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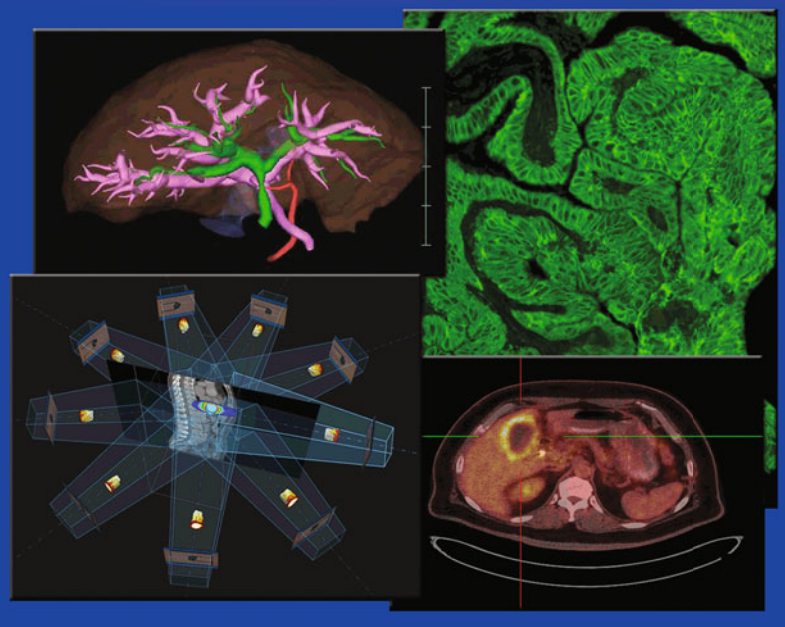
Timothy M. Pawlik
Charles R. Thomas, Jr.
*Editors*

# Biliary Tract and Gallbladder Cancer

## A Multidisciplinary Approach

*Second Edition*



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## A Multidisciplinary Approach

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*Editors*
Joseph M. Herman
Department of Radiation Oncology and
Molecular Radiation Sciences
Johns Hopkins University
Baltimore, MD
USA

Timothy M. Pawlik
Department of Surgery
Johns Hopkins Hospital
Baltimore, MD
USA

Charles R. Thomas, Jr.
Department of Radiation Oncology
Oregon Health and Science University
Portland, OR
USA

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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 — Luther W. Brady
Hamburg — Hans-Peter Heilmann
München — Michael Molls
Bodø — 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.

# Contents

# Contributors

**Domenico Alvaro** Division of Gastroenterology, Department of Medico-Surgical Sciences and Biotechnologies, Pontino, Fondazione Eleonora Lorillard Spencer Cenci, Sapienza University of Rome, Rome, Italy

**Ijeoma A. Azodo** Division of Hepatobiliary and Pancreas Surgery, Department of Clinical Surgery, University of Edinburgh, Edinburgh, UK

**Gory Ballester-Ortiz** Section of Diagnostic Radiology, University of Puerto Rico School of Medicine, San Juan, PR, USA

**Edgar Ben-Josef** Department of Radiation Oncology, Perelman Center for Advanced Medicine, Hospital of the University of Pennsylvania, Philadelphia, PA, USA

**Susanne Bonekamp** The Russell H. Morgan Department of Radiology and Radiological Sciences, The Johns Hopkins School of Medicine, Baltimore, USA

**Kimberly M. Brown** Department of Surgery, University of Texas Medical Branch, Galveston, TX, USA

**Vincenzo Cardinale** Division of Gastroenterology, Department of Medico-Surgical Sciences and Biotechnologies, Pontino, Fondazione Eleonora Lorillard Spencer Cenci, Sapienza University of Rome, Rome, Italy

**Héber Salvador de Castro Ribeiro** Surgical Oncology, Department of Abdominal Surgery, AC Camargo Cancer Center, São Paulo, Brazil

**Muhammad A. Chaudhry** School of Medicine and Medical Director, Tawam Molecular Imaging Centre, Johns Hopkins University, Al-Ain, United Arab Emirates; Division of Radiology, School of Medicine, Johns Hopkins University, Baltimore, USA

**Felipe José Fernández Coimbra** Surgical Oncology, Department of Abdominal Surgery, AC Camargo Cancer Center, São Paulo, Brazil

