to the microspheres, median survival was 10.5 months, compared to 4.5 months for non-responders. There were no cases of grade 4 or 5 toxicity, veno-occlusive disease, or RILD. The most common side effects were fatigue, brief nausea, and transient elevation of liver enzymes. Maximal response occurred at 12 weeks as measured by CT scan and the nadir of the tumor marker carcinoembryonic antigen (CEA).

Prospective clinical trials have also shown promising results for the use of ⁹⁰Y microspheres. One such study, published by Gray et al. [84], was a phase III trial studying the use of resin ⁹⁰Y microspheres in chemotherapy-naïve colorectal cancer patients with metastases to the liver. Patients were randomized to hepatic artery infusion of FUDR alone or FUDR plus a single whole liver treatment of microspheres. Each arm of the study included 32 patients, and partial or complete tumor response rates were higher for the patients receiving the microspheres (44 vs. 17.6 %; $p = 0.01$). The median time to progression in the liver was longer for the patients receiving microspheres (15.9 vs. 9.7 months, $p = 0.04$), and survival was also improved for the patients receiving microspheres (5-year survival: 3.5 vs. 0 %). Quality of life and toxicity were found to be similar for the two groups.

The use of ⁹⁰Y microspheres for neuroendocrine primary tumors in the liver has been examined retrospectively by 10 institutions [85]. Kennedy et al. reports a total of 148 patients that were treated with 185 separate procedures. The median age was 58 years (26–95 years) at treatment with median performance status of ECOG (0). There were no acute or delayed toxicity of Common Toxicity Criteria (CTC) 3.0 grade 3 in 67 % of patients, with fatigue (6.5 %) being the most common side effect. Imaging response was stable in 22.7 %, partial response in 60.5 %, complete in 2.7 %, and progressive disease in 4.9 %. No radiation liver failure occurred. The median survival was 70 months. After review of local therapy in the liver, including surgery, embolization, and radiation (systemic and external beam), it was concluded that ⁹⁰Y microspheres compared very favorably to these other

treatments. Microsphere therapy to the whole liver or lobe with single or multiple fractions were safe and produced high response rates, even with extensive tumor replacement of normal liver and/or heavy pretreatment.

4.2.2 Cholangiocarcinoma

Cholangiocarcinoma has been studied in two prospective trials, one each for resin and glass microspheres [86, 87]. Saxena et al. completed a phase II study using resin microspheres in 25 patients with unresectable cholangiocarcinoma. Each patient received only one treatment and endpoints of the study were toxicity, radiographic response and overall survival. No patient was lost to follow-up. The median follow-up was 8.1 (range, 0.4–56) months and the median survival after ^{90}Y radioembolization was 9.3 months. Two patients died within 1 month of treatment; the median follow-up for the remaining 23 was 8.9 (range, 1.5–56) months. On imaging follow-up of 23 patients, a partial response to treatment was observed in 6 patients (26 %), stable disease in 11 patients (48 %), and progressive disease in 5 patients (20 %). One patient (4 %) who had a partial response to treatment was downstaged to resection after treatment. Two factors were associated with an improved survival: peripheral tumor type (vs. infiltrative, $P = .004$) and Eastern Cooperative Oncology Group performance status of 0 (vs. 1 and 2, $P < .001$) [86].

Ibrahim et al. reported the use of glass microspheres to treat unresectable cholangiocarcinoma in 24 patients in segmental or lobar treatments but not the whole liver. Therefore, each patient received more than one treatment. Fatigue and transient abdominal pain were reported in 18 patients (75 %) and 10 patients (42 %), respectively. WHO criteria were used for imaging follow-up of 22 patients, with a partial response in 6 patients (27 %), stable disease in 15 patients (68 %), and progressive disease in 1 patient (5 %). Using EASL guidelines, 17 patients (77 %) showed >50 % tumor necrosis on imaging follow-up. Two patients (9 %) demonstrated 100 % tumor necrosis. The median overall survival for the entire cohort ($n = 24$) was 14.9 months. The median survival for patients with an ECOG performance status of 0, 1, and 2 was 31.8 months, 6.1 months, and 1 month, respectively ($p < 0.0001$); the median survival for patients without and with PVT was 31.8 and 5.7 months, respectively ($p = 0.0003$); and the median survival for patients with peripheral versus periductal-infiltrative tumors was 31.8 and 5.7 months, respectively ($p = 0.0005$) [87].

Although radioembolization with ^{90}Y microspheres is a relatively novel treatment for nearly untreatable tumors such as those of the biliary tract, recent evidence has suggested encouraging results in patients with biliary cancers. Preliminary data reveal that ^{90}Y radioembolization is an

efficacious and safe treatment for unresectable intrahepatic cholangiocarcinoma and other cancers of the biliary tract [38]; however, further prospective analyses with a large selection of patients are necessary to determine the benefits of radioembolization as first-line therapy and to establish distinct prognostic factors that influence patient outcome.

5 Conclusions

Brachytherapy is a very useful modality in the treatment of unresectable liver tumors. Solitary or a limited number of localized tumors can be treated with interstitial permanent ^{125}I seeds, HDR brachytherapy, or intraluminal ^{192}Ir to the unresected tumor. Image-guided HDR biliary brachytherapy is a promising minimally invasive approach for patients with unresectable tumors or as part of a protocol for transplant candidates. Intraoperative radiation therapy (electron beam, LDR or HDR) can be used when patients are at risk of having a positive margin at the time of surgical resection. Radioembolization of diffuse or multiple primary or metastatic liver tumors with ^{90}Y glass or resin microspheres can be used to slow disease progression and improve survival. These benefits are more realized when radioembolization is done early in the treatment program or in combination with appropriate chemotherapy. Currently, ^{90}Y glass microspheres are generally used for HCCs, while ^{90}Y resin microspheres are used to treat metastases from colorectal carcinomas. However, studies are underway to treat various primary or metastatic liver tumors with either glass or resin microspheres. The role of these therapies must be investigated further in controlled clinical trials to integrate and quantify the benefit when combined with other therapies.

References

1. Russell AH, Clyde C, Wasserman TH, Turner SS, Rotman M (1993) Accelerated hyperfractionated hepatic irradiation in the management of patients with liver metastases: results of the RTOG dose escalating protocol. *Int J Radiat Oncol Biol Phys* 27:117–123
2. Reed GB Jr, Cox AJ Jr (1966) The human liver after radiation injury. A form of veno-occlusive disease. *Am J Pathol* 48:597–611
3. Dawson LA, Ten Haken RK (2005) Partial volume tolerance of the liver to radiation. *Semin Radiat Oncol* 15:279–283
4. Pawlik TM, Keyomarsi K (2004) Role of cell cycle in mediating sensitivity to radiotherapy. *Int J Radiat Oncol Biol Phys* 59:928–942
5. Haie-Meder C, Potter R, Van Limbergen E et al (2005) Recommendations from Gynaecological (GYN) GEC-ESTRO working group (I): concepts and terms in 3D image based 3D treatment planning in cervix cancer brachytherapy with emphasis on MRI assessment of GTV and CTV. *Radiother Oncol* 74:235–245 (journal of the European Society for Therapeutic Radiology and Oncology)

6. Conroy RM, Shahbazian AA, Edwards KC et al (1982) A new method for treating carcinomatous biliary obstruction with intracatheter radium. *Cancer* 49:1321
7. Fletcher MS, Brinkley D, Dawson JL, Nunnerley H, Wheeler PG, Williams R (1981) Treatment of high bile duct carcinoma by internal radiotherapy with iridium-192 wire. *Lancet* 2:172–174
8. Loigman BI, Mattern RF, Healey RF, Hayes TH (1981) Nonsurgical evaluation and management of malignant biliary tract obstruction. *J Med Soc NJ* 78:883–886
9. McLean GK, Ring EJ, Freiman DB (1982) Therapeutic alternatives in the treatment of intrahepatic biliary obstruction. *Radiology* 145:289–295
10. Mornex F, Gerard JP, Bret P, Partensky C (1981) Iridium wire radiotherapy for high bile duct carcinoma. *Lancet* 2:479
11. Wheeler PG, Dawson JL, Nunnerley H, Brinkley D, Laws J, Williams R (1981) Newer techniques in the diagnosis and treatment of proximal bile duct carcinoma—an analysis of 41 consecutive patients. *Q J Med* 50:247–258
12. Nag S, Tai DL, Gold RE (1984) Biliary tract neoplasms: a simple management technique. *South Med J* 77:593–595
13. Ikeda H, Kuroda C, Uchida H et al (1979) Intraluminal irradiation with iridium-192 wires for extrahepatic bile duct carcinoma—a preliminary report (author's transl). *Nihon Igaku Hoshasen Gakkai zasshi Nippon acta radiologica* 39:1356–1358
14. Koster R, Schmidt H, Greuel H (1982) Afterloading method for the irradiation of malignant bile duct obstructions]. *Strahlentherapie* 158:678–680
15. Nunnerley HB, Karani JB (1990) Interventional radiology of the biliary tract. Intraductal radiation. *Radiol Clin North Am* 28:1237–1240
16. Pakisch B, Klein GE, Stucklschweiger G et al (1992) Metallic mesh endoprosthesis and intraluminal high dose rate 192Ir brachytherapy in the palliative treatment of malignant bile duct obstruction. Initial results]. *RoFo : Fortschritte auf dem Gebiete der Röntgenstrahlen und der Nuklearmedizin* 156:592–595
17. Trodella L, Mantini G, Barina M, Montemaggi P (1991) External and intracavitary radiotherapy in the management of carcinoma of extrahepatic biliary tract. *Rays* 16:71–75
18. Urban MS, Siegel JH, Pavlou W et al (1990) Treatment of malignant biliary obstruction with a high-dose rate remote afterloading device using a 10 F nasobiliary tube. *Gastrointest Endosc* 36:292–296
19. Montemaggi P, Costamagna G, Döbelbower RR et al (1995) Intraluminal brachytherapy in the treatment of pancreas and bile duct carcinoma. *Int J Radiat Oncol Biol Phys* 32:437–443
20. Montemaggi P, Morganti AG, Döbelbower RR Jr et al (1996) Role of intraluminal brachytherapy in extrahepatic bile duct and pancreatic cancers: is it just for palliation? *Radiology* 199:861–866
21. Morganti AG, Trodella L, Valentini V et al (2000) Combined modality treatment in unresectable extrahepatic biliary carcinoma. *Int J Radiat Oncol Biol Phys* 46:913–919
22. Prempre T, Cox EF, Sewchand W, Tang CK (1983) Cholangiocarcinoma. A place for brachytherapy. *Acta Radiol Oncol* 22:353–359
23. Siegel JH, Lichtenstein JL, Pullano WE et al (1988) Treatment of malignant biliary obstruction by endoscopic implantation of iridium 192 using a new double lumen endoprosthesis. *Gastrointest Endosc* 34:301–306
24. Phillip J, Hagenmuller F, Manegold K, Szepesi S, Classen M (1984) Endoscopic intraductal radiotherapy of high bile-duct carcinoma]. *Dtsch Med Wochenschr* 109:422–426
25. Classen M, Hagenmuller F (1987) Endoprosthesis and local irradiation in the treatment of biliary malignancies. *Endoscopy* 19(Suppl 1):25–30
26. Venu RP, Geenen JE, Hogan WJ, Johnson GK, Klein K, Stone J (1987) Intraluminal radiation therapy for biliary tract malignancy—an endoscopic approach. *Gastrointest Endosc* 33:236–238
27. Singh V, Kapoor R, Solanki KK, Singh G, Verma GR, Sharma SC (2007) Endoscopic intraluminal brachytherapy and metal stent in malignant hilar biliary obstruction: a pilot study. *Liver Int* 27:347–352 (official journal of the International Association for the Study of the Liver)
28. Fletcher MS, Brinkley D, Dawson JL, Nunnerley H, Williams R (1983) Treatment of hilar carcinoma by bile drainage combined with internal radiotherapy using 192iridium wire. *Br J Surg* 70:733–735
29. Karani J, Fletcher M, Brinkley D, Dawson JL, Williams R, Nunnerley H (1985) Internal biliary drainage and local radiotherapy with iridium-192 wire in treatment of hilar cholangiocarcinoma. *Clin Radiol* 36:603–606
30. Shinohara ET, Guo M, Mitra N, Metz JM (2010) Brachytherapy in the treatment of cholangiocarcinoma. *Int J Radiat Oncol Biol Phys* 78:722–728
31. Valek V, Kysela P, Kala Z, Kiss I, Tomasek J, Petera J (2007) Brachytherapy and percutaneous stenting in the treatment of cholangiocarcinoma: a prospective randomised study. *Eur J Radiol* 62:175–179
32. Gonzalez D, Gerard JP, Maners AW et al (1990) Results of radiation therapy in carcinoma of the proximal bile duct (Klatskin tumor). *Semin Liver Dis* 10:131–141
33. Verbeek PC, Van Leeuwen DJ, Van Der Heyde MN, Gonzalez D (1991) Does additive radiotherapy after hilar resection improve survival of cholangiocarcinoma? An analysis in sixty-four patients. *Ann Chir* 45:350–354
34. Pitt HA, Nakeeb A, Abrams RA et al (1995) Perihilar cholangiocarcinoma. Postoperative radiotherapy does not improve survival. *Annals Surg* 221:788–797 (discussion 97–98)
35. Cameron JL, Pitt HA, Zinner MJ, Kaufman SL, Coleman J (1990) Management of proximal cholangiocarcinomas by surgical resection and radiotherapy. *Am J Surg* 159:91–97 (discussion 7–8)
36. Kraybill WG, Lee H, Picus J et al (1994) Multidisciplinary treatment of biliary tract cancers. *J Surg Oncol* 55:239–245
37. Simmons DT, Baron TH, Petersen BT, Gostout CJ, Haddock MG, Gores GJ, Yeakel PD, Topazian MD, Levy MJ (2006) A novel endoscopic approach to brachytherapy in the management of Hilar cholangiocarcinoma. *Am J Gastroenterol* 101(8):1792–1796
38. Hoffmann RT, Paprottka PM, Schön A, Bamberg F, Haug A, Dürr EM, Rauch B, Trumm CT, Jakobs TF, Helmberger TK, Reiser MF, Kolligs FT (2012) Transarterial hepatic yttrium-90 radioembolization in patients with unresectable intrahepatic cholangiocarcinoma: factors associated with prolonged survival. *Cardiovasc Intervent Radiol* 35(1):105–116. doi: [10.1007/s00270-011-0142-x](https://doi.org/10.1007/s00270-011-0142-x)
39. Heimbach JK, Gores GJ, Haddock MG, Alberts SR, Nyberg SL, Ishitani MB, Rosen CB (2004) Liver transplantation for unresectable perihilar cholangiocarcinoma. *Semin Liver Dis* 24(2):201–207
40. Nag S, DeHaan M, Scruggs G, Mayr N, Martin EW (2006) Long-term follow-up of patients of intrahepatic malignancies treated with iodine-125 brachytherapy. *Int J Radiat Oncol Biol Phys* 64:736–744
41. Anderson LL (1976) Spacing nomograph for interstitial implants of 125I seeds. *Med Phys* 3:48–51
42. Thomas DS, Nauta RJ, Rodgers JE et al (1993) Intraoperative high-dose rate interstitial irradiation of hepatic metastases from colorectal carcinoma. Results of a phase I-II trial. *Cancer* 71:1977–1981
43. Ricke J, Wust P, Stohlmann A et al (2004) CT-guided interstitial brachytherapy of liver malignancies alone or in combination with