**Celia Pamela Corona-Villalobos** The Russell H. Morgan Department of Radiology and Radiological Sciences, The Johns Hopkins School of Medicine, Baltimore, USA

**Wilson Luiz da Costa Junior** Surgical Oncology, Department of Abdominal Surgery, AC Camargo Cancer Center, São Paulo, Brazil

**Vikram Deshpande** Department of Pathology, Massachusetts General Hospital, Boston, USA

**John DiGiovanni** Dell Pediatric Research Institute, College of Pharmacy, The University of Texas at Austin, Austin, TX, USA

**Igor Correia de Farias** Surgical Oncology, Department of Abdominal Surgery, AC Camargo Cancer Center, São Paulo, Brazil

**Yuman Fong** Department of Surgery, Murray F. Brennan Chair in Surgery, Memorial Sloan–Kettering Cancer Center, New York, NY, USA

**O. James Garden** Division of Hepatobiliary and Pancreas Surgery, Department of Clinical Surgery, University of Edinburgh, Edinburgh, UK

**David A. Geller** Liver Cancer Center, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

**André Luis de Godoy** Surgical Oncology, Department of Abdominal Surgery, AC Camargo Cancer Center, São Paulo, Brazil

**Vivek Gowdra Halappa** The Russell H. Morgan Department of Radiology and Radiological Sciences, The Johns Hopkins School of Medicine, Baltimore, USA

**Joseph M. Herman** Department of Radiation Oncology and Molecular Radiation Sciences, Johns Hopkins University, Baltimore, MD, USA

**Daniel J. Holzwanger** Department of Radiology, Division of Interventional Radiology, New York-Presbyterian Hospital, Weill Cornell Medical Center, New York, NY, USA

**Theodore S. Hong** Department of Radiation Oncology, Massachusetts General Hospital, Boston, MA, USA

**Lujaien Al-Rubaiey Kadhim** Research Consultant, Tawam Molecular Imaging Centre, Al-Ain, United Arab Emirates

**Anusha Kalbasi** Department of Radiation Oncology, Perelman Center for Advanced Medicine, Hospital of the University of Pennsylvania, Philadelphia, PA, USA

**Ihab R. Kamel** The Russell H. Morgan Department of Radiology and Radiological Sciences, The Johns Hopkins School of Medicine, Baltimore, USA

**Andrew S. Kennedy** Radiation Oncology, Sarah Cannon Research Institute, Nashville, TN, USA

**Kaoru Kiguchi** Dell Pediatric Research Institute, College of Pharmacy, The University of Texas at Austin, Austin, TX, USA

**Laura Lambert** Division of Surgical Oncology and Palliative Medicine, UMass Memorial Medical Center, Worcester, MA, USA

**David C. Madoff** Department of Radiology, Division of Interventional Radiology, New York-Presbyterian Hospital, Weill Cornell Medical Center, New York, NY, USA

**Leonardo Marcal** Department of Diagnostic Radiology, Unit 1473, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

**L. Matthew Scala** Kaiser Permanente Radiation Oncology, Santa Clara, CA, USA

**Taofic Mounajjed** Division of Anatomic Pathology, Mayo Clinic, Rochester, MN, USA

**Subir Nag** Kaiser Permanente Radiation Oncology, Santa Clara, CA, USA

**David M. Nagorney** Department of Surgery, Mayo Clinic, Rochester, MN, USA

**Heljä Oikarinen** Department of Diagnostic Radiology, Oulu University Hospital (OYS), Oulu, Finland

**Rowan W. Parks** Division of Hepatobiliary and Pancreas Surgery, Department of Clinical Surgery, University of Edinburgh, Edinburgh, UK

**John G. Phillips** Harvard Radiation Oncology Program, Boston, MA, USA

**Andrea Primiani** Harvard Medical School, Massachusetts General Hospital, Boston, USA

**Amudhan Pugalenthi** Department of Surgery, Memorial Sloan–Kettering Cancer Center, New York, NY, USA

**Lauren M. Rosati** Department of Radiation Oncology, Johns Hopkins University, Baltimore, MD, USA

**Charles B. Rosen** Department of Surgery, Mayo Clinic, Rochester, MN, USA

**Laura Rubbia-Brandt** Division of Clinical Pathology, Faculty of Medicine of Geneva, Geneva University Hospital, Geneva, Switzerland