- thermal ablation: phase I-II results of a novel technique. *Int J Radiat Oncol Biol Phys* 58:1496–1505
44. Pech M, Wieners G, Kryza R et al (2008) CT-guided brachytherapy (CTGB) versus interstitial laser ablation (ILT) of colorectal liver metastases: an intraindividual matched-pair analysis. *Strahlentherapie und Onkologie* 184:302–306 (Organ der Deutschen Röntgengesellschaft [et al])
 45. Wieners G, Mohnike K, Peters N et al (2011) Treatment of hepatic metastases of breast cancer with CT-guided interstitial brachytherapy—a phase II-study. *Radiother Oncol* 100:314–319 (journal of the European Society for Therapeutic Radiology and Oncology)
 46. Wieners G, Pech M, Hildebrandt B et al (2009) Phase II feasibility study on the combination of two different regional treatment approaches in patients with colorectal “liver-only” metastases: hepatic interstitial brachytherapy plus regional chemotherapy. *Cardiovasc Intervent Radiol* 32:937–945
 47. Tselis N, Chatzikonstantinou G, Kolotas C, Milickovic N, Baltas D, Chung TL, Zamboglou N (2012) Hypofractionated accelerated computed tomography-guided interstitial high-dose-rate brachytherapy for liver malignancies. *Brachytherapy* 11:507–514
 48. Ackerman NB, Lien WM, Kondi ES et al (1970) The blood supply of experimental liver metastases I: the distribution of hepatic artery and portal vein blood to “small” and “large” tumors. *Surgery* 66(6):1067–1072
 49. Breedis C, Young G (1954) The blood supply of neoplasms in the liver. *Am J Pathol* 30:969–984
 50. Lien WM, Ackerman NB (1970) The blood supply of experimental liver metastases II: a microcirculatory study of the normal and tumor vessels of the liver with the use of perfused silicone rubber. *Surgery* 68(2):334–340
 51. Roth M (1961) Carcinoid of the rectum. A case report with observations on radiosensitivity of nodular metastases to the skin. *Am J Roentgenol Radium Ther Nucl Med*. 86:97–102
 52. Ya PM, Guzman T, Loken MK, Perry JF (1961) Isotope localization with tagged microspheres. *Surgery* 49(5):644–650
 53. Kennedy AS, Nutting C, Coldwell D, Gaiser J, Drachenberg C (2004) Pathologic response and microdosimetry of (90Y) microspheres in man: review of four explanted whole livers. *Int J Radiat Oncol Biol Phys* 60(5):1552–1563
 54. Kennedy A, Nag S, Salem R et al (2007) Recommendations for radioembolization of hepatic malignancies using yttrium-90 microsphere brachytherapy: a consensus panel report from the radioembolization brachytherapy oncology consortium. *Int J Radiat Oncol Biol Phys* 68(1):13–23
 55. Di Matteo G, Gennarelli L, Lenti R (1962) Una nuova metodica per la fissazione elettiva dell' Au198 adsorbio su carbonio in terriori lobari e sublobari. *Gazz Intern Med Chir* 67:1875
 56. Muller JH, Rossier PH (1947) Treatment of cancer of the lungs by artificial radioactivity. *Experientia* 3:75
 57. Muller JH, Rossier PH (1951) A new method for the treatment of cancer of the lungs by means of artificial radioactivity (Zn63 and Au198). *Acta Radiol* 35:449–468
 58. Goin JE, Salem R, Carr BI et al (2005) Treatment of unresectable hepatocellular carcinoma with intrahepatic yttrium 90 microspheres: a risk-stratification analysis. *J Vasc Interv Radiol* 16(2 Pt 1):195–203
 59. Kennedy AS, Coldwell D, Nutting C et al (2006) Resin 90Y-microsphere brachytherapy for unresectable colorectal liver metastases: modern USA experience. *Int J Radiat Oncol Biol Phys* 65(2):412–425
 60. Lewandowski RJ, Thurston KG, Goin JE et al (2005) 90Y microsphere (TheraSphere) treatment for unresectable colorectal cancer metastases of the liver: response to treatment at targeted doses of 135–150 Gy as measured by [18F]fluorodeoxyglucose positron emission tomography and computed tomographic imaging. *J Vasc Interv Radiol* 16(12):1641–1651
 61. Salem R, Lewandowski RJ, Atassi B et al (2005) Treatment of unresectable hepatocellular carcinoma with use of 90Y microspheres (TheraSphere): safety, tumor response, and survival. *J Vasc Interv Radiol* 16(12):1627–1639
 62. Kennedy AS, Coldwell D, Sangro B, Wasan H, Salem R (2012) Integrating radioembolization (90Y microspheres) into current treatment options for liver tumors: introduction to the international working group report. *Am J Clin Oncol* 35:81–90
 63. Coldwell D, Sangro B, Wasan H, Salem R, Kennedy A (2011) General selection criteria of patients for radioembolization of liver tumors: an international working group report. *Am J Clin Oncol* 34(3):337–341
 64. Kennedy A, Coldwell D, Sangro B, Wasan H, Salem R (2012) Radioembolization for the treatment of liver tumors general principles. *Am J Clin Oncol* 35(1):91–99
 65. Wasan H, Kennedy A, Coldwell D, Sangro B, Salem R (2012) Integrating radioembolization with chemotherapy in the treatment paradigm for unresectable colorectal liver metastases. *Am J Clin Oncol* 35(3):293–301
 66. Sangro B, Salem R, Kennedy A, Coldwell D, Wasan H (2011) Radioembolization for hepatocellular carcinoma: a review of the evidence and treatment recommendations. *Am J Clin Oncol* 34(4):422–431
 67. Kennedy A, Coldwell D, Sangro B, Wasan H, Salem R (2012) Integrating radioembolization into the treatment paradigm for metastatic neuroendocrine tumors in the liver. *Am J Clin Oncol* 35(4):393–398
 68. Coldwell D, Sangro B, Salem R, Wasan H, Kennedy A (2012) Radioembolization in the treatment of unresectable liver tumors: experience across a range of primary cancers. *Am J Clin Oncol* 35(2):167–177
 69. Carr BI (2004) Hepatic arterial 90Yttrium glass microspheres (Therasphere) for unresectable hepatocellular carcinoma: interim safety and survival data on 65 patients. *Liver Transpl* 10(2 Suppl 1):S107–S110
 70. Dancey JE, Shepherd FA, Paul K et al (2000) Treatment of nonresectable hepatocellular carcinoma with intrahepatic 90Y-microspheres [in process citation]. *J Nucl Med* 41(10):1673–1681
 71. Geschwind JF, Salem R, Carr BI et al (2004) Yttrium-90 microspheres for the treatment of hepatocellular carcinoma. *Gastroenterology* 127(5 Suppl 1):S194–S205
 72. Kim DY, Kwon DS, Salem R et al (2006) Successful embolization of hepatocellular carcinoma with yttrium-90 glass microspheres prior to liver transplantation. *J Gastrointest Surg* 10(3):413–416
 73. Kulik LM, Mulcahy MF, Hunter RD et al (2005) Use of yttrium-90 microspheres (TheraSphere) in a patient with unresectable hepatocellular carcinoma leading to liver transplantation: a case report. *Liver Transpl* 11(9):1127–1131
 74. Kulik LM, Atassi B, van Holsbeeck L et al (2006) Yttrium-90 microspheres (TheraSphere) treatment of unresectable hepatocellular carcinoma: downstaging to resection, RFA and bridge to transplantation. *J Surg Oncol* 94(7):572–586
 75. Salem R, Lewandowski RJ, Mulcahy MF, Riaz A, Ryu RK, Ibrahim S et al (2010) Radioembolization for hepatocellular carcinoma using Yttrium-90 microspheres: a comprehensive report of long-term outcomes. *Gastroenterology* 138(1):52–64
 76. Sangro B, Carpanese L, Cianni R, Golfieri R, Gasparini D, Ezziddin S et al (2011) Survival after yttrium-90 resin microsphere radioembolization of hepatocellular carcinoma across Barcelona clinic liver cancer stages: a European evaluation. *Hepatology* 54(3):868–878
 77. Goin JE, Dancey JE, Roberts CA et al (2004) Comparison of post-embolization syndrome in the treatment of patients with

- unresectable hepatocellular carcinoma: trans-catheter arterial chemo-embolization versus yttrium-90 glass microspheres. *World J Nucl Med* 3(1):49–56
78. Goin JE, Salem R, Carr BI et al (2005) Treatment of unresectable hepatocellular carcinoma with intrahepatic yttrium 90 microspheres: factors associated with liver toxicities. *J Vasc Interv Radiol* 16(2 Pt 1):205–213
 79. Ho S, Lau WY, Leung TW et al (1996) Partition model for estimating radiation doses from yttrium-90 microspheres in treating hepatic tumours. *Eur J Nucl Med* 23(8):947–952
 80. Steel J, Baum A, Carr B (2004) Quality of life in patients diagnosed with primary hepatocellular carcinoma: hepatic arterial infusion of Cisplatin versus 90-Yttrium microspheres (Therasphere). *Psychooncology* 13(2):73–79
 81. Thamboo T, Tan KB, Wang SC et al (2003) Extra-hepatic embolisation of Y-90 microspheres from selective internal radiation therapy (SIRT) of the liver. *Pathology* 35(4):351–353
 82. Kennedy AS, McNeillie P, Dezarn WA, Nutting C, Sangro B, Wertman D et al (2009) Treatment parameters and outcome in 680 treatments of internal radiation with resin 90Y-microspheres for unresectable hepatic tumors. *Int J Radiat Oncol Biol Phys* 74(5):1494–1500
 83. Leung TW, Lau WY, Ho SK et al (1995) Radiation pneumonitis after selective internal radiation treatment with intraarterial 90yttrium-microspheres for inoperable hepatic tumors. *Int J Radiat Oncol Biol Phys* 33(4):919–924
 84. Gray BN, Van Hazel G, Hope M et al (2001) Randomised trial of SIR-Spheres plus chemotherapy vs. chemotherapy alone for treating patients with liver metastases from primary large bowel cancer. *Ann Oncol* 12(12):1711–1720
 85. Kennedy AS, Dezarn WA, McNeillie P et al (2008) Radioembolization for unresectable neuroendocrine hepatic metastases using resin ⁹⁰Y-microspheres: early results in 148 patients. *Amer J Clin Oncol* 31(3):271–279
 86. Saxena A, Bester L, Chua TC et al (2010) Yttrium-90 radiotherapy for unresectable intrahepatic cholangiocarcinoma: a preliminary assessment of this novel treatment option. *Ann Surg Oncol* 17(2):484–491
 87. Ibrahim SM, Mulcahy MF, Lewandowski RJ, Sato KT, Ryu RK, Masterson EJ et al (2008) Treatment of unresectable cholangiocarcinoma using yttrium-90 microspheres: results from a pilot study. *Cancer* 113(8):2119–2128
 88. Elschot M, Vermolen BJ, Lam MG, de Keizer B, van den Bosch MA, de Jong HW (2013) Quantitative comparison of PET and Bremsstrahlung SPECT for imaging the in vivo yttrium-90 microspheredistribution after liver radioembolization. *PLoS One* 8(2):e55742

Emerging Techniques in Image-Guided Radiation Therapy and Stereotactic Body Radiation Therapy

John G. Phillips, John A. Wolfgang, and Theodore S. Hong

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Abstract

Locoregional recurrence is the primary failure pattern for biliary tract and gallbladder cancers. However, the use of external beam radiation therapy in the treatment for these cancers, particularly cholangiocarcinoma, has had limited efficacy historically due to the high risk of toxicity with curative doses. While a range of anatomic locations and thus dose-limiting structures exist, proximity to the duodenum, risk of biliary stricture, and radiation-induced liver disease (RILD) have posed special challenges to achieving a dose capable of preventing local recurrence or achieving cure. Over the last decade, the rapid development and integration of technologies such as advanced onboard imaging (OBI) integrated with stereotactic body radiation therapy (SBRT) have created renewed interest in the use of external beam radiation in both the adjuvant and palliative setting for these cancers. In this chapter, we review the relevant advances, particularly the growing experience with SBRT in intrahepatic cholangiocarcinoma (ICC).

1 The Liver as a Dose-limiting Structure

Historically, the use of radiation therapy for liver tumors, including intrahepatic cholangiocarcinoma (ICC), was limited by the inability to deliver a curative dose without the development of radiation-induced liver disease (RILD). RILD is a clinical syndrome of fatigue, ascites, hepatomegaly, and markedly elevated liver function tests. In a classic study from Stanford University in 1966, 12 patients were irradiated to the liver with 3,000–5,900 rads over 6 weeks for a variety of metastatic and primary tumors. Pathologic specimens were then sampled from the liver at various time points including at autopsy. They described a pattern of venoocclusive disease driven by fibrous collagen deposition in veins. For several patients, this proved fatal. If patients lived longer than 4 months, RILD seemed to resolve and improve with time [1].

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Table 1 Projected doses for partial liver tolerance in the Emami report 1991

	1/3 liver (Gy)	2/3 liver (Gy)	Whole liver (Gy)
TD 5/5	50	35	30
TD 50/5	55	45	40

TD 5/5 = dose at which 5 % of patients will have a complication at 5 years

TD 50/5 = dose at which 50 % of patients will have a complication at 5 years [2]

This pattern was seen in many of the initial studies using radiation in liver tumors. The risk of RILD appears to be driven by two treatment factors: dose of radiation to the liver and the volume of liver irradiated. In the two-dimensional (2D) radiation therapy era, radiation was delivered using plain film X-rays to guide treatment. Without the anatomic information derived from more advanced imaging techniques, such as CT scans, radiation fields had to be quite large to avoid missing tumors. Guidelines for radiation delivery were limited as there was no accurate way to calculate the volume of liver irradiated. The advent of CT-based treatment planning, so called 3D conformal radiation, allowed for both more accurate definition of tumor volumes as well as accurate calculation of both radiation dose and volume in the liver.

Radiation dose parameters which predict for RILD have evolved over time. The Emami [2] report in 1991 established largely projected doses for partial liver tolerance as shown above in Table 1.

This tolerance is considerably less than the dose needed for durable control of biliary tumors. This model has been further adapted, particularly through work done at the University of Michigan [3–6], and a mean dose to the liver of less than 32 in 2 gray (Gy) fractions was established as a more accurate TD 5/5. Additionally, they have shown that there may be no upper limit on dose in volumes less than 25 % of the liver [4, 5]. The quantitative analyses of normal tissue effects in the clinic (QUANTEC) initiative published in 2010 established similar dose tolerances based in large part on the efforts from the Michigan group [7]. They also included a tolerance dose reduction in 4–6 Gy for patients with mild pre-existing hepatic dysfunction. This improved the understanding of the threshold for RILD and has allowed for doses capable of controlling tumors, particularly with the use of stereotactic body radiation therapy (SBRT) (Table [2]).

2 Tumor Motion and the Use of Image-guided Adaptive Radiation

The advent of 3D conformal therapy and intensity-modulated radiation therapy has allowed for radiation treatment plans that shape dose around the target with a rapid

fall off in surrounding tissues. This has allowed for treatment volumes to be vastly decreased compared to traditional 2D radiation (Fig. 1). As treatment volumes have decreased, the possibility of missing the target has vastly increased. Modern radiation treatments are typically designed first to target the visible tumor or gross tumor volume (GTV). This is then expanded to encompass microscopic disease in a clinical target volume (CTV). Finally, this CTV is expanded to account for day-to-day setup error, intra-fractional motion, and uncertainty in the planning target volume (PTV). The expansion to PTV and the ratio of treated volume to GTV and CTV increases with uncertainty. In an ideal system, PTV would equal CTV.

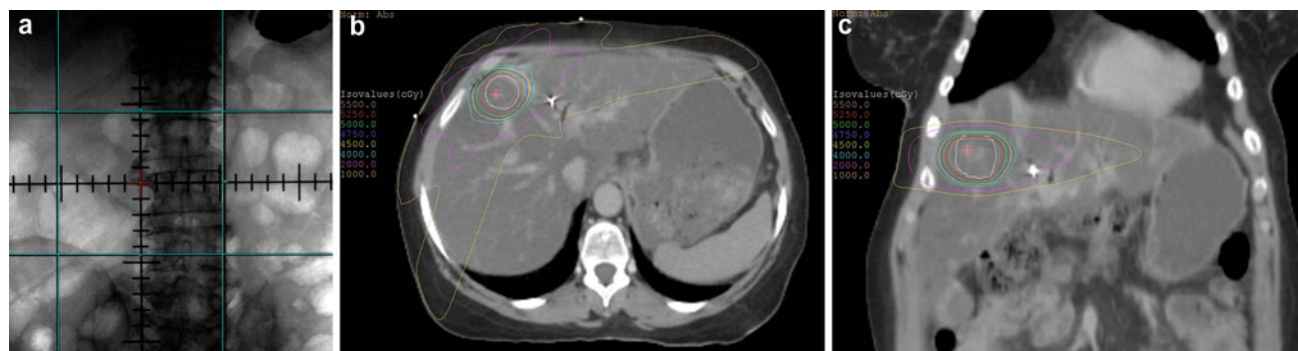
3 Four-dimensional CT Scanning

The introduction of CT scanning through the entire range of the respiratory cycle, 4D-CT scans, revealed that respiratory motion and its effect on abdominal tumors are exceptionally variable [8–10]. This uncertainty in upper abdominal tumors is primarily driven by tumor motion from breathing but is also affected by the filling of hollow organs such as the stomach and duodenum. In a review of organ motion by Langen and Jones [11], liver motion (using a variety of techniques such as scintigraphy, MRI, and ultrasound) was found to average between 8 and 25 mm under normal breathing conditions and up to 37–55 mm on deep breathing.

This motion must be accounted for and traditionally this meant expanding margins and increasing radiation dose to normal tissues. The use of 4D-CT imaging has led to the use of internal target volumes (ITVs) in several disease sites. ITV is defined as a target which accounts for physiologic tumor motion. 4D-CT scans are created by acquiring several datasets at each CT slice position throughout the entire respiratory cycle. Reconstruction of this data creates a series of CT scans at predefined positions in the respiratory cycle. Tumor motion can be assessed by viewing these scans in the axial, coronal, and sagittal planes, as a movie or “cine-mode review” which displays the entire tumor range-of-motion (Fig. 2). The tumor can then be targeted throughout its entire range-of-motion.

Table 2 Summary of control rates and reported survival in SBRT for intrahepatic cholangiocarcinoma

Study	Patients	Fractionation	System	Local control	OS	Notes
Blomgren et al. [1] Karolinska Hospital	23 with intrahepatic cancers (1 IHCC)	Mean does to the PTV from 8–63 Gy	Traditional linear accelerator with 4–8 non-coplanar uncollimated octagaonal beam	14 patients had a measurable response with five of those with stable disease	Not reported	First three patients treated with 7.7–20 Gy in one fraction and a fourth patient received two 20 Gy fractions separated by 72 days. All for of these patients progressed
Tse et al. [2] Princess Margaret Hospital	41 patients with intrahepatic cancers (ten with IHCC)	Median dose of 36 Gy (24–54 Gy) in 6 fractions	Individualized dose-escalation strategy integrating cine MRI and breath hold technique	One-year in-field control rate—65 %	Median survival—13.4 months for all patients	No RILD observed
					IHCC patient median survival—15.0 months	One patient had a tumor-duodenal fistula 15 months after SBRT and died of a GI bleed
					One-year survival—58 %	One patient developed a small bowel obstruction requiring surgery at 15 months
Goodman et al. [3] Stanford University	26 patients with intrahepatic tumors (5 with IHCC)	Dose-escalation single-fraction study	CyberKnife with implanted fiducials	One-year local failure rate—23 %	Median survival—28.6 months	No Grade 3 toxicities. Three patients developed duodenal ulcers (one early, two late)
		18 Gy (3 pts)				
		22 Gy (9 pts)				
		30 G (8 pts)			Two-year OS—50.4 %	Two patients developed chest wall pain near the site of sbrt
Ibarra et al. [4] Multi-institutional	32 patients with primary intrahepatic tumors (11 patients with IHCC)	22–37.5 Gy in varying fractionations	Varied	FFLP of 55.5 % for IHCC	One-year OS—45 %	39.5 % had Grades 1–2 toxicities (mainly nausea)
Barney et al. [5] Mayo Clinic	Ten patients with unresectable or recurrent cholangiocarcinoma	3–5 fractions to a dose of 45–60 Gy	IMRT or 3D conformal planning delivered with a standard linac	No in-field failures at median follow-up 14 months	Median OS—14 months	One patient had liver failure resulting in death

**Fig. 1** Comparison of 2D radiation fields with SBRT fields. **a** Traditional 2D fields relied on bony targets without any adjustment for motion or target localizer such as fiducials. **b** Axial CT scan showingSBRT plan with very high radiation doses using multiple beam angles. **c** Coronal CT from same SBRT plan

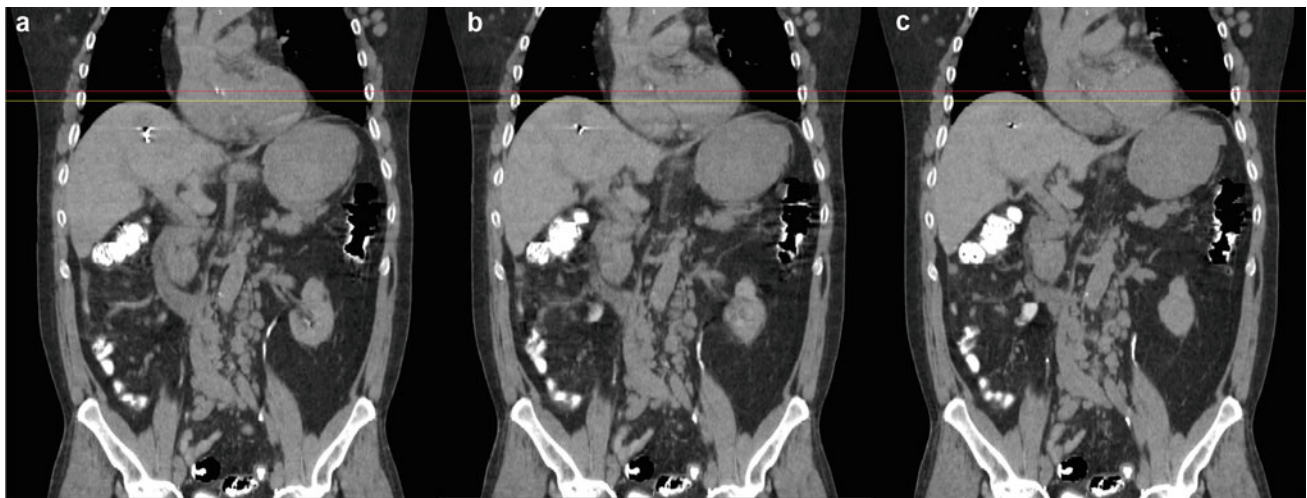


Fig. 2 Coronal CT demonstrating tumor motion throughout the respiratory cycle in the same patient. **a** End inspiration **b** mid-cycle and **c** end-expiratory. Red and yellow lines represent same plane at

each point and allow for measurement of tumor motion. The artifact within the liver represents a gold seed fiducial

4 Reducing Organ Motion

While 4D-CT scanning describes tumor and organ motion, several techniques have been developed to try to reduce this motion, thus decreasing the volume treated. Abdominal compression techniques employ a device to apply a high amount of pressure to the abdomen. This increases intra-abdominal pressure, reducing diaphragmatic motion. Initial studies in abdominal SBRT from the Karolinska Institute in Sweden describe their abdominal compression methods which reduced organ motion to 5–10 mm [12, 13]. Several additional studies have shown that liver tumor motion can be reduced to <1 cm using this technique [14, 15]. In a study from the University of Texas Southwestern, 4D-CT scans were performed on 10 patients using either no compression, medium compression, or high compression [14]. A special load cell which calculated the force applied was used to define and reproduce the two levels of compression similarly in each patient. Medium compression and high compression reduced tumor motion in the superior-inferior direction by a mean of 40 and 50 %, respectively. One pitfall of this technique is the possibility for liver shape deformation. However, a recent study from the Princess Margaret Hospital (PMH) demonstrated that inter-fraction liver-to-liver misregistrations due to deformation were less than 5 mm for most patients [16].

Active breathing control (ABC) is a method of controlled breathing which requires patient training to attempt to turn the beam on only during a favorable point in the breathing cycle. This can be difficult due to patient co-morbidities which limit their control over respiration. Respiratory gating utilizes

computerized video tracking, typically of the chest wall, to time the beam activation to a certain point in the breathing cycle. Several early studies demonstrated the feasibility of this technique with very good inter-fraction reproducibility [5, 17]. Further advancing this technique, a study from PMH utilized both daily imaging with ABC [18]. Adjustments and repositioning was performed for any positioning errors greater than 3 mm. A total of 109 of 120 fractions delivered to a total of 20 patients required this repositioning. Using this technique, they demonstrated an average reduction in absolute systematic errors from 4.1 mm (cranial–caudal), 2.4 mm (anterior–posterior), and 3.1 mm (medial–lateral) to 1.1 mm (CC), 1.3 mm (AP), and 1.6 mm (ML).

5 Image-Guidance

Accounting for motion with an ITV invariably increases the volume of treated liver. One method of increasing accuracy of the treatment delivered and reducing treated tissue is the use of adaptive image-guidance to reduce systematic uncertainty. There are, generally, two forms of adaptive image-guidance: offline and online. Offline image-guidance consists of daily or weekly review by the physician of images taken at the time of treatment delivery. Adjustments are then made in the next day treatment as needed. These typically consist of small shifts in patient position but can be as drastic as creation of a new radiation plan. While this can be a very useful tool, it does not allow for real-time adjustments prior to delivery. If daily fractions are small, this is likely of little consequence.

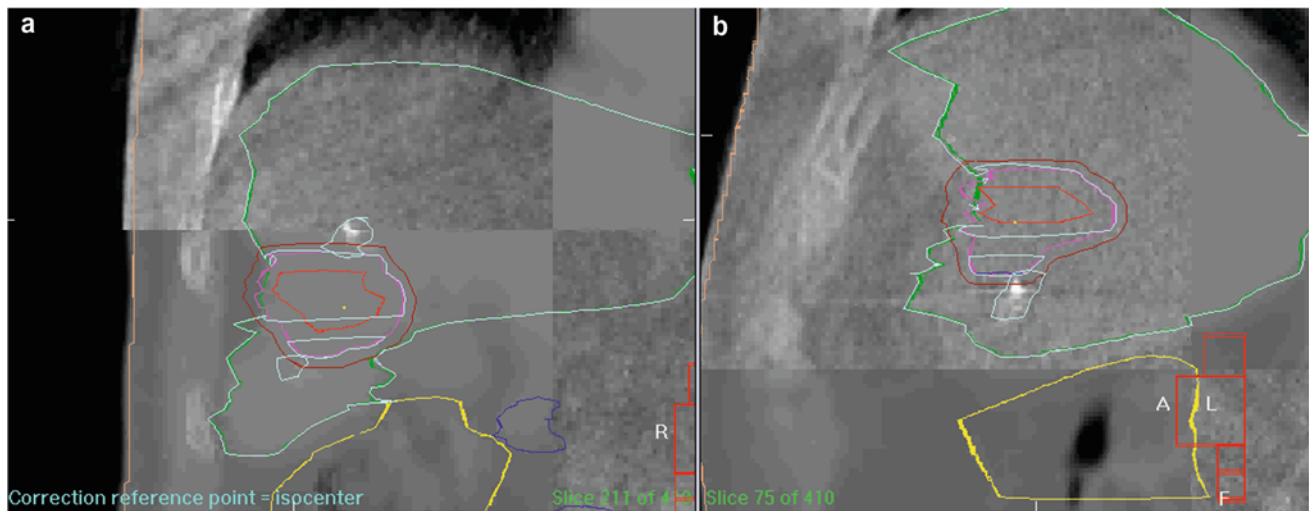


Fig. 3 **a** Coronal and **b** sagittal cone-beam CT overlays from online imaging prior to a liver SBRT treatment. Quadrants **a** and **c** in each film represent the planning CT scan, while quadrants **b** and **d** represent the cone-beam CT taken at the time of delivery. The liver contour (light

green) is primarily aligned using a fiducial contour (light blue). The physician can view these overlays in a variety of contrasts and change the focus from planning scan to treatment scan in real time

As daily doses have increased to as high as 30 Gy per day, the need for accurate imaging in real time at delivery has become essential. Online imaging consists of imaging done at treatment which is reviewed by the physician prior to delivery. While this can be as simple as non-diagnostic megavoltage imaging, newer treatment machines come equipped with onboard imaging (OBI) capable of diagnostic X-rays and even CT. In the era of 2D radiation therapy, daily or weekly megavoltage portal imaging was used to ensure whether the radiation was being delivered to the target. MV imaging, while useful in other disease sites, has limited utility in the upper abdomen as tracking to bony anatomy does not provide for respiratory motion or daily changes in hollow viscera.

The development of treatment machines capable of diagnostic-quality kilovoltage CT scanning (CT-on-rails, cone-beam CT scans, fan beam CT) has expanded this capability tremendously. While most liver tumors do not appear on CT images, gold fiducials implanted near the tumor can be easily tracked each day prior to treatment with image-guidance (Fig. 3). In a series of studies from Hokkaido University, gold seed implantation in the liver was found to be both feasible and highly accurate [19, 20] with interobserver variability of 2.5 mm for the distance from the center of mass of the liver and the tumor. Several studies have demonstrated the effectiveness of gold seed tracking in liver tumors [21–23]. When using this technique, communication between the treating radiation oncologist and the physician implanting the gold seed markers is critical for assessment of marker proximity to tumors.

6 Emergence of Stereotactic Body Radiation Therapy for Cholangiocarcinoma

6.1 Introduction

Safe delivery of very high single or few doses of radiation to a highly conformal target has, until very recently, been beyond the limits of our technology. Traditional radiation treatments target both the tumor and a margin of normal tissue. This margin helps account for uncertainty in daily treatment such as respiratory motion, changes in hollow organ motion, and technical factors such as limitations of the linear accelerator or in-room positioning system. Radiation predominantly kills cells through induction of double-stranded DNA breaks which are highly cytotoxic. Normal tissues, for the most part, are able to repair these double-stranded breaks much more efficiently than tumor cells. As radiation oncologists, we capitalize on this gradient by delivering radiation at doses which kill tumor cells but allow for repair of normal tissues. This is highly dependent on both the dose per day and the total dose.

The differential between tumor kill and normal cell kill decreases in a continuous fashion with increasing dose. Through clinical experimentation at each disease site, our field has established generally accepted daily and total doses for each disease site, which balance the probability of tumor control with both severe acute and long-term toxicities. In any treatment which includes a significant amount of normal tissue, standard fractionation for gastrointestinal malignancies uses treatment regimens of around 1.5–3.0 Gy



Fig. 4 Sample patient undergoing radiation planning using a vacuum bag. This bag is placed around the patient and the air removed via vacuum. This both immobilizes the patient and prevents deep inspiration reducing respiratory motion

per day for 25–28 treatments to total doses of ~ 50 Gy. Above these doses, moderate-to-severe toxicities limit any benefit of treatment.

The ability to deliver higher daily doses for fewer total treatments was beyond the limits of our technology for most of the history of radiation oncology. This technique was first applied successfully in 1951 with the development of the Gamma Knife. This system uses a bolted-on skull frame to create a 3D coordinate system known as stereotactic space—hence, the technique was named stereotactic radiosurgery (SRS). Benefitting from the bony landmarks of the skull and lack of movement in intracranial contents, SRS successfully ablated intracranial tumors using a few very high-dose fractions despite the limitations in targeting in the pre-MRI/CT era.

It would take almost 50 years for the technical advances necessary to apply this technique to extracranial targets. The advances described above, particularly image-guidance and adjustments for respiratory motion, have allowed for accurate targeting while advances in beam shaping in the linear accelerator have allowed for increasingly conformal treatments. The American Society for Radiation Oncology (ASTRO) defines SBRT as a highly conformal extracranial high-dose radiation treatment delivered in five or fewer fractions designed to kill all living tissue within a target [24]. SBRT uses regimens such as 18–30 Gy in a single fraction or 45–60 Gy given over three to five fractions. At these daily doses, it is theorized that the radiation has more of an ablative effect on the tumor, rather than relying on

gradual cell death from double-stranded DNA breaks as in standard fractionation.

6.2 Technique

There are several commercially available SBRT systems in use today. Common among these systems are three key features: stereotactic target localization reference frame, immobilization, and tumor motion tracking. The creation of a stereotactic coordinate system to target a lesion typically consists of a body-frame which appears on the planning CT scan at marked intervals. Frameless SBRT does exist in the form of the Elekta CyberKnife system [25]. This system uses onboard kilovoltage (kV) imaging to track tumor motion using tumor surrogates such as the spine, skull, or implanted fiducials. Other systems such as Novalis, Gamma Knife, and traditional linear accelerator-based systems require a frame (Fig. 4).

6.3 Dose Delivery

Dose is delivered via many low-dose beamlets targeting a common disease site, but delivered from different patient entry points. These beamlets allow for summation at the tumor but are typically too low dose to induce side-effects in intervening tissue. Generally, by increasing the number of beamlets for a given treatment site, the target

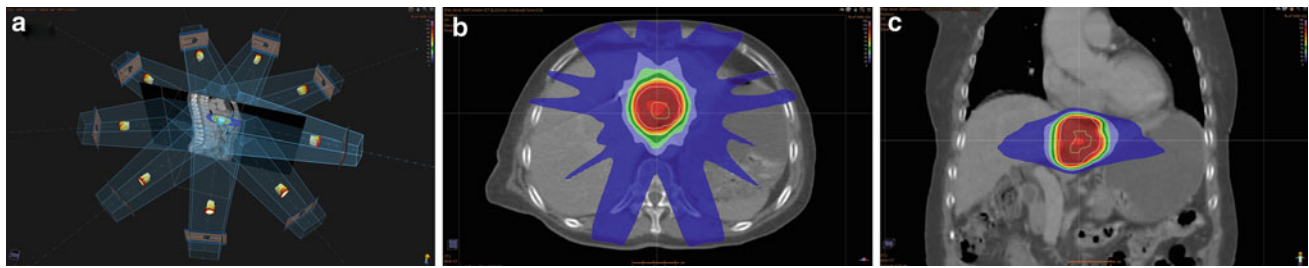


Fig. 5 Representative SBRT plan for an intrahepatic tumor. A total of 50 Gy was delivered over 5 separate 10 Gy fractions. **a** Radiation planning representation of a nine-beam SBRT plan. **b** Axial CT slice through the isocenter showing the GTV (yellow unfilled central

contour) being covered by the 105 % isodose line. Each successive isodose line represents a lesser radiation dose. This demonstrates the rapid drop-off in dose. **c** Coronal view of same plan

conformality may be improved. The use of many beams (in Cyberknife >100 beams are typical) creates a very conformal plan in the high-dose region (Fig. 5).

6.4 Stereotactic Body Radiation Therapy for Cholangiocarcinoma

Cholangiocarcinoma can be either ICC or extrahepatic cholangiocarcinoma (ECC). Though only 5–10 % of cholangiocarcinomas are intrahepatic [26], ICCs are the focus of most existing literature as they are typically included in trials using SBRT for hepatocellular carcinoma (HCC) or liver metastases. While a margin-negative resection (R0) is the only curative option, most patients present with unresectable disease due to vascular invasion or risk of hepatobiliary compromise. Patients with unresectable disease treated with standard fractionated radiotherapy and/or chemotherapy have traditionally had dismal outcomes. SBRT allows for the delivery of very high doses, especially relative to conventional equivalents, that have shown some promise with respect to local recurrence. Distant failure remains high, underscoring a need for more effective systemic agents. Here, we review the literature describing SBRT for both ICC and HCC.

7 Stereotactic Body Radiation Therapy for Intrahepatic Cholangiocarcinoma

7.1 Initial Experiences

Data on the use of SBRT for ICC exists within several studies which included cholangiocarcinoma, HCC, and metastatic disease. Because cholangiocarcinoma is a rare tumor, data on technique and dose-limiting structures are primarily extrapolated from studies using SBRT for HCC and metastases. Blomgren et al. at the Karolinska Institute in Sweden were the first to describe SBRT for intrahepatic

lesions [13]. In a study conducted in the early 1990s, they treated a single patient with ICC and 23 total with intrahepatic cancers. Their mean dose to the PTV ranged from 8 to 63 Gy. Treatment was delivered using 4–8 non-coplanar octagonal beams without the use of multi-leaf collimation. Treatment was prescribed with up to a 50 % hotspot in the isocenter. The first three patients treated received 7.7–20 Gy in a single fraction due to concern for RILD. A fourth patient received two 20 Gy fractions separated by 72 days. All four of these patients developed progression at the site of disease. One patient died two days after a single 30 Gy fraction to the PTV. However, this patient was cirrhotic with ascites prior to treatment. Other notable toxicity included fever and nausea of a few hours duration immediately after treatment in all patients. Fourteen tumors showed measurable response with an additional five with stable disease. Survival was not reported.

In a more modern series, Dawson et al. at PMH in Toronto reported the results of a dose-escalation study with 10 patients with unresectable ICC in a series of 41 patients treated with SBRT from 2003 to 2006 for intrahepatic cancers [3, 27]. In this series, patients underwent two training sessions to learn the ABC system [17]. Patient diaphragm motion was assessed using kV fluoroscopy with the vertebral bodies as a reference point. Reproducibility and necessity of ABC were determined for each case. Simulation consisted of a tri-phasic CT for tumor delineation along with a planning CT scan at end expiration for patients using ABC. Liver tumor motion in patients who were free breathing was assessed by 4D CT. GTV was defined on end exhalation breath hold with an 8-mm margin expansion to CTVs. For free breathing patients, an ITV encompassing the entire tumor range-of-motion was created using 4D cines confirmed by corresponding fluoroscopy and MRI. The Lyman–Kutcher–Burman normal tissue complication probability model (NTCP) [28], initially developed at the University of Michigan, was employed to individualize each patient's dose as previously established in the PMH group's conformal radiation series [4]. Normal/functional

liver tumor volume (Veff) irradiated was calculated by subtracting the GTV from the total liver volume. Patients were not treated if Veff was greater than 0.8. Image-guidance for each fraction consisted of orthogonal megavoltage imaging, kV fluoroscopy, and kV cone-beam CT. Patients were repositioned for offsets greater than 3 mm. SBRT was delivered using three to ten coplanar or non-coplanar beams from 6 to 18 MV and given over six fractions during 2 weeks. A dose-escalation algorithm was used based on the Veff. For the low range of Veff (≤ 0.2), patients received 9, 9.5, or 10 Gy per fraction for six fractions with higher-risk patient dosages determined by the NTCP model. The median tumor volume was 173 ml, and median Veff was 0.48. The median dose delivered was 36 Gy (range 24–54 Gy). At a median follow-up of 17.6 months, there was no dose-limiting toxicity or RILD. Two patients developed transient biliary obstruction during treatment. Other side-effects included elevated liver enzymes, thrombocytopenia, and right-sided pleural effusion. Two patients had late GI toxicity (tumor-duodenal fistula and a small bowel obstruction at 15 and 17 months, respectively). The median survival was 13.4 months with a one-year survival of 51 % for the group. For the IHC patients, median survival and one-year survival were 15.0 months and 58 %, respectively. The one-year local control rate was 65 % for all patients with IHC results not individually reported. Additionally, they found that cranial caudal tumor motion averaged 17 mm with a maximum of 29 mm. Anterior-posterior and medial-lateral motion averaged 9 and 8 mm, respectively.