**Junichi Shindoh** The University of Texas, MD Anderson Cancer Center, Texas, USA

**Ross C. Smith** Department of Surgery, University of Sydney, Sydney, NSW, Australia

**Janio Szklaruk** Department of Diagnostic Radiology, Unit 1473, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

**Adam D. Talenfeld** Department of Radiology, Division of Interventional Radiology, New York-Presbyterian Hospital, Weill Cornell Medical Center, New York, NY, USA

**Benoit Terris** Service de pathologie, Hôpital Cochin Assistance Publique-Hôpitaux de Paris, Université Paris Descartes, Paris, France

**Melanie B. Thomas** Charleston, SC, USA

**Chitra Viswanathan** Department of Diagnostic Radiology, Unit 1473, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

**Richard L. Wahl** Division of Radiology, School of Medicine, Johns Hopkins University, Baltimore, USA

**John A. Wolfgang** Department of Radiation Oncology, Massachusetts General Hospital, Boston, MA, USA

**Tsung-Teh Wu** Division of Anatomic Pathology, Mayo Clinic, Rochester, MN, USA

**Victor M. Zaydfudim** Department of Surgery, Mayo Clinic, Rochester, MN, USA

**Giuseppe Zimmitti** The University of Texas, MD Anderson Cancer Center, Texas, USA

# Epidemiology of Cholangiocarcinoma and Gallbladder Carcinoma

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

## Contents




**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 GBCA 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 GBCA include longstanding cholelithiasis,



I. A. Azodo · R. W. Parks (✉) · O. J. Garden
Division of Hepatobiliary and Pancreas Surgery, Department of Clinical Surgery, University of Edinburgh, Edinburgh, UK
e-mail: r.w.parks@ed.ac.uk


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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.