Goodman et al. [29] reported on a phase I dose-escalation study at Stanford University. A total of 26 patients with intrahepatic lesions less than 5 cm were treated between 2004 and 2008 with single-fraction SBRT including five patients with ICCs. A total of 26 patients were treated with a single fraction of 18 ($n = 3$), 22 ($n = 6$), 26 ($n = 9$), or 30 Gy ($n = 8$). Maximum tolerated dose was defined as more than 50 % of patients experiencing dose-limiting toxicity, particularly gastrointestinal dysfunction or hepatic dysfunction. One patient developed an acute duodenal ulcer, while two patients developed late duodenal ulcers. Of note, one patient that developed a duodenal ulcer received a single-fraction maximum dose to the duodenum of 29 Gy (BED2 = 187.7 Gy). Risk of local failure at 12 months was 23 %. Median survival and two-year actuarial overall survival were 28.6 months and 50.4 %, respectively.

Ibarra et al. [30] treated 11 patients with ICC with SBRT at four institutions with doses ranging from 22 to 37.5 Gy in varying fractionation schedules. With a median follow-up of 7.8 months, the freedom from local progression was

55.5 %. Barney et al. [31] treated ten patients with unresectable primary or recurrent cholangiocarcinoma at the Mayo Clinic Rochester from 2009 to 2011. Most ($n = 10$) patients had intrahepatic lesions. Radiation was prescribed to a median dose of 55 Gy in three to five fractions. At a median follow-up of 14 months, freedom from local progression was 100 %. One Grade 3 biliary stenosis and a single Grade 5 (death) liver failure were observed.

8 Extrahepatic Cholangiocarcinoma

Kopek et al. [32] reported the results of 27 patients with unresectable cholangiocarcinoma at Aarhus University in Denmark from 1999 to 2006. The majority ($n = 26$) of patients had Klatskin tumors. Patients were immobilized using a stereotactic body-frame and a vacuum pillow. GTV was determined by CT with additional information from ERCP, MRCP, and PET/CT. The PTV was expanded more CC (10 mm) than radially (5 mm) based on the observed tumor motion in the Karolinska study. The prescription dose was 45 Gy in three fractions given over 5–8 days with at least the 95 % isodose covering the CTV and 67 % isodose covering the PTV. The actuarial one-year local control rate was 84 %. Median progression-free survival and overall survival were 6.7 months and 10.6 months. Significant toxicities (greater than Grade 3) included six patients with duodenal/pyloric ulcerations and two with duodenal stenosis.

Polistina et al. [33] reported on a series of two patients with unresectable Klatskin tumors treated with combination SBRT and gemcitabine from 2004 to 2009 in Venice. Patients were treated with intravenous gemcitabine weekly at a dose of 1,000 mg/m². Prescribed dose was 30 Gy in three consecutive daily fractions to the 80 % isodose covering 90 % of the PTV. Notable dose limits included <15 Gy to at least 700 ml of normal liver and less than 24 Gy to the duodenum. Six patients developed local progression and six developed distant metastases. Two-year survival was 80 %, and four-year survival was 30 % with a median survival of 35.5 months.

9 Conclusions

The integration of advanced imaging and treatment technologies has improved the capabilities of SBRT for biliary tract cancers from the early series at Karolinska using octagonal beams to advanced techniques integrating real-time fiducial tracking, abdominal compression, respiratory synchronization, and advanced collimation. A developing

body of literature supports the use of SBRT in unresectable or medically inoperable patients with both ICC and ECC. However, these data are gleaned predominantly from small, single-institution retrospective or phase I studies. The rarity of cholangiocarcinoma and the lack of clear guidelines underscore the need for large cooperative group, multi-institution randomized trials.

References

1. Reed GB Jr, Cox AJ Jr (1966) The human liver after radiation injury. A form of veno-occlusive disease. *Am J Pathol* 48(4):597–611
2. Emami B, Lyman J, Brown A et al (1991) Tolerance of normal tissue to therapeutic irradiation. *Int J Radiat Oncol Biol Phys* 21(1):109–122
3. Dawson LA, Eccles C, Craig T (2006) Individualized image guided iso-NTCP based liver cancer SBRT. *Acta Oncol* 45(7):856–864
4. Dawson LA, Normolle D, Balter JM, McGinn CJ, Lawrence TS, Ten Haken RK (2002) Analysis of radiation-induced liver disease using the Lyman NTCP model. *Int J Radiat Oncol Biol Phys* 53(4):810–821
5. Dawson LA, Ten Haken RK, Lawrence TS (2001) Partial irradiation of the liver. In: *Seminars in radiation oncology*, vol 11(3). Jul 2001 pp 240–246
6. Ben-Josef E, Normolle D, Ensminger WD et al (2005) Phase II trial of high-dose conformal radiation therapy with concurrent hepatic artery floxuridine for unresectable intrahepatic malignancies. *J Clin Oncol* 23(34):8739–8747 (official journal of the American Society of Clinical Oncology)
7. Marks LB, Yorke ED, Jackson A et al (2010) Use of normal tissue complication probability models in the clinic. *Int J Radiat Oncol Biol Phys* 76(Suppl 3):S10–S19
8. Pan T, Lee TY, Rietzel E, Chen GT (2004) 4D-CT imaging of a volume influenced by respiratory motion on multi-slice CT. *Med Phys* 31(2):333–340
9. Rietzel E, Chen GT, Choi NC, Willet CG (2005) Four-dimensional image-based treatment planning: target volume segmentation and dose calculation in the presence of respiratory motion. *Int J Radiat Oncol Biol Phys* 61(5):1535–1550
10. Rietzel E, Pan T, Chen GT (2005) Four-dimensional computed tomography: image formation and clinical protocol. *Med Phys* 32(4):874–889
11. Langen KM, Jones DT (2001) Organ motion and its management. *Int J Radiat Oncol Biol Phys* 50(1):265–278
12. Lax I, Blomgren H, Naslund I, Svanstrom R (1994) Stereotactic radiotherapy of malignancies in the abdomen: methodological aspects. *Acta Oncol* 33(6):677–683
13. Blomgren H, Lax I, Naslund I, Svanstrom R (1995) Stereotactic high dose fraction radiation therapy of extracranial tumors using an accelerator: clinical experience of the first thirty-one patients. *Acta Oncol* 34(6):861–870
14. Heinzerling JH, Anderson JF, Papiez L et al (2008) Four-dimensional computed tomography scan analysis of tumor and organ motion at varying levels of abdominal compression during stereotactic treatment of lung and liver. *Int J Radiat Oncol Biol Phys* 70(5):1571–1578
15. Wunderink W, Mendez Romero A, De Kruijf W, de Boer H, Levendag P, Heijmen B (2008) Reduction of respiratory liver tumor motion by abdominal compression in stereotactic body frame, analyzed by tracking fiducial markers implanted in liver. *Int J Radiat Oncol Biol Phys* 71(3):907–915
16. Eccles CL, Dawson LA, Moseley JL, Brock KK (2011) Interfraction liver shape variability and impact on GTV position during liver stereotactic radiotherapy using abdominal compression. *Int J Radiat Oncol Biol Phys* 80(3):938–946
17. Wong JW, Sharpe MB, Jaffray DA et al (1999) The use of active breathing control (ABC) to reduce margin for breathing motion. *Int J Radiat Oncol Biol Phys* 44(4):911–919
18. Dawson LA, Eccles C, Bissonnette JP, Brock KK (2005) Accuracy of daily image guidance for hypofractionated liver radiotherapy with active breathing control. *Int J Radiat Oncol Biol Phys* 62(4):1247–1252
19. Kitamura K, Shirato H, Shimizu S et al (2002) Registration accuracy and possible migration of internal fiducial gold marker implanted in prostate and liver treated with real-time tumor-tracking radiation therapy (TRTR). *Radiother Oncol* 62(3):275–281 (journal of the European Society for Therapeutic Radiology and Oncology)
20. Shirato H, Harada T, Harabayashi T et al (2003) Feasibility of insertion/implantation of 2.0 mm-diameter gold internal fiducial markers for precise setup and real-time tumor tracking in radiotherapy. *Int J Radiat Oncol Biol Phys* 56(1):240–247
21. Wunderink W, Mendez Romero A, Seppenwoolde Y, de Boer H, Levendag P, Heijmen B (2010) Potentials and limitations of guiding liver stereotactic body radiation therapy set-up on liver-implanted fiducial markers. *Int J Radiat Oncol Biol Phys* 77(5):1573–1583
22. Seppenwoolde Y, Wunderink W, Wunderink-van Veen SR, Storchi P, Mendez Romero A, Heijmen BJ (2011) Treatment precision of image-guided liver SBRT using implanted fiducial markers depends on marker-tumour distance. *Phys Med Biol* 56(17):5445–5468
23. Park JC, Park SH, Kim JH et al (2012) Liver motion during cone beam computed tomography guided stereotactic body radiation therapy. *Med Phys* 39(10):6431–6442
24. Potters L, Kavanagh B, Galvin JM et al (2010) American Society for Therapeutic Radiology and Oncology (ASTRO) and American College of Radiology (ACR) practice guideline for the performance of stereotactic body radiation therapy. *Int J Radiat Oncol Biol Phys* 76(2):326–332
25. Adler JR Jr, Chang SD, Murphy MJ, Doty J, Geis P, Hancock SL (1997) The Cyberknife: a frameless robotic system for radiosurgery. *Stereotact Funct Neurosurg* 69(1–4 Pt 2):124–128
26. Aljiffry M, Walsh MJ, Molinari M (2009) Advances in diagnosis, treatment and palliation of cholangiocarcinoma: 1990–2009. *World J Gastroenterol* 15(34):4240–4262
27. Tse RV, Hawkins M, Lockwood G et al (2008) Phase I study of individualized stereotactic body radiotherapy for hepatocellular carcinoma and intrahepatic cholangiocarcinoma. *J Clin Oncol* 26(4):657–664 (official journal of the American Society of Clinical Oncology)
28. Kutcher GJ, Burman C, Brewster L, Goitein M, Mohan R (1991) Histogram reduction method for calculating complication probabilities for three-dimensional treatment planning evaluations. *Int J Radiat Oncol Biol Phys* 21(1):137–146
29. Goodman KA, Wiegner EA, Maturen KE et al (2010) Dose-escalation study of single-fraction stereotactic body radiotherapy for liver malignancies. *Int J Radiat Oncol Biol Phys* 78(2):486–493

30. Ibarra RA, Rojas D, Snyder L et al (2012) Multicenter results of stereotactic body radiotherapy (SBRT) for non-resectable primary liver tumors. *Acta Oncol* 51(5):575–583
31. Barney BM, Olivier KR, Miller RC, Haddock MG (2012) Clinical outcomes and toxicity using stereotactic body radiotherapy (SBRT) for advanced cholangiocarcinoma. *Radiat Oncol* 7:67
32. Kopek N, Holt MI, Hansen AT, Hoyer M (2010) Stereotactic body radiotherapy for unresectable cholangiocarcinoma. *Radiother Oncol* 94(1):47–52 (journal of the European Society for Therapeutic Radiology and Oncology)
33. Polistina FA, Guglielmi R, Baiocchi C et al (2011) Chemoradiation treatment with gemcitabine plus stereotactic body radiotherapy for unresectable, non-metastatic, locally advanced hilar cholangiocarcinoma. Results of a five year experience. *Radiother Oncol* 99(2):120–123 (journal of the European Society for Therapeutic)

Symptomatic Management and Palliation

Laura Lambert

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Abstract

For most patients with biliary cancer effective treatment options are limited, side effects are common, and the long-term prognosis is grim. A majority of biliary cancer patients are diagnosed with advanced-stage cancer. Many have comorbidities that preclude major hepatic surgery, and it is estimated that up to 25 % of patients who are considered surgical candidates after a thorough preoperative work-up are unable to undergo a complete surgical resection. Of those who do undergo curative resection, 5-year survival is less than 50%. Patients with metastatic disease have limited chemotherapy options and on average survive only 3–6 months. Given the poor prognoses of biliary tract and gallbladder cancers, it is important that caregivers are educated on symptom management and palliation for this patient population.

Despite the novel treatments and increased understanding of biliary cancers described in the other chapters of this book, for most patients with biliary cancer, meaningful treatment options are limited, the efficacy of treatment poor, and the prognosis grim. More than half of all biliary cancer patients are diagnosed with advanced-stage cancer. Many have comorbidities that preclude major hepatic surgery, and it is estimated that up to 25 % of patients who are considered surgical candidates after a thorough preoperative work-up are unable to undergo a complete surgical resection [1, 2]. Of those who do undergo curative resection, 5-year survival is only 30–40 % without lymph node involvement and only 10–15 % with lymph node involvement [3, 4]. Patients with metastatic disease (up to 30 % at the time of diagnosis) have an average survival of 3–6 months [5].

In addition to the dismal prognosis associated with biliary cancer, patients also suffer from both disease-related and treatment-related symptoms and are at high risk for experiencing a “bad death.” Not only do patients with biliary cancer experience the protean symptoms of advanced

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cancer (pain, nausea, cachexia, fatigue, anxiety, and depression), but also biliary cancers often create a unique set of challenging symptoms such as biliary obstruction with resultant jaundice, pruritis, cholangitis, gastric outlet and duodenal obstruction, liver failure, ascites, and intestinal dysmotility or obstruction from peritoneal carcinomatosis. This chapter discusses palliation of both biliary cancer-specific symptoms and some of the more common symptoms associated with advanced cancer. Additionally, the rationale and indications for early palliative care intervention and the role of hospice are reviewed.

To many cancer patients, their families, and clinicians, the words “palliative care” signify that there are no more options for treating the cancer. Most equate a palliative care consult with “giving up,” a lack of hope, and imminent death. However, in actuality, palliative care is a woefully underutilized and effective adjunct to ongoing cancer treatment. Recent studies have shown that patients who receive early palliative care in addition to standard oncologic therapy live significantly longer with a better quality of life, often despite receiving less chemotherapy than patients who do not have an early palliative care intervention. In a recent study by Temel et al., patients with advanced lung cancer who were randomized to receive standard chemotherapy plus palliative care lived an average of 2.7 months longer (11.6 vs. 8.9 months, $p = 0.05$), despite receiving less aggressive end-of-life care, than patients randomized to receive chemotherapy alone [6]. Although patients in both arms received a statistically equivalent amount of chemotherapy, patients who received early palliative care were 50 % less likely to receive systemic chemotherapy in the last 60 days of life, had a significantly longer interval between the last chemotherapy dose and death, and were more likely to spend more than a week in hospice [7].

A common misunderstanding is that cancer patients cannot receive palliative care while actively being treated for their cancer. While this is true for patients who choose to use their hospice benefit, any patient with symptoms is eligible for and should receive palliative care (either by their primary physicians or by a palliative care specialist). Furthermore, for patients with advanced cancer, even those who are asymptomatic, a palliative care consult at the time of diagnosis is now recommended by the American Society of Clinical Oncology (ASCO) and National Cancer Center Network (NCCN) guidelines [8, 9]. The goal of such a consult for the asymptomatic patient is to establish a relationship with the palliative care team who can help with difficult discussions around goals of care, code status, and provide end-of-life care options and education. For the symptomatic patient, a palliative care consult also provides expert management of disease-specific and treatment-related symptoms. Another benefit of early palliative care referral is that it provides the treating medical, surgical, and

radiation oncologists with clinical and emotional support and allows them to keep their focus on their primary role in the treatment of the patient. When the time comes for an alteration of goals of care from cancer treatment to symptom control and maximizing quality of life, an established relationship with the palliative care team can facilitate this transition in a most effortless manner.

1 Biliary Obstruction

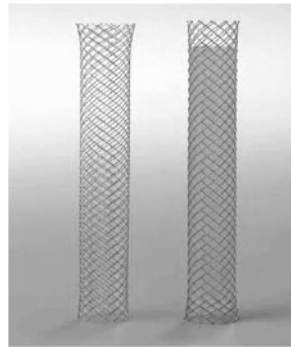
Most patients with biliary cancers present with some manifestation of biliary obstruction—jaundice, hepatic atrophy, or cholangitis. For patients with resectable tumors, surgery offers both a therapeutic and a palliative benefit. For patients with unresectable or metastatic tumors, biliary decompression is essential for palliation.

Biliary decompression can be achieved through a number of approaches including surgical, percutaneous, and endoscopic. In the setting of an extrahepatic biliary obstruction, surgical decompression can be achieved by the performance of a choledochoduodenostomy or choledochojunostomy. These are major surgical procedures that require technical expertise, particularly in the setting of an advanced cancer and frail patient. Due to the significant risk of morbidity (14–48 %) and mortality (3–16 %) associated with these procedures and with the advent of equally effective and less invasive methods of biliary decompression, surgery is used much less frequently than previously [10–14].

For many years, percutaneous drainage was the preferred method for both distal and more proximal biliary obstructions and for patients who were not surgical candidates. These procedures are typically performed by interventional radiologists. Until recently, the percutaneous approach allowed placement of larger plastic stents with greater rates of patency than the endoscopic approach which was limited by the access channel of the duodenoscope. Percutaneous drainage has been shown to be equivalent to surgical decompression in terms of survival and better in terms of morbidity and 30-day mortality [15]. Percutaneous drainage can be performed with either external drainage or internal drainage. The advantage of external drainage is that it is a one-step procedure that involves percutaneous access of typically dilated peripheral biliary radicals. The disadvantages of external drainage are the need for a drainage bag, pain, irritation at the drain site, risk of infection, leakage of ascites and/or bile, and fluid loss. Internalization of the stent requires a second procedure, and most patients require additional interventions and follow-up.

Currently, the preferred method for biliary decompression of distal malignant obstruction is endoscopic stent placement. The development of larger access channels for the duodenoscope and self-expanding metal stents (SEMS) has

Fig. 1 Uncovered (*left*) and covered (*right*) self-expandable metal stents (SEMS) (from first edition of book—needs permission)



significantly improved the patency of endoscopic stents (Fig. 1). Endoscopic stents are available in both plastic and metal. Plastic stents have the advantage of being easy to place, are less expensive than metal stents, and can be removed if necessary. The main disadvantage of plastic stents is their relatively short patency of 3–4 months [16–19]. Obstruction of plastic stents is usually caused by biofilm (the deposition of a proteinaceous material) and bacterial overgrowth, rather than tumor infiltration [20–22]. Consequently, patients undergoing palliative stent placement with a life expectancy longer than 3–4 month will likely need additional procedures for stent replacement and also run the risk of developing cholangitis due to stent obstruction [23].

SEMS have a number of advantages over plastic stents and are the recommended stent type for palliation of malignant biliary obstruction. First, they can expand up to three times the internal diameter of plastic stents. Consequently, they tend not to be obstructed by biofilm or bacterial overgrowth. Second, their duration of patency is 8–9 months, which is often longer than the life expectancy of patients with malignant biliary obstruction. Unlike plastic stents, if metal stents, which are constructed of an alloy mesh, become obstructed, it is usually tumor in-growth and overgrowth [24–26]. In an effort to combat this issue, SEMS with a membrane covering the alloy mesh were developed (covered SEMS). Studies have shown that covered SEMS do have a significantly longer patency, but are associated with increased risk of complications such as cholecystitis and pancreatitis as compared to uncovered SEMS [27].

In summary, endoscopically placed SEMS are the recommended first line of therapy for non-hilar malignant biliary obstructions. These stents offer a better quality of life for patients through the avoidance of additional procedures, increased symptom-free periods, potentially improved survival, and lower cost due to fewer endoscopies. Percutaneous drainage is the preferred approach for patients with hilar tumors, patients undergoing brachytherapy, or those who are not candidates for endoscopy.

Potential adjuvant therapies to stenting or bypass for palliation of symptomatic, unresectable biliary cancers include radiation and photodynamic therapy. The use of

radiation has been shown to help with pain, stent patency, and survival (in the absence of metastatic disease) [28, 29]. Radiation therapy can be delivered as external beam radiation, brachytherapy, or a combination of the two. The majority of the studies showing a benefit of radiation used a combination of both modalities with patient survival ranging from 9 to 14 months [30–33]. Because radiation is often associated with higher rates of complications including cholangitis, longer hospital stays, and gastroduodenitis, it must be used selectively. Another approach to radiation therapy is radioembolization with Yttrium-90 microspheres. This approach is actually associated with fewer side effects and equivalent outcomes and is becoming the approach of choice in centers that offer this technology [34].

Another novel approach is photodynamic therapy. Photodynamic therapy involves the intravenous administration of a photosensitizing agent that is preferentially taken up by cancer cells. The cells are then exposed to light of specific wavelengths that activates the photosensitizer and kills the cells. The depth of tumor necrosis is 4–6 mm. Improvements in quality of life, biliary drainage, and survival have been reported with this approach [35–37]. Other regional therapy options for palliation also include transarterial chemoembolization (TACE) and radiofrequency ablation (RFA), which are discussed in other chapters of this text.

Symptoms associated with malignant biliary obstruction can include jaundice, pruritus, cholangitis, biliary colic, tender hepatomegaly, fatigue, weakness, anorexia, and cachexia. Previous studies have shown that relief of biliary obstruction can significantly improve the jaundice, pruritus, anorexia, indigestion, sleep and global health scores [38–41]. As patients with biliary cancers are also at increased risk for hepatic dysfunction, other sources of jaundice must also be investigated such as medications (including non-prescription), intrahepatic cholestasis, intraparenchymal tumor burden, and hemolysis.

2 Pruritus

One of the most frustrating symptoms of biliary obstruction and cholestasis is pruritus. If the cholestasis is due to biliary obstruction, successful relief of the obstruction is usually associated with complete relief of the pruritus. However, if the cholestasis is due to intrahepatic infiltration of tumor, pruritus can be extremely challenging to treat. Because this symptom impacts every aspect of a person's life, management efforts are a priority for palliation. Despite significant research, the true cause of the pruritus remains unknown and the optimal management is not clearly defined. One prominent theory is that the pruritus of cholestasis is mediated centrally by endogenous opioids [42]. This theory has been supported by findings of increased opioidergic tone in

animal models, as well as through clinical evidence of symptom relief from opioid antagonists such as naloxone, naltrexone, and nalmefene [43–49]. Administration of these agents must be used with caution due to the significant incidence of early and short-term opioid withdrawal-like symptoms including nausea, hallucinations, and dysphoria—even in opioid-naïve patients. Many of these side effects can be avoided with the initial use of ultra-low doses of intravenous naloxone before advancing to oral naltrexone for maintenance [50, 51]. Obviously, one concern is the prevalent use of opioid analgesics in biliary cancer patient. Fortunately, other studies in postoperative patients have shown that low-dose naloxone can improve the opioid side effects, including pruritus, without loss of the analgesic effects [52, 53].

Other potential options to treat pruritus of cholestasis include medications with central, opioid-related effects (e.g., GABA agonism by propofol), serotonin antagonists (e.g., ondansetron, mirtazapine, sertraline), intravenous lidocaine, and dronabinol. Other efforts have focused on removal of the pruritogens by such means as anion exchange resins (e.g., cholestyramine), plasmapheresis, and other extracorporeal techniques (e.g., hemodialysis). However, the use of such methods may not be feasible in patients with advanced cancers due to unpalatable side effects. Drugs that induce hepatic enzymes such as rifampin and phenobarbital have also had some success. Because there is no demonstrated role for histamines in the pruritus of cholestasis, it is not surprising that antihistamines have not proven hugely effective outside of the potential sedative effect. Topical therapies such as bathing with sodium bicarbonate can be helpful [54]. Consultation with a dermatologist to identify potential primary skin lesions and for topical medication recommendations is also advisable. Finally, in terminally ill patients with refractory pruritus, sedation may be helpful.

3 Gastric Outlet and Duodenal Obstruction

Due to their proximity to the distal stomach and duodenum, obstruction of the gastric outlet and/or duodenum is a common occurrence in patients with biliary cancer. Most patients present with nausea and vomiting of undigested food. Many will have upper abdominal distention and tympani. Imaging studies often reveal a distended stomach with retained enteric contents. Acute symptoms can be managed with a nasogastric tube, bowel rest, and intravenous resuscitation, including aggressive electrolyte repletion. For patients with chronic gastric outlet obstruction, special attention should be paid to chloride replacement which is lost in large quantities with emesis of gastric

secretions. If significant weight loss is present or there may be a delay in definitive treatment, it may be reasonable to consider parenteral nutrition while planning additional palliative measures.

Options for managing these obstructions include intraluminal stenting, surgical bypass, and decompression gastrostomy with possible feeding jejunostomy. The potential benefits of stenting include immediate palliation of nausea and vomiting with a less invasive procedure than surgical bypass and earlier resumption of oral nutrition [55, 56]. Stents may be particularly useful in patients with advanced metastatic disease, who are poor surgical risk or who are technically inoperable. Flexible SEMS can be placed using endoscopic or fluoroscopic techniques. Stenting provides a comparable survival outcome with equivalent morbidity and mortality to surgical bypass [57]. In a systematic review of the literature comparing endoscopic stenting with open surgical bypass from 1990 to 2008, Ly et al. found that endoscopic stenting was more likely to result in tolerance of oral intake (OR 2.6; $p = 0.002$), in a shorter period of time (mean difference of 6.9 days, $p < 0.001$) and with a shorter hospitalization (mean difference of 11.8 days, $p < 0.001$) as compared to open surgical bypass. Similar findings were reported by Zheng et al. [58]. Based on these findings, it is also likely that stenting is less expensive than surgical bypass. The major limiting factor for the endoscopic approach is being unable to pass the scope through the obstruction. The major complications reported are gastric ulceration, bowel perforation, biliary obstruction, stent dysfunction, and stent migration. Stent placement would be contraindicated in patients with multiple sites of obstruction and should be considered carefully in patients with peritoneal carcinomatosis who are at risk for more distal obstructions.

For patients in whom stenting is not an option, surgical bypass can relieve both the symptoms of the obstruction and allow the patient to resume enteral nutrition. Surgical bypass, most commonly in the form of a gastroenterostomy, can either be performed laparoscopically or through a relatively small upper midline incision. The estimated risk of morbidity and mortality from these procedures is 25–60 and 0–25 %, respectively [57, 58]. While surgical bypass is usually technically successful, patient selection with regard to preoperative nutritional status and life expectancy is imperative to the palliative success of this approach. For example, in addition to general surgical risks such as bleeding or infection, a patient with chronic gastric outlet or duodenal obstruction who is malnourished is at increased risk for a leak from the intestinal anastomosis. Such a life-threatening complication could rob the patient of both time and any remaining quality of life. Other potential complications specific to gastric bypass include dumping syndrome, alkaline reflux gastritis, and delayed gastric emptying.

Placement of a gastrostomy tube for decompression is another option for palliation of gastric outlet, duodenal and non-operable small bowel obstruction, or profound gastrointestinal dysmotility from carcinomatosis. Gastrostomy tubes can be placed endoscopically, fluoroscopically, or surgically (either laparoscopic or open). Decompression gastrostomy tubes provide patients the ability to drain the stomach as needed for nausea and to avoid vomiting. It also allows them to drink liquids and eat some soft foods for pleasure and comfort. It does not allow for the enteric maintenance of nutrition. Many endoscopists, surgeons, and international radiologists are leery of placing gastrostomy tubes in the setting of malignant ascites. They are concerned about the risk of infecting the ascites, intraperitoneal leakage from the stomach due to poor apposition to the anterior abdominal wall, as well as leakage of ascites from around the tube. However, there is a growing body of literature demonstrating the safety of placing gastrostomy tubes in patients with malignant ascites from a variety of tumors [59–61]. Paracentesis prior to or concurrent with gastrostomy placement is advisable. Also, consideration of placing a peritoneal drainage catheter at the time of gastrostomy may also help lower any risk associated with the ascites. As gastrostomy may be the only viable palliative option for these patients, all efforts to manage the ascites and increase the safety of gastrostomy placement are warranted.

4 Pain

Pain is another symptom commonly experienced by patients with advanced biliary cancers. The type, location, and size of the biliary cancer will determine the intensity and quality of pain experienced. For example, a peripheral cholangiocarcinoma may only cause pain after growing to a size large enough to cause capsular stretching of the liver (hepatic distention syndrome), whereas a patient with gallbladder cancer may present with pain due to the presence of stones or an obstructed bile duct. Others may develop pain due to metastatic disease within the peritoneum. The pain can have a number of different qualities. The pain of hepatic distention is often described as a discomfort in the right subcostal region that may radiate to the right neck, shoulder, or scapula. This may be accompanied by acute subcostal pain often worsened by respiration. On the other hand, the pain of a bile duct obstruction may be more intense and colicky in nature. Regardless of the origin of the pain, relief is essential.

While a comprehensive review of cancer pain management is beyond the scope of this chapter, some guiding principles are provided. For patients with more refractory pain, or patients who are more difficult to palliate due to chronic opioid use or other medical comorbidities, either a

Fig. 2 Representation of World Health Organization analgesic ladder

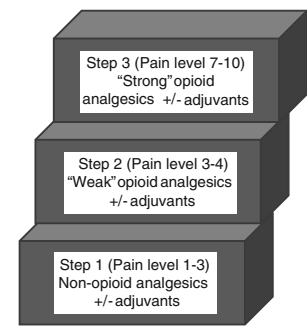


Table 1 The World Health Organization (WHO) analgesic ladder to assess pain

Rating	Numeric rating for intensity of pain
0	No pain
1–3	Mild pain (nagging, annoying, interfering little with ADL*s)
4–6	Moderate pain (interferes significantly with ADLs)
7–10	Severe pain (disabling; unable to perform ADLs)

*Activities of daily living

pain service or palliative care consult is appropriate. However, for 80–90 % of patients, the approach proposed by the World Health Organization (WHO) analgesic ladder should be effective [62]. This simple, three-step ladder focuses on the intensity of the pain, rather than the etiology, as the main consideration in analgesic selection (Fig. 2). The goal is complete relief of the patient's pain. For patients with mild cancer pain (1–3 on the numeric rating scale) (Table 1), the WHO analgesic ladder recommends starting with non-opioid analgesics (aspirin, ibuprofen, acetaminophen), which can be combined with adjuvant drugs if a specific indication exists (i.e., a tricyclic antidepressant in addition to acetaminophen for a neuropathic pain component). Caution is advised in using these medications in patients with advanced biliary or gastrointestinal cancers if there are any concerns for gastrointestinal bleeding or liver dysfunction. If non-opioid analgesics do not provide adequate relief or the pain worsens, then the patient moves to level two of the ladder and is considered to have moderate pain (4–6 on the numeric rating scale). These patients should be treated with opioids such as oxycodone, codeine, propoxyphene, or the opioid-like drug, tramadol. These opioids can be given as combination drugs with a non-opioid analgesic such as acetaminophen or separately but concurrently. The advantage of giving them separately is that the opioid dose can be increased beyond the dose-limiting maximum of the acetaminophen. These drugs can also be given concurrently with non-steroidal anti-inflammatory agents in patients without contraindications. Patients who present with severe pain (7–10 on numeric rating scale) or whose pain is not relieved by the appropriate

administration of the drugs on the second step should receive an opioid used for moderate to severe pain (step three). The gold standard in this group is morphine. Other options include hydromorphone, fentanyl, methadone, di-amorphine, phenazocine, levorphanol, and oxymorphone. These drugs can also be combined with non-opioid analgesics and other adjuvant agents.

Another important consideration in the management of pain in patient with advanced biliary cancer is the route of administration. Many of these patients may be approaching a transition in goals of care to hospice care, and the logistics of pain management need to be considered. Oral pain medications are the easiest to manage but may not be an option for patients with a gastric outlet or duodenal obstruction. Sublingual and transdermal formulations and rectal suppositories are other options for patients who cannot use oral medications. Subcutaneous administration is also effective and not particularly uncomfortable. Finally, patients on hospice can be set up at home with intravenous access and a patient-controlled analgesia (PCA) device.

Since relief of the patient's pain is the goal, pain medications should be administered "around the clock" and the next dose should be given before the pain returns. By definition, waiting for the patient's pain to return or increase in intensity does not achieve the goal of pain relief. Also, the appropriate dose of the medication is one that relieves the pain. It must be remembered that patients with liver failure may have a higher bioavailability and decreased clearance and therefore may require a lower dose than expected. Thus, it is advisable to start out at lower doses for these patients. Also, because opioids are largely renally excreted, patients with renal failure can be at risk for buildup of opioid metabolites. This can manifest as myoclonus and hypesthesias, which can be confused for worsening pain leading to administration of more opioid [63]. For patients in renal failure, fentanyl is the opioid of choice due to the buildup of fewer metabolites. All opioid-based pain regimens need to be accompanied by a bowel regimen containing a stool softener and laxative. Finally, all of these efforts should be combined with non-pharmacological methods of cancer pain where possible including radiotherapy, surgery, chemotherapy, hormone therapy, regional anesthetic techniques, physiotherapy, and psychological, cognitive, and spiritual approaches.

5 Nausea

Nausea is one of the most common and debilitating symptoms experienced by patients with advanced cancers. Patients with biliary cancer are at particularly high risk to suffer from nausea as both of the main brain centers involved in nausea and vomiting, the chemoreceptor trigger

zone (CTZ) and vomiting center (VC), may be involved. It is important to have a basic understanding of these pathways in order to have the best chance to treat the nausea [64, 65]. The CTZ is located in the area postrema in the floor of the fourth ventricle, where there is essentially no blood-brain barrier. Chemoreceptors in this area are triggered by drugs, toxins, and metabolites in the systemic circulation. The VC is a diffuse interconnecting neural network that integrates emetogenic stimuli with parasympathetic and motor efferent activity to produce the vomiting reflex. In addition to receiving afferents from other regions of the brain and vestibular system, the VC receives afferents from the vagus and splanchnic nerves, the gastrointestinal tract (including chemo- and mechanoreceptors in the liver and gut), and peritoneum. The principle receptors in the CTZ are dopamine type 2 (D2). The principle receptors in the VC are muscarinic cholinergic (AChm) and histamine type 1 (H1). Both sites have serotonin type 3 (5HT3), and the VC also has serotonin type 2 (5HT2) [66]. Finally, neurokinin type 1 (NK1) receptors are concentrated in areas of the brainstem involved in emesis [67]. Understanding these pathways can guide antiemetic selection.

Before prescribing any medications for nausea, there are some non-pharmacologic efforts that may be beneficial. All efforts should be made to reduce any stimulus to nausea such as cooking smells, unpleasant odors, or strong scents. Small, appealing, bland meals and cool, carbonated drinks are also likely to be more palatable. To select an antiemetic, an effort should be made to identify the most likely cause of the nausea—i.e., a drug or toxin versus stretched mechanoreceptors in the stomach due to obstruction. This will help identify the most likely pathway—CTZ versus VC—and the most likely neurotransmitter receptor involved (Table 2). The most potent antagonist of the suspected receptor should then be selected and administered by a route that ensures that the drug will reach its target. The dose should be titrated, the response reviewed frequently, and the medication should be given regularly. If the symptoms persist, it is important to review the likely causes. If a new cause becomes apparent or if there are multiple causes identified, multiple agents may be necessary to target each receptor involved [68].

In addition to chemical (chemotherapy, initiation, or escalation of opioids) and gastrointestinal causes of nausea (including severe constipation), other causes of nausea need to be considered in advanced cancer patients in order to direct therapy at the cause. These causes might include bowel obstruction, metabolic disturbances, or central nervous system metastases. In these circumstances, other therapies such as corticosteroids, radiation, or surgery may also be indicated. Anxiety-induced nausea and anticipatory nausea are also very common in cancer patients. Often, the patients or caregivers can identify anxiety as the trigger;

Table 2 Types of antiemetics used to relieve nausea

Common causes of nausea	Receptors	Site	Drug class	Drug example
Opioid induced	D2	CTZ	Butyrophenones	Haloperidol
Gastric stasis	D2	Intestine CTZ	Prokinetics	Metoclopramide, domperidone
Intestinal obstruction/peritoneal irritation	D2 H1 ACHm	CTZ VC Intestine	Phenothiazines Antihistamines Anticholinergics	Prochlorperazine Diphenhydramine Hyoscine
Chemotherapy/PONV	5-HT3	Intestine CTZ	5-HT3 antagonists	Ondansetron
Late-onset chemotherapy related	NK1	Widespread	NK1 antagonist	Aprepitant

however, if not, other sources must be ruled out before ascribing the nausea to anxiety. Psychotherapeutic techniques to address the anxiety combined with a long-acting benzodiazepine for a short period of time can be helpful [69]. The addition of an antidepressant with secondary anxiolytic properties may also be helpful. Anticipatory emesis is a conditioned response in patients who have suffered nausea and vomiting with cytotoxic agents. Any memory can trigger it. Psychotherapy combined with alprazolam has been shown to be helpful [70].

6 Cancer Cachexia and Fatigue

Perhaps one of the most emotionally distressing symptoms of advanced cancer is cachexia. The latest working definition of cancer cachexia is “a multifactorial syndrome defined by a negative protein and energy balance driven by a variable combination of a reduced food intake and abnormal metabolism. A key defining feature is ongoing loss of skeletal muscle mass, which cannot be fully reversed by conventional nutritional support, leading to progressive functional impairment” [71]. Cancer cachexia is diagnosed by involuntary weight loss greater than 10 % over a 6-month period with ongoing weight loss in the previous 1–2 months. It is important to recognize that in some patients such as those who are obese, have large tumors, or have significant fluid retention, muscle mass may be lost without loss of weight. More recently, it is recognized that cachexia is a spectrum ranging from preclinical cachexia (patients with cancer who manifest early clinical signs of cachexia without significant weight loss) to patients with late (irreversible) cachexia (advanced muscle wasting, a low performance status, and life expectancy of less than three months) [72]. Identifying cachexia in its preclinical stage may allow providers to intervene earlier and delay its progression.

Clinical assessment of a patient for cancer cachexia should include (1) evaluating for anorexia and decreased food intake; (2) determining the presence of a catabolic drive caused by progressive disease and inflammation;

(3) identifying decreased muscle mass and reduced strength; and (4) reduced physical, psychosocial, and social function [71]. There are both primary and secondary causes of cachexia. The pathogenesis of primary anorexia–cachexia is a combination of decreased food intake due to neurohormonal (brain–gut axis) eating dysregulation (anorexia), catabolic inflammation (muscle–liver axis), and anabolic dysbalance with muscle loss (brain–muscle axis) [73–75]. Secondary anorexia and secondary cachexia can also be divided into three groups: (1) starvation due to impaired oral intake or malabsorption (secondary anorexia); (2) loss of muscle mass (i.e., due to prolonged immobility or protein loss (due to comorbidities such as nephritic syndrome or repeat paracenteses) (secondary cachexia); and (3) a catabolic state and comorbidities associated with cachexia (chronic infection, poorly controlled diabetes, hyperthyroidism) [71].

Optimal management of cancer cachexia is still evolving. The best therapeutic option for treating cancer cachexia is treatment of the underlying disease [76]. Unfortunately, this is not always an option. For patients and families, the obvious explanation for the patient’s weight loss and fatigue is poor nutrition. This often prompts families to aggressively feed the patient despite his or her anorexia. These well-intended efforts can lead to significant eating-related distress for both the patient and the family. Unfortunately, efforts to provide adequate caloric support either parenterally or enterally for a patient with advanced cancer have not been shown to improve lean body weight or fatigue [77, 78]. In fact, the lack of improvement with adequate protein nutrition is part of the definition of cancer cachexia as described above and supports the notion that this is not just an issue of inadequate calories, but of a systemic process impacting the entire protein–energy balance and metabolism. Hopefully this understanding will help improve efforts to treat cancer cachexia. For example, for patients with primary cachexia, either disease-directed (chemotherapy if feasible) or pharmacologic measures are also required in addition to adequate calories. For patients with cancer cachexia, currently only progestins and corticosteroids have

been shown in randomized controlled trials to have sufficient evidence to support their use [79]. With growing recognition of the role of inflammation in cancer cachexia, low-dose non-steroidal anti-inflammatory drugs and fish oil are also commonly used to try to break the cycle, particularly in the earlier stages.

Fatigue is often associated with cancer cachexia and has a profound impact on a patient's quality of life. The exact relationship between cachexia and fatigue remains undetermined, and fatigue can occur for a number of non-cachexia-related reasons. For example, patients with advanced cancers can have fatigue without having cancer cachexia, while patients with anorexia nervosa can be profoundly cachectic without having fatigue. Fatigue is gaining recognition as a complex multidimensional symptom. As such, it requires a thorough therapeutic approach to identify and manage the contributing factors. Fatigue may be primarily related to tumor progression, but it may also be related to antineoplastic treatments (chemotherapy, radiation, surgery), comorbid conditions (infection, inflammation), or deconditioning. Because fatigue is a subjective symptom, and because of its multidimensional nature, it is difficult to quantitate. There are a number of assessment approaches for fatigue (performance status, functional capacity, fatigue scale), but as of now, there is no "gold standard" [80, 81]. To quote Dr. Cassell [82], "people go to the doctors and become patients when they cannot pursue their goals or purposes because of impairments that they believe are in the province of a physician." If solely reducing fatigue allows a patient to pursue one more goal, even with a stable residual level of fatigue, then the effort is worthwhile.

Interventions for fatigue can include both non-pharmacologic and pharmacologic approaches. Counseling can be very effective for both patients and families. Providing information about the different causes of and treatments for fatigue and what to expect from cancer treatment and disease progression can help patients and families better manage fatigue. With better understanding, patients can change schedules according to the fatigue pattern, decrease the burden of daily activities, or increase the level of activity if the cause is related to deconditioning. Physiotherapists and occupational therapists can be very helpful in making these lifestyle changes to combat fatigue. Interventions for fatigue that are supported by one or more well-designed randomized control trials include exercise, psychoeducational interventions, measures to optimize sleep quality, correction of anemia less than 10 mg/dL, relaxation, massage, and healing touch [83]. Pharmacologic approaches that are used include interventions similar to those for cancer cachexia, namely corticosteroids, progestins, and psychostimulants. A number of studies are currently ongoing looking at alternative theories such as L-

carnitine, testosterone, melatonin, yoga, and meditation. Both cancer cachexia and fatigue are appropriate indications for a palliative care consult.

7 Hospice Means Hope

Another appropriate indication for a palliative care consult is difficult transitions in care—such as the transition from cancer-directed therapy to quality of life and goals of life-directed therapy. One of the greatest challenges for providers is balancing encouraging the fight for life and the knowledge that the patient is likely to die soon of his or her disease. Maintaining this balance becomes imperative when further treatment is likely to do more harm than good. No provider wants to admit that there is nothing more that he or she can offer the patient to treat the disease and no patient or family member wants to hear those words. How this information is conveyed has a profound impact on the patient and family's reaction and emotional response. For example, an empathic statement such as, "I wish things were different, but we have come to a point where there are no other treatments against the cancer that will help you live longer or better... however, there are still many ways that I can care for you and help you achieve other goals" can be much more effective than a non-empathic statement such as, "we could try one more line of treatment, but it is not likely to help." Compared to the first statement, it is easy to see how the second statement could leave the patient and family feeling hopeless and abandoned. It is also at this point that considering hospice should be discussed.

There are many misconceptions about hospice that cause people to reject it without any consideration (Table 3). The only mandated requirement for hospice eligibility is for the attending physician and hospice medical director to certify that a patient is terminally ill with a prognosis of less than six months if the disease follows its usual course. It does require that the patient is no longer receiving disease-directed therapy except for palliation of symptoms. Hospice programs seek to neither prolong life nor hasten death. Their primary goal is to provide comfort and dignity and optimize the quality of time that is left. Hospice provides support to both the patient and the family through an interdisciplinary team of providers, including physicians, nurses, social workers, chaplains, and volunteers. Hospice programs also help prepare families for their loss and offer bereavement programs after the death. They also provide a continuum of care from home to the in-patient setting. A recent study showed that more than half of older Americans presented to an emergency room within one month of their death, and most died in the hospital. Patients who were on hospice were less likely to visit the emergency room [84]. While there are no randomized control trials to demonstrate

Table 3 Common misconceptions about hospice

Misconception: <i>Patients will lose contact with their primary physician</i>
Truth: The referring physician (i.e., primary care provider or oncologist) continues to play an important role in the patients' care
Misconception: <i>Hospice patients cannot go to the hospital</i>
Truth: Hospice patients can be hospitalized as needed for symptoms that cannot be managed at home or at a hospice house
Misconception: <i>Patients can use up their hospice eligibility</i>
Truth: Hospice eligibility does not expire. Patients who live longer than 6 months will continue to receive hospice services, provided they still meet the eligibility requirements
Misconception: <i>Patients cannot come off hospice if a new treatment comes available</i>
Truth: Hospice is not a one-way street. If a new treatment directed at the patient disease becomes available that may be helpful to the patient, the patient can come off hospice to receive treatment
Misconception: <i>Patients enrolling in hospice have to choose not to be resuscitated</i>
Truth: While it is typically recommended that patients receiving hospice care choose not to be resuscitated and imperative for providers and patients to discuss the role of resuscitation, it is not required for hospice eligibility
Misconception: <i>Patients on hospice cannot receive parenteral nutrition</i>
Truth: While it depends on the benefits covered by an individual's insurance benefit, patients receiving hospice can receive parenteral nutrition, although the actual benefit in terms of symptom control and survival is unclear
Misconception: <i>Hospice patients cannot participate in research projects</i>
Truth: Hospice patients can participate in research projects that are consistent with the mission of hospice

that receiving hospice at the end of life is “better” than without, studies that have tried to assess the impact of hospice at the end of life suggest that families of patients who enrolled earlier felt that they had received all of the care that they needed and did not feel that they had enrolled too late [85, 86]. Starting this conversation early in the patient and family's journey with cancer will make it easier when the appropriate time comes to consider these options.

In summary, patients with biliary cancer often present with advanced disease. Consequently, much of the therapy they receive is palliative in nature. Because of the location and nature of these tumors, the symptoms that they create can be complex and challenging to treat. Multimodality approaches are often necessary (including medicinal, chemotherapy, radiation, surgery, and psychosocial). Most importantly, these patients need to feel that they will not be abandoned as their disease progresses. Open, honest, and compassionate communication is essential to helping both the patients and their families cope with an impossible situation. Early consultation with a palliative care and hospice team can be of great help to both the patient and family and involved providers.

References

1. Nakeeb A et al (1996) Cholangiocarcinoma. A spectrum of intrahepatic, perihilar, and distal tumors. *Ann Surg* 224(4):463–473 (discussion 473–475)
2. Forsmo HM et al (2008) Survival and an overview of decision-making in patients with cholangiocarcinoma. *Hepatobiliary Pancreat Dis Int* 7(4):412–417
3. He P et al (2002) Multivariate statistical analysis of clinicopathologic factors influencing survival of patients with bile duct carcinoma. *World J Gastroenterol* 8(5):943–946
4. Kitagawa Y et al (2001) Lymph node metastasis from hilar cholangiocarcinoma: audit of 110 patients who underwent regional and paraaortic node dissection. *Ann Surg* 233(3):385–392
5. Jarnagin WR et al (2001) Staging, resectability, and outcome in 225 patients with hilar cholangiocarcinoma. *Ann Surg* 234(4):507–517 (discussion 509–517)
6. Temel JS et al (2010) Early palliative care for patients with metastatic non-small-cell lung cancer. *N Engl J Med* 363(8):733–742
7. Greer JA et al (2012) Effect of early palliative care on chemotherapy use and end-of-life care in patients with metastatic non-small-cell lung cancer. *J Clin Oncol* 30(4):394–400
8. Smith TJ et al (2012) American society of clinical oncology provisional clinical opinion: the integration of palliative care into standard oncology care. *J Clin Oncol* 30(8):880–887
9. Levy MH et al (2012) Palliative care. *J Natl Compr Canc Netw* 10(10):1284–1309
10. Hamade AM et al (2005) Therapeutic, prophylactic, and preresection applications of laparoscopic gastric and biliary bypass for patients with periaampullary malignancy. *Surg Endosc* 19(10):1333–1340
11. Jarnagin WR et al (1998) Intrahepatic biliary enteric bypass provides effective palliation in selected patients with malignant obstruction at the hepatic duct confluence. *Am J Surg* 175(6):453–460
12. Kuhlmann KF et al (2007) Initial and long-term outcome after palliative surgical drainage of 269 patients with malignant biliary obstruction. *Eur J Surg Oncol* 33(6):757–762
13. Mann CD et al (2009) Combined biliary and gastric bypass procedures as effective palliation for unresectable malignant disease. *ANZ J Surg* 79(6):471–475
14. Moss AC et al (2007) Malignant distal biliary obstruction: a systematic review and meta-analysis of endoscopic and surgical bypass results. *Cancer Treat Rev* 33(2):213–221
15. Bornman PC et al (1986) Prospective controlled trial of transhepatic biliary endoprosthesis versus bypass surgery for incurable carcinoma of head of pancreas. *Lancet* 1(8472):69–71
16. Miura Y et al (2001) Adjuvant therapies using biliary stenting for malignant biliary obstruction. *J Hepatobiliary Pancreat Surg* 8(2):113–117
17. Harris J, Bruckner H (2001) Adjuvant and neoadjuvant therapies of pancreatic cancer: a review. *Int J Pancreatol* 29(1):1–7

18. England RE et al (2000) A prospective randomised multicentre trial comparing 10 Fr Teflon Tannenbaum stents with 10 Fr polyethylene Cotton-Leung stents in patients with malignant common duct strictures. *Gut* 46(3):395–400
19. Costamagna G, Pandolfi M (2004) Endoscopic stenting for biliary and pancreatic malignancies. *J Clin Gastroenterol* 38(1):59–67
20. Groen AK et al (1987) Characterization of the content of occluded biliary endoprostheses. *Endoscopy* 19(2):57–59
21. Dowidar N, Kolmos HJ, Matzen P (1992) Experimental clogging of biliary endoprostheses. Role of bacteria, endoprosthesis material, and design. *Scand J Gastroenterol* 27(1):77–80
22. Libby ED, Leung JW (1996) Prevention of biliary stent clogging: a clinical review. *Am J Gastroenterol* 91(7):1301–1308
23. Frakes JT, Johanson JF, Stake JJ (1993) Optimal timing for stent replacement in malignant biliary tract obstruction. *Gastrointest Endosc* 39(2):164–167
24. Davids PH et al (1992) Randomised trial of self-expanding metal stents versus polyethylene stents for distal malignant biliary obstruction. *Lancet* 340(8834–8835):1488–1492
25. Knyrim K et al (1993) A prospective, randomized, controlled trial of metal stents for malignant obstruction of the common bile duct. *Endoscopy* 25(3):207–212
26. O'Brien S et al (1995) A three year follow up of self expanding metal stents in the endoscopic palliation of longterm survivors with malignant biliary obstruction. *Gut* 36(4):618–621
27. Isayama H et al (2004) A prospective randomised study of “covered” versus “uncovered” diamond stents for the management of distal malignant biliary obstruction. *Gut* 53(5):729–734
28. Ohnishi H et al (1995) External radiotherapy for biliary decompression of hilar cholangiocarcinoma. *Hepatogastroenterology* 42(3):265–268
29. Cameron JL et al (1990) Management of proximal cholangiocarcinomas by surgical resection and radiotherapy. *Am J Surg* 159(1):91–97 (discussion 97–98)
30. Ishii H et al (2004) Relief of jaundice by external beam radiotherapy and intraluminal brachytherapy in patients with extrahepatic cholangiocarcinoma: results without stenting. *Hepatogastroenterology* 51(58):954–957
31. Golfieri R et al (2006) Unresectable hilar cholangiocarcinoma: multimodality approach with percutaneous treatment associated with radiotherapy and chemotherapy. *In Vivo* 20(6A):757–760
32. Takamura A et al (2003) Intraluminal low-dose-rate ¹⁹²Ir brachytherapy combined with external beam radiotherapy and biliary stenting for unresectable extrahepatic bile duct carcinoma. *Int J Radiat Oncol Biol Phys* 57(5):1357–1365
33. Kuvshinov BW et al (1995) Palliation of irresectable hilar cholangiocarcinoma with biliary drainage and radiotherapy. *Br J Surg* 82(11):1522–1525
34. Ibrahim SM et al (2008) Treatment of unresectable cholangiocarcinoma using yttrium-90 microspheres: results from a pilot study. *Cancer* 113(8):2119–2128
35. Ortner MA (2004) Photodynamic therapy in cholangiocarcinomas. *Best Pract Res Clin Gastroenterol* 18(1):147–154
36. Berr F et al (2000) Photodynamic therapy for advanced bile duct cancer: evidence for improved palliation and extended survival. *Hepatology* 31(2):291–298
37. Wiedmann M et al (2003) Neoadjuvant photodynamic therapy as a new approach to treating hilar cholangiocarcinoma: a phase II pilot study. *Cancer* 97(11):2783–2790
38. Ballinger AB et al (1994) Symptom relief and quality of life after stenting for malignant bile duct obstruction. *Gut* 35(4):467–470
39. Abraham NS, Barkun JS, Barkun AN (2002) Palliation of malignant biliary obstruction: a prospective trial examining impact on quality of life. *Gastrointest Endosc* 56(6):835–841
40. Barkay O et al (2013) Effect of endoscopic stenting of malignant bile duct obstruction on quality of life. *J Clin Gastroenterol* 47:526–531
41. Luman W, Cull A, Palmer KR (1997) Quality of life in patients stented for malignant biliary obstructions. *Eur J Gastroenterol Hepatol* 9(5):481–484
42. Jones EA, Bergasa NV (1990) The pruritus of cholestasis: from bile acids to opiate agonists. *Hepatology* 11(5):884–887
43. Bergasa NV et al (1995) Effects of naloxone infusions in patients with the pruritus of cholestasis. A double-blind, randomized, controlled trial. *Ann Intern Med* 123(3):161–167
44. Bergasa NV et al (1993) Plasma from patients with the pruritus of cholestasis induces opioid receptor-mediated scratching in monkeys. *Life Sci* 53(16):1253–1257
45. Bergasa NV et al (1992) A controlled trial of naloxone infusions for the pruritus of chronic cholestasis. *Gastroenterology* 102(2):544–549
46. Carson KL et al (1996) Pilot study of the use of naltrexone to treat the severe pruritus of cholestatic liver disease. *Am J Gastroenterol* 91(5):1022–1023
47. Bergasa NV et al (1998) Open-label trial of oral nalmefene therapy for the pruritus of cholestasis. *Hepatology* 27(3):679–684
48. Terg R et al (2002) Efficacy and safety of oral naltrexone treatment for pruritus of cholestasis, a crossover, double blind, placebo-controlled study. *J Hepatol* 37(6):717–722
49. Wolfhagen FH et al (1997) Oral naltrexone treatment for cholestatic pruritus: a double-blind, placebo-controlled study. *Gastroenterology* 113(4):1264–1269
50. Jones EA, Zylicz Z (2005) Treatment of pruritus caused by cholestasis with opioid antagonists. *J Palliat Med* 8(6):1290–1294
51. Jones EA, Neuberger J, Bergasa NV (2002) Opiate antagonist therapy for the pruritus of cholestasis: the avoidance of opioid withdrawal-like reactions. *QJM* 95(8):547–552
52. Cepeda MS et al (2004) Addition of ultralow dose naloxone to postoperative morphine PCA: unchanged analgesia and opioid requirement but decreased incidence of opioid side effects. *Pain* 107(1–2):41–46
53. Maxwell LG et al (2005) The effects of a small-dose naloxone infusion on opioid-induced side effects and analgesia in children and adolescents treated with intravenous patient-controlled analgesia: a double-blind, prospective, randomized, controlled study. *Anesth Analg* 100(4):953–958
54. Bergasa NV (2004) Pruritus in chronic liver disease: mechanisms and treatment. *Curr Gastroenterol Rep* 6(1):10–16
55. Mosler P et al (2005) Palliation of gastric outlet obstruction and proximal small bowel obstruction with self-expandable metal stents: a single center series. *J Clin Gastroenterol* 39(2):124–128
56. Holt AP, Patel M, Ahmed MM (2004) Palliation of patients with malignant gastroduodenal obstruction with self-expanding metallic stents: the treatment of choice? *Gastrointest Endosc* 60(6):1010–1017
57. Ly J et al (2010) A systematic review of methods to palliate malignant gastric outlet obstruction. *Surg Endosc* 24(2):290–297
58. Zheng B et al (2012) Endoscopic stenting versus gastrojejunostomy for palliation of malignant gastric outlet obstruction. *Dig Endosc* 24(2):71–78
59. Pothuri B et al (2005) Percutaneous endoscopic gastrostomy tube placement in patients with malignant bowel obstruction due to ovarian carcinoma. *Gynecol Oncol* 96(2):330–334
60. Ryan JM, Hahn PF, Mueller PR (1998) Performing radiologic gastrostomy or gastrojejunostomy in patients with malignant ascites. *AJR Am J Roentgenol* 171(4):1003–1006
61. Shaw C et al (2013) Palliative venting gastrostomy in patients with malignant bowel obstruction and ascites. *Ann Surg Oncol* 20(2):497–505

62. Organization WH (1996) Cancer pain relief, 2nd edn. WHO, Geneva
63. Murtagh FE et al (2007) The use of opioid analgesia in end-stage renal disease patients managed without dialysis: recommendations for practice. *J Pain Palliat Care Pharmacother* 21(2):5–16
64. Borison HL, Wang SC (1953) Physiology and pharmacology of vomiting. *Pharmacol Rev* 5(2):193–230
65. Sanger GJ, Andrews PL (2006) Treatment of nausea and vomiting: gaps in our knowledge. *Auton Neurosci* 129(1–2):3–16
66. Lang IM (1999) Noxious stimulation of emesis. *Dig Dis Sci* 44(8 Suppl):58S–63S
67. Edwards CM (1988) Chemotherapy induced emesis—mechanisms and treatment: a review. *J R Soc Med* 81(11):658–662
68. Mannix K (2010) Palliation of nausea and vomiting. In: Hanks G, Cherny NI, Christakis NA, Fallon M, Kaasa S, Portenoy RK (eds) *Oxford textbook of palliative medicine*. Oxford University Press Inc, New York, p 803
69. Burish TG, Tope DM (1992) Psychological techniques for controlling the adverse side effects of cancer chemotherapy: findings from a decade of research. *J Pain Symptom Manage* 7(5):287–301
70. Razavi D et al (1993) Prevention of adjustment disorders and anticipatory nausea secondary to adjuvant chemotherapy: a double-blind, placebo-controlled study assessing the usefulness of alprazolam. *J Clin Oncol* 11(7):1384–1390
71. Strasser F (2010) Weight loss in palliative medicine. In: Hanks G, Cherny NI, Christakis NA, Fallon M, Kaasa S, Portenoy RK (eds) *Oxford textbook of palliative medicine*. Oxford University Press, New York, pp 889–900
72. Fearon KC (2008) Cancer cachexia: developing multimodal therapy for a multidimensional problem. *Eur J Cancer* 44(8):1124–1132
73. Meguid MM, Yang ZJ, Gleason JR (1996) The gut-brain brain-gut axis in anorexia: toward an understanding of food intake regulation. *Nutrition* 12(1 Suppl):S57–S62
74. Argiles JM, Busquets S, Lopez-Soriano FJ (2001) Metabolic interrelationships between liver and skeletal muscle in pathological states. *Life Sci* 69(12):1345–1361
75. Laviano A et al (2008) Neural control of the anorexia-cachexia syndrome. *Am J Physiol Endocrinol Metab* 295(5):E1000–E1008
76. Geels P et al (2000) Palliative effect of chemotherapy: objective tumor response is associated with symptom improvement in patients with metastatic breast cancer. *J Clin Oncol* 18(12):2395–2405
77. Tisdale MJ (2002) Cachexia in cancer patients. *Nat Rev Cancer* 2(11):862–871
78. Koretz RL, Lipman TO, Klein S (2001) AGA technical review on parenteral nutrition. *Gastroenterology* 121(4):970–1001
79. Yavuzsen T et al (2005) Systematic review of the treatment of cancer-associated anorexia and weight loss. *J Clin Oncol* 23(33):8500–8511
80. Piper BF et al (1998) The revised Piper Fatigue Scale: psychometric evaluation in women with breast cancer. *Oncol Nurs Forum* 25(4):677–684
81. Mendoza TR et al (1999) The rapid assessment of fatigue severity in cancer patients: use of the Brief Fatigue Inventory. *Cancer* 85(5):1186–1196
82. Cassell E (2013) *The nature of healing*. Oxford University Press, New York, p 1
83. Mitchell SA et al (2007) Putting evidence into practice: evidence-based interventions for fatigue during and following cancer and its treatment. *Clin J Oncol Nurs* 11(1):99–113
84. Smith AK et al (2012) Half of older Americans seen in emergency department in last month of life; most admitted to hospital, and many die there. *Health Aff (Millwood)* 31(6):1277–1285
85. Kapo J et al (2005) Are we referring patients to hospice too late? Patients' and families' opinions. *J Palliat Med* 8(3):521–527
86. Rickerson E et al (2005) Timing of hospice referral and families' perceptions of services: are earlier hospice referrals better? *J Am Geriatr Soc* 53(5):819–823

Future Directions

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Abstract

Improving the late detection and poor prognosis associated with gallbladder and biliary tract cancers remains a monumental task for physicians today. Recent developments in genome sequencing, pathology, functional imaging, and therapeutic approaches have resulted in enhanced diagnosis, staging, and management techniques of these cancers. However, to maximize the efficacy of these advancements, several considerations must be taken into account. Universal terminology should be defined because standard management guidelines cannot be established without uniform diagnosis and staging criteria. Multidisciplinary efforts among surgical oncology, medical oncology, radiation oncology, interventional radiology, pathology, and palliative care are fundamental to effective patient care as the collaboration of resources allows for more accurate treatment recommendations and favorable patient outcomes. Additional prospective clinical trials with large patient populations are needed to validate these findings and optimize full clinical potential in order to establish a standard of care for these patients.

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Biliary tract malignancies consist of rare cancers that have detrimental impacts on the lives of their victims. The first report of biliary tract carcinoma dates back to the 1840 [1], prior to the modern era of scientific discoveries and technological innovations. Despite advancements in medicine and our recognition of biliary tract cancers for centuries, biliary tract carcinomas are frequently associated with late detection and poor prognosis. Incidence rates of biliary cancers in the United States have doubled within the past 10 years, with an increase of 7,100 diagnoses in 2002 to a projection of approximately 14,000 new cases in the year 2013 [2, 3].

Due to the rare origin and anatomical, histological, and biological heterogeneity of biliary tract and gallbladder

cancers, scientific discovery in this field is fairly scarce. Although known risk factors exist, patients with these cancers are commonly asymptomatic, consequently causing them to present with advanced disease. Accurate classification and differentiation of morphological and histological tissue are essential to determine the exact incidence and risk factors of these cancers. No standard of care treatment for biliary tract carcinomas has been accepted, and large clinical trials are required to optimize full clinical potential to make historic advances in this field of study.

Fortunately, however, with increased incidence comes increased technology. Current findings have made an immense contribution to the diagnosis and management of biliary tract cancers; from herbal medicine to exome sequencing, innovative ideas are becoming reality. The latest discoveries are discussed here to suggest what lies ahead in the future efforts to improve outcomes of patients with biliary tract and gallbladder cancers.

1 Genome Sequencing

Recent advances in cancer genomics have been significant in identifying biological processes to improve prognostication and evidence-based management to ultimately lead to personalized medicine. Prevention of tumor formation and early diagnosis is difficult with biliary tract carcinomas due to their poor sensitivity to conventional therapies. Therefore, progressive efforts have been made to understand the underlying cellular mechanisms among abnormal cells and to develop innovative therapies accordingly. Technological advances in molecular profiling have conveyed common molecular deviations in biliary tract cancers, allowing for a better understanding of the biology of the cancer and possible drug therapy.

Biliary tract cancers arise from the epithelial lining, due to overexpression of epidermal growth factors or mutated signaling pathways. Precise clarification of these cellular pathways, in accordance with the advancement of technology, is paramount to developing efficient expansion in molecular profiling techniques. Several complex technologies are being used to complete mutation analysis of biliary tract cancers including mass spectrometry, SNaPshot genotyping, and whole exome sequencing [4]. Recent analysis of recurrent mutations in routine diagnostic pathology specimens, using SNaPshot technology, revealed a distinct signature involving IDH1/2 gene mutation in intrahepatic cholangiocarcinoma, suggesting a possible therapeutic target in the future [5]. Additionally, a new computational technology called heterogeneous expression profile analysis (HEPA) has been developed to highlight specific genes that are heterogeneously overexpressed by cancer cells in comparison with their normal counterparts

using an innovative mathematical scheme to prioritize clinically relevant tumor-specific antigens [6].

The heterogenic nature of biliary tract malignancies signifies more intricate subtyping, therefore requiring personalized tumor profiling and treatment therapy [7]. The introduction of molecular-targeted therapy for biliary tract carcinomas has increased our understanding of the molecular behavior of tumors in order to introduce novel therapeutic agents. There are two possible options in using molecular targeting agents—as first-line therapy used simultaneously with standard chemotherapy or as second-line therapy alone. Currently, there are limited findings on molecular–biological characteristics of biliary tract carcinomas, but further research on EGFR and anti-angiogenic inhibitors such as erlotinib, lapatinib, and sorafenib is soon expected [8]. Recently discovered pathways and/or mutations where molecular targeting agents could be effective include the PIK3CA/AKT/mTOR pathway, MET receptor tyrosine kinase inhibitors, ROS-1 translocations, and BRAF mutations [7]. Additionally, current studies report the significant role that zinc finger X-chromosomal protein (ZFX) has in gallbladder cancer cell proliferation and migration, which could potentially lead to therapies targeting the transcription factor [9]. With the rapid evolution of genome technology, the technical and economic feasibility of complete molecular profiling of a patient's tumor has increased substantially [10].

2 Diagnostic and Staging Technique

2.1 Pathology

Specific dissection of the histopathology and pathogenesis of bile tract carcinomas must be undertaken to advance current diagnostic, prognostic, and staging efforts. Complete characterization of the pathogenesis and prediction of metastatic involvement are of utmost concern in regard to the nature of these heterogeneous malignant cells. Can routine biopsies prevent advanced disease? Recently, the role of laparoscopic intraaortocaval (IAC) lymph node biopsy and frozen-section histopathological examination in gallbladder cancer is being studied to determine the potential effects on surgical management [11]. Studies of pathogenesis also reveal the possible role of hepatic progenitor cells in the malignant progression of intrahepatic cholangiocarcinoma, inherently adding clarification to the heterogeneity of such tumors [12]. Additionally, there is supporting data illustrating a direct correlation between microRNA (miRNA) and hepatobiliary neoplasia, which can lead to potential diagnostic biomarkers and new miRNA-based therapies in biliary tract cancers [13].

Because of the heterogeneity of biliary tract and gallbladder carcinomas, many biomarkers are expected to exist. For example, nemo-like kinase (NLK) has recently been associated with advanced tumor progression and poor clinical outcome in gallbladder cancer patients, which provides support of its potential to be used as a prognostic marker of gallbladder cancer [14]. Proteomic analysis of biliary carcinomas frequently serves as an approach to discover new biomarkers. According to Mulvenna et al. [15], cutting-edge technology in proteomics such as laser capture microdissection (LCM), isotopic labeling, sophisticated hypothesis-driven targeted mass spectrometric analyses, and the combination of these technologies can be used to detect and identify novel protein biomarkers. One such proteomic technique used recently was matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) combined with weak cation-exchange (WCX) magnetic beads and pattern recognition to rapidly detect cancer-related proteins [16]. Additionally, a pursuit for a serological method to noninvasively diagnose cholangiocarcinoma has been put forth. WFA-positive LICAM has been found to be a highly specific cholangiocarcinoma marker in bile and serum, and further investigation is being explored to enhance the reliability of the present system [17].

2.2 Imaging

Within the past few decades, the introduction of advanced image-guided diagnostic tools has made a large impact on the diagnosis and treatment of biliary tract and gallbladder carcinomas. Ultrasonography (US), computed tomography (CT), positron emission tomography (PET), magnetic resonance imaging (MRI), and cholangiography are beneficial imaging modalities currently being used. However, each modality has its advantages and disadvantages, and continued investigation is necessary to discover the most efficient diagnostic and staging techniques.

Most often, contrast-enhanced and endoscopic US are used in the early detection of bile duct tumors. A modern investigation confirmed that endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) is highly specific (100 %) with sensitivity rates of 87 and 80 % in the diagnosis of biliary tract and gallbladder cancer, respectively [18]. EUS-FNA has also recently been shown to be more accurate than CT or PET for the evaluation of regional lymph node metastases in patients with cholangiocarcinoma [19]. Additional studies are seeking the potential usefulness of new contrast agents in endoscopic and contrast-enhanced US. Finally, recent advances in contrast-enhanced US are expected to increase capabilities for early diagnosis.

A recent multicenter study investigated the usefulness of contrast-enhanced ultrasound (CEUS) in the diagnosis

of gallbladder carcinoma, and ultimately concluded that CEUS is undoubtedly superior to other ultrasound techniques in distinguishing biliary sludge from gallbladder lesions [20]. Additionally, Doppler and color Doppler ultrasound have been identified as beneficial to assessing vascularity of hepatobiliary lesions and vascular anatomy [21] as well as indicating expansive lesions by ruling out biliary sludge and cholesterol polyps [22]. However, more investigation into the imaging modality of ultrasound is essential. Despite the numerous advancements in ultrasound imaging, the majority of gallbladder masses identified preoperatively have been found to be “pseudo-masses” on final pathology [23]. Further investigation seeking high frequency probes [24], color Doppler, and contrast agents will dramatically enhance the effectiveness of ultrasound imaging.

In recent years, state-of-the-art cross-sectional imaging such as CT, MRI, and magnetic cholangiopancreatography (MRCP) has provided high-resolution imaging in contribution to the planning and treatment process of biliary tract cancers. Until recently, endoscopic retrograde cholangiopancreatography (ERCP) has been the gold standard in diagnosing pancreaticobiliary malignancies, though recent findings suggest MRCP is a more effective noninvasive method for obtaining quality images of the biliary tract [25]. Additionally, recent advances in multidetector row-computed tomography (MDCT) have demonstrated increased accuracy rates of differential diagnosis of gallbladder polyps [26]. MRI modifications such as an increased flip angle have resulted in significantly enhanced visualization of the biliary tree [27]. Newly improved instrumentation of MRI has greatly increased spatial and contrast resolution as well as signal-to-noise ratio [26] although improvements of more intervention-compatible technology would have great clinical potential.

Simultaneous PET/CT imaging has great potential in the staging, restaging, and determination of recurrence in biliary tract cancers although current studies are in search of alternate PET radiotracers to ^{18}F -FDG that are tumor-specific. For example, ^{68}Ga -NOTA-RGD has been recently discovered as a PET radiotracer to visualize angiogenesis and is a promising candidate for cancer imaging [28]. Very recently, however, combined PET/MRI has become increasingly popular. PET/MRI is believed to effectively integrate individual molecular, functional, and anatomical imaging techniques to be used in radiotherapy treatment planning [29]. Although current research on the advantage of PET/MRI in biliary tract tumors is limited, separately acquired data from PET/CT and MRI fusion images suggest that PET/MRI has an advantage over PET/CT [30]. Further investigations must be performed to determine the benefit of the precise tumor volume delineation of biliary tract cancers associated with PET/MRI.

3 Therapy

Many novel treatment options have been introduced to improve the multifaceted management of bile duct and gallbladder cancer patients. Modern advances in transarterial and percutaneous therapies within the last decade have improved treatment effects while minimizing morbidity from systemic exposure. Transarterial chemoembolization (TACE), for example, has recently been confirmed to be an effective palliation intervention with overall survival benefits without negatively impacting the patient's overall quality of life [31]. TACE may be applied in combination or in sequences to improve outcome achieved by systemic chemotherapy [32]. Moreover, irinotecan-eluting beads (iDEB-TACE) are shown to be an effective treatment in patients with intrahepatic cholangiocarcinoma, safely improving progression-free survival, and overall survival rates [32]. Doxorubicin drug-eluting beads and transarterial hepatic Yttrium-90 radioembolization are also found to be effective in prolonging survival in patients with unresectable intrahepatic cholangiocarcinoma [33].

Surgical resection resulting in margin negativity remains the best predictor of long-term survival for both bile duct and gallbladder cancers. With the latest advances, more aggressive surgical approaches have emerged and minimally invasive surgery has become more prominent. Laparoscopic liver resection techniques have improved and have recently been reported as a superior option to open surgery, without compromising oncological outcomes [34]. To date, staging laparoscopy has been shown to be very effective at detecting unresectable gallbladder tumors and identify metastases that were not identified on standard imaging [35]. In cases of unresectable cholangiocarcinoma, a recent study reports robotic palliation as a safe and effective, minimally invasive approach to treatment, offering low morbidity, mortality and postoperative pain, short hospital stays, and fewer readmissions [36]. A recent investigation on advances in systemic and radiotherapy in gallbladder cancer reports that less than 5 % of gallbladder cancer patients are receiving the appropriate surgery (hepatectomy and lymphadenectomy) and advocates overcoming this misfortune prior to assessing additional effects of adjuvant chemotherapy and radiation to improve survival [37].

Advances in systemic therapy and radiotherapy are currently under investigation as well. Based on recent randomized clinical trials, chemotherapy combining gemcitabine with a platinum agent (cisplatin or oxaliplatin) is considered the standard first-line treatment for patients with advanced biliary tract cancer and additional studies are being conducted with varying combinations of gemcitabine, capecitabine, and irinotecan [7]. A recent report determined

that inhibition of gemcitabine-induced NF- κ B activation may be a new chemotherapeutic approach against malignant tumors in the gallbladder [38]. Recently, a novel oral fluoropyrimidine agent called S-1 was combined with gemcitabine in a chemotherapeutic approach, reporting a synergistic effect with promising survival benefit in patients with advanced biliary tract cancer [39]. Current studies are also being conducted on second-line treatment and possible regimens if first-line chemotherapy resulted in a failure. Evidently, new drugs and chemotherapy regimens, and eventually standard treatment regimens, are necessary to manage bile duct and gallbladder cancers and the development of these regimens will result as soon as molecular mechanisms in the preclinical setting are better understood.

Lately, the role of vaccines in the treatment of biliary tract cancers has been evaluated. One study has suggested the use of dendritic cell-based immunotherapy pulsed with WT1 and/or MUC1 as a safe way to manage patients with advanced biliary tract cancer who do not have the option of curative surgery [40]. Additionally, a novel immunotherapeutic approach called personalized peptide vaccine (PPV) has been developed to provide better antigen-specific immune responses [41]. Repetitive, change to "furthermore" a recent study concludes that all-trans retinoic acid (RA)-incorporated glycol chitosan (GC) nanoparticles demonstrate anti-proliferative effects against human cholangiocarcinoma cells, inhibiting tumor cell invasion and migration [42].

Although prospective data on radiation therapy is limited, retrospective literature has suggested a benefit in adjuvant and definitive settings in terms of local control and even survival. Stereotactic body radiation therapy (SBRT) has been associated with a high rate of late grade 3–4 gastrointestinal complications, but a recent clinical investigation indicates that SBRT up to a cumulative dose of 40 Gy in five fractions was a feasible treatment for liver tumors adjacent to the central biliary system with minimal biliary toxicity [43]. Furthermore, particle beam, such as proton and carbon ion beam, radiation therapy may allow for dose escalation of unresectable bile duct and gallbladder cancers while reducing dose to normal tissues when compared with photon therapy [44]. This accuracy will potentially reduce side effects that participants would normally experience using photon radiation therapy. Further studies regarded toxicity and efficacy levels are needed to understand dose tolerances of organs at risk and improved conformality of treatment modalities.

Effective neoadjuvant or adjuvant treatments have not yet been demonstrated, but this remains a subject of ongoing investigation. In fact, we anxiously await the results of the first prospective study led by Southwest Oncology Group, evaluating the role of adjuvant capecitabine, gemcitabine, and oxaliplatin chemotherapy and modern radiation therapy in patients with resected bile duct or gallbladder tumors.

A study conducted by Horgan et al. represents the largest pooled analysis of available data on the role of adjuvant therapy in the treatment of biliary tract cancers and presented evidence that chemotherapy or chemoradiation was better than radiation alone in patients with lymph node- or margin-positive resections [2]. Very recently, a retrospective review looking at locally advanced cholangiocarcinoma compared traditional resection with orthotopic liver transplantation (OLT), demonstrating that neoadjuvant chemotherapy and OLT were a superior option to radical resection and adjuvant therapy [45]. However, it is difficult to make final recommendations given the small population of patients.

Remarkably enough, plant-derived compounds are gaining increased consideration as potential cancer therapies. One study demonstrated that the crude ethanolic extract of ginger had cytotoxic, anticancer activities in cholangiocarcinoma cell lines in vitro at dose levels that may be acceptable for clinical trials [46]. Additionally, the ethanolic extract of *Atractylodes lancea* (AL) has been shown to possess potent anti-cholangiocarcinoma activity with the reduction in tumor mass and prolongation of survival time at all dose levels as well as the extracts of Pra-Sa-Prao-Yhai formulation (PPF) and curcumin (CUR) at high dose levels [47]. Studies combining these therapies with standard therapies are needed, given that they may have non-overlapping toxicities.

4 Conclusion

While significant advances have been made regarding biliary tract and gallbladder carcinomas, results from an increased number of long-term prospective clinical trials are crucial. A multidisciplinary approach with collaborative efforts among physicians, medical physicists, dosimetrists, nurses, and therapists in cancer centers worldwide in addition to the respective treatment center is paramount to deciphering the unresolved issues and ultimately overcoming the biological and therapeutic challenges of biliary tract and gallbladder cancers. An optimistic, systematic, and multi-targeted approach to close the gaps in our understanding of the disease and its pathogenesis, standardized terminology, and the limited number of management strategies is essential to pave the way for a better future.

References

1. Cleary S, Dawson L, Knox J, Gallinger S (2007) Cancer of the gallbladder and extrahepatic bile ducts. *Curr Probl Surg* 44(7):396–482
2. Ciombor K, Goff L (2013) Current therapy and future directions in biliary tract malignancies. *Curr Treat Options Oncol* 14(3):1–13
3. Michaud D (2002) The epidemiology of pancreatic, gallbladder, and other biliary tract cancers. *Gastrointest Endosc* 56(6 Suppl):S195–S200
4. Voss J, Holtegaard L, Kerr S, Barr Fritcher E, Roberts L, Gores G et al (2013) Molecular profiling of cholangiocarcinoma shows potential for targeted therapy treatment decisions. *Hum Pathol* 44(7):1216–1222
5. Borger D, Iafrate A, Zhu A (2013) A new signature for bile duct cancer. *Adv Mass Gen Cancer Cent* 3(1):10–11
6. Xu Q, Zhang Y, Wang X (2013) HEPA and PARSE: systematic discovery of clinically relevant tumor-specific antigens. *Oncoimmunology* 2(3):0–1
7. Geynisman D, Catenacci D (2012) Toward personalized treatment of advanced biliary tract cancers. *Disc Med* 14(74):41–57
8. Furuse J, Okusaka T (2011) Targeted therapy for biliary tract cancer. *Cancers* 3(2):2243–2254
9. Tan Z, Zhang S, Li M, Wu X, Weng H, Ding Q et al (2013) Regulation of cell proliferation and migration in gallbladder cancer by zinc finger X-chromosomal protein. *Gene* 528(2):261–266
10. Chin L, Andersen J, Futreal P (2011) Cancer genomics: from discovery science to personalized medicine. *Nat Med* 17(3):297–303
11. Agarwal AK, Kalayarasan R, Javed A, Sakhuja P (2013) Role of routine 16b1 lymph node biopsy in the management of gallbladder cancer: an analysis. *HPB (Oxford)*. doi:10.1111/hpb.12127. (in press)
12. Oishi N, Kumar M, Roessler S, Ji J, Forgues M, Budhu A et al (2012) Transcriptomic profiling reveals hepatic stem-like gene signatures and interplay of miR-200c and epithelial-mesenchymal transition in intrahepatic cholangiocarcinoma. *Hepatology* 56(5):1792–1803
13. Papaconstantinou I, Karakatsanis A, Gazouli M, Polymeneas G, Voros D (2012) The role of microRNAs in liver cancer. *Eur J Gastroenterol Hepatol* 24(3):223–229
14. Li M, Zhang S, Wang Z, Zhang B, Wu X, Weng H et al (2013) Prognostic significance of nemo-like kinase (NLK) expression in patients with gallbladder cancer. *Tumor Biol* 34(6):3995–4000
15. Mulvenna J, Yonglitthipagon P, Sripa B, Brindley P, Loukas A, Bethony J (2012) Banking on the future: biobanking for “omics” approaches to biomarker discovery for opisthorchis-induced cholangiocarcinoma in Thailand. *Parasitol Int* 61(1):173–177
16. Mou Y, Xing R, Liu C (2012) Diagnosis of gallbladder cancer using matrix-assisted laser desorption/ionization time-of-flight profiling. *Am J Med Sci* 343(2):119–123
17. Matsuda A, Kuno A, Matsuzaki H, Kawamoto T, Shikanai T, Nakanuma Y et al (2013) Glycoproteomics-based cancer marker discovery adopting dual enrichment with *Wisteria floribunda* agglutinin for high specific glyco-diagnosis of cholangiocarcinoma. *J Proteomics* 85:1–11
18. Wu L, Jiang X, Gu H, Xu X, Zhang W, Lin L et al (2011) Endoscopic ultrasound-guided fine-needle aspiration biopsy in the evaluation of bile duct strictures and gallbladder masses: a systematic review and meta-analysis. *Eur J Gastroenterol Hepatol* 23(2):113–120
19. Anderson M, Appalaneni V, Ben-Menachem T, Decker G, Early D, Evans J et al (2013) The role of endoscopy in the evaluation and treatment of patients with biliary neoplasia. *Gastrointest Endosc* 77(2):167–174
20. Liu L, Xu H, Lu M, Xie X, Wang W, Hu B et al (2012) Contrast-enhanced ultrasound in the diagnosis of gallbladder diseases: a multi-center experience. *PloS One* 7(10):1–13
21. Faingold R, Albuquerque P, Carpineta L (2011) Hepatobiliary tumors. *Radiol Clin North Am* 49:679–687
22. Barretta M, Catalano O, Setola S, Granata V, Marone U, Gallipoli A (2011) Gallbladder metastasis: spectrum of imaging findings. *Abdom Imaging* 36(6):729–734

23. Konstantinidis I, Bajpai S, Kambadakone A, Tanabe K, Berger D, Zheng H et al (2012) Gallbladder lesions identified on ultrasound. Lessons from the last 10 years. *J Gastrointest Surg* 16(3):549–553
24. Dutta U (2012) Gallbladder cancer: can newer insights improve the outcome? *J Gastroenterol Hepatol* 27(4):642–653
25. Takuma K, Kamisawa T, Tabata T, Hara S, Kuruma S, Inaba Y et al (2012) Importance of early diagnosis of pancreaticobiliary maljunction without biliary dilatation. *World J Gastroenterol* 18(26):3409–3414
26. Inui K, Yoshino J, Miyoshi H (2011) Diagnosis of gallbladder tumors. *Intern Med* 50(11):1133–1136
27. Stelter L, Grieser C, Perez Fernandes C, Rothe J, Streithparth F, Seehofer D et al (2012) Flip angle modulations in late phase of gd-EOB-DTPA MRI improve the identification of the biliary system. *Eur J Radiol* 81(11):e991–e995
28. Kim J, Lee J, Kang K, Lee H, Han S, Kim T et al (2012) Whole-body distribution and radiation dosimetry of 68 Ga-NOTA-RGD, a positron emission tomography agent for angiogenesis imaging. *Cancer Biother Radiopharm* 27(1):65–71
29. Leibfarth S, Monnich D, Welz S, Siegel C, Schwenzer N, Schmidt H et al (2013) A strategy for multimodal deformable image registration to integrate PET/MR into radiotherapy treatment planning. *Acta Oncol* 52(7):1353–1359
30. Jadvar H, Colletti P (2013) Competitive advantage of PET/MRI. *Eur J Radiol* 83(1):84–94
31. Eltawil K, Berry R, Abdoell M, Molinari M (2012) Quality of life and survival analysis of patients undergoing transarterial chemoembolization for primary hepatic malignancies: a prospective cohort study. *HPB* 14(5):341–350 (Oxford)
32. Kuhlmann J, Euringer W, Spangenberg H, Breidert M, Blum H, Harder J et al (2012) Treatment of unresectable cholangiocarcinoma: conventional transarterial chemoembolization compared with drug eluting bead-transarterial chemoembolization and systemic chemotherapy. *Eur J Gastroenterol Hepatol* 24(4):437–443
33. Hoffman R, Paprottka P, Schon A, Bamberg F, Haug A, Durr E et al (2012) Transarterial hepatic yttrium-90 radioembolization in patients with unresectable intrahepatic cholangiocarcinoma: factors associated with prolonged survival. *Cardiovasc Intervent Radiol* 35(1):105–116
34. Lee W, Chan C, Wang B (2013) Recent advances in laparoscopic surgery. *Asian J Endosc Surg* 6(1):1–8
35. Zhuang P, Tang Z, Liu Y, Quan Z, Zhang Y (2012) Diagnostic and prognostic role of laparoscopic staging for gallbladder carcinoma. *Dig Surg* 29(5):367–373
36. Buchs N, Addeo P, Bianco F, Elli E, Ayloo S, Giulianotti P (2011) Robotic palliation for unresectable pancreatic cancer and distal cholangiocarcinoma. *Int J Med Robot* 7(1):60–65
37. Pilgrim C, Groeschl R, Quebbeman E, Gamblin T (2013) Recent advances in systemic therapies and radiotherapy for gallbladder cancer. *Surg Oncol* 22(1):61–67
38. Iwase R, Haruki K, Fujiwara Y, Furukawa K, Shiba H, Uwagawa T et al (2013) Combination chemotherapy of nafamostat mesylate with gemcitabine for gallbladder cancer targeting nuclearfactor-kB activation. *J Surg Res* 184(1):605–612
39. Lim J, Ryu J, Choi Y, Kwon J, Kim J, Lee Y et al (2013) A case of common bile duct cancer that completely responded to combination chemotherapy of gemcitabine and TS-1. *Gut Liver* 7(3):371–376
40. Kobayashi M, Sakabe T, Abe H, Tani M, Takahashi H, Chiba A et al (2013) Dendritic cell-based immunotherapy targeting synthesized peptides for advanced biliary tract cancer. *J Gastrointest Surg* 17(9):1609–1617
41. Takahashi R, Yoshitomi M, Yutani S et al (2013) Current status of immunotherapy for the treatment of biliary tract cancer. *Hum Vacc Immunother* 9(5):1069–1072
42. Chung K, Jeong Y, Chung C, Kim D, Kang D (2012) Anti-tumor activity of all-trans retinoic acid-incorporated glycol chitosan nanoparticles against HuCC-T1 human cholangiocarcinoma cells. *Int J Pharm* 422(1–2):454–461
43. Eriguchi T, Takeda A, Sanuki N et al (2013) Acceptable toxicity after stereotactic body radiation therapy for liver tumors adjacent to the central biliary system. *Int J Radiat Oncol Biol Phys* 85(4):1006–1011
44. Proton Beam Irradiation for the Treatment of Unresectable Hepatocellular Cancer and Cholangiocarcinoma (2013). Available from: ClinicalTrials.gov [Internet]
45. Valero V, Cosgrove D, Herman J, Pawlik T (2012) Management of perihilar cholangiocarcinoma in the era of multimodal therapy. *Expert Rev Gastroenterol Hepatol* 6(4):481–495
46. Plengsuriyakarn T, Viyanant V, Eursitthichai V, Tesana S, Chaijaroenkul W, Itharat A et al (2012) Cytotoxicity, toxicity, and anticancer activity of *Zingiber officinale* Roscoe against cholangiocarcinoma. *Asian Pacific J Cancer Prev* 13(9):4597–4606
47. Plengsuriyakarn T, Viyanant V, Eursitthichai V et al (2012) Anticancer activities against cholangiocarcinoma, toxicity and pharmacological activities of thai medicinal plants in animal models. *BMC Complement Alt Med* 12(23). doi: [10.1186/1472-6882-12-23](https://doi.org/10.1186/1472-6882-12-23)

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