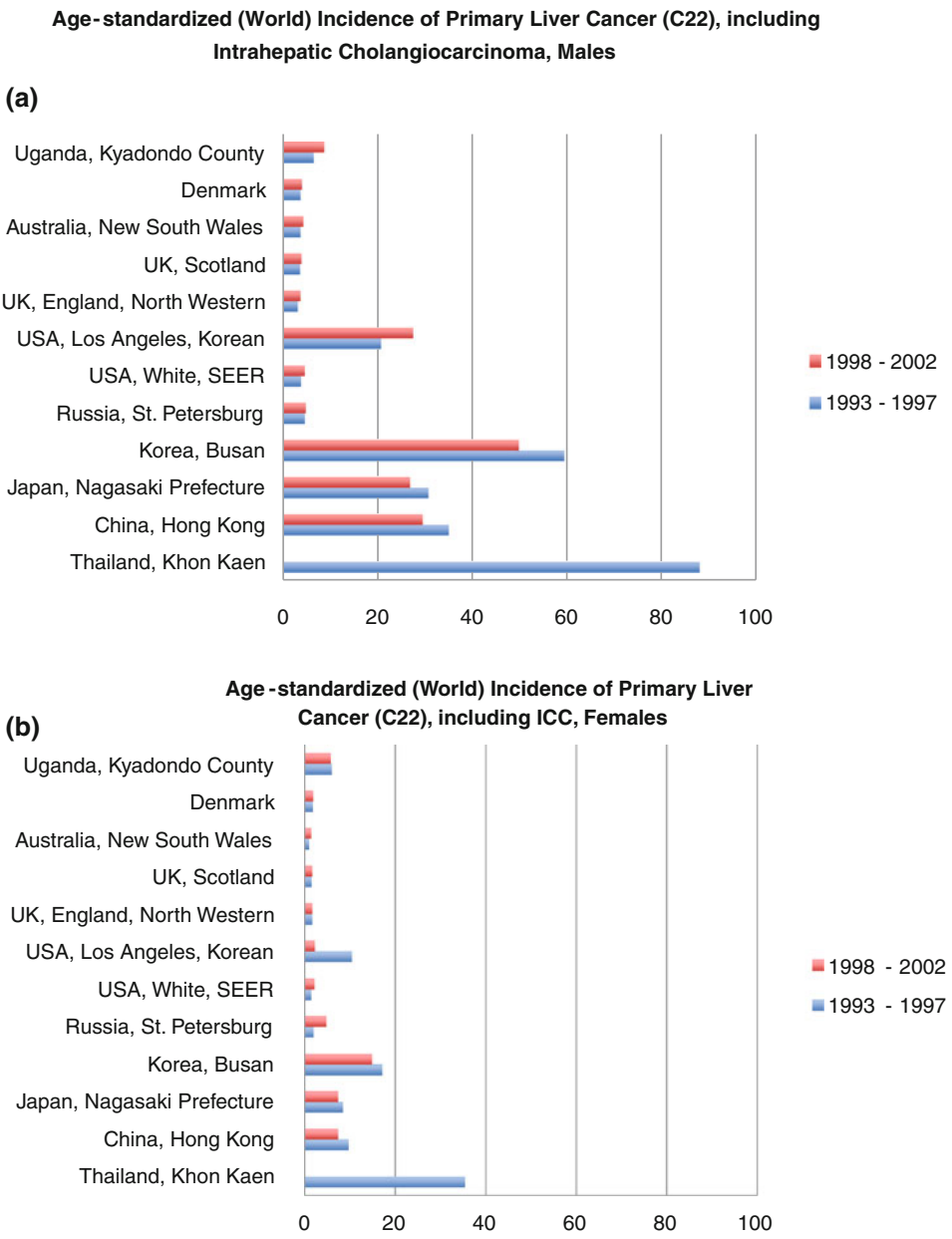



**Fig. 1** Age-standardized (World) incidence (per 100,000) of primary liver cancer (C22) including ICC (C22.1) in high-risk, low-risk and endemic population areas, **a** males, **b** females. Rates shown on *x*-axis are per 100,000 person-years. *Source* WHO IARC [5, 6]

## 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]

**Table 2** Incidence rates (per 100,00) 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]

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 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 gallbladder 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 (gallbladder, 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 'Klatskin' 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

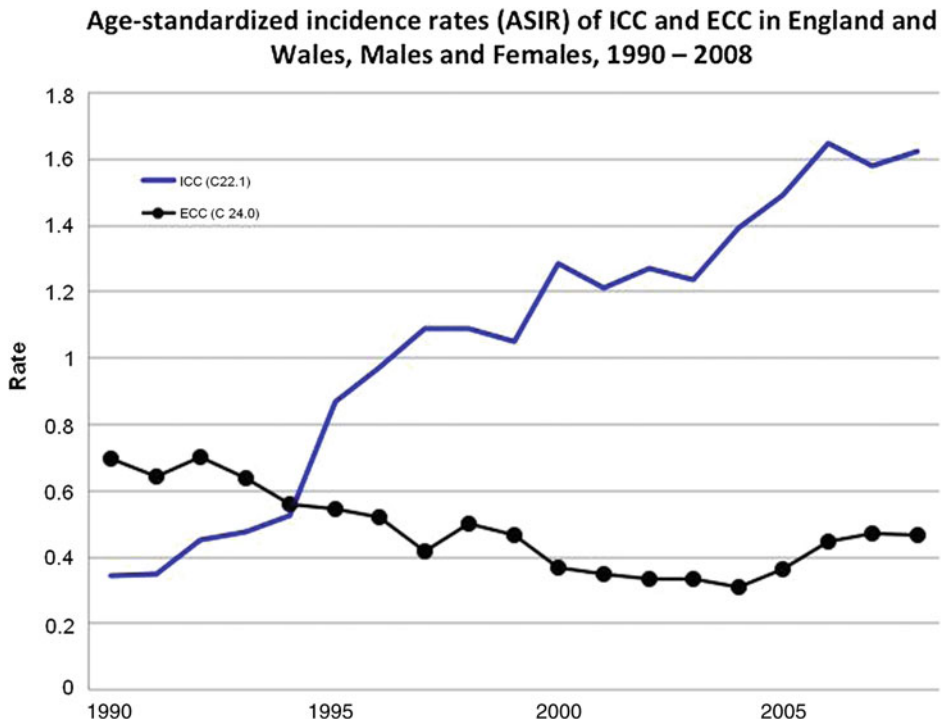



**Fig. 2** Age-standardized incidence rates (ASIR) per 100,000 of ICC and ECC in England and Wales, 1990–2008, Males and Females combined. ICC (C22.1), excludes Klatskin tumors (M8162/3). ECC (C24.0), includes Klatskin tumors (M8162.3). Modified and reprinted with permission from Khan et al. 2012 [33]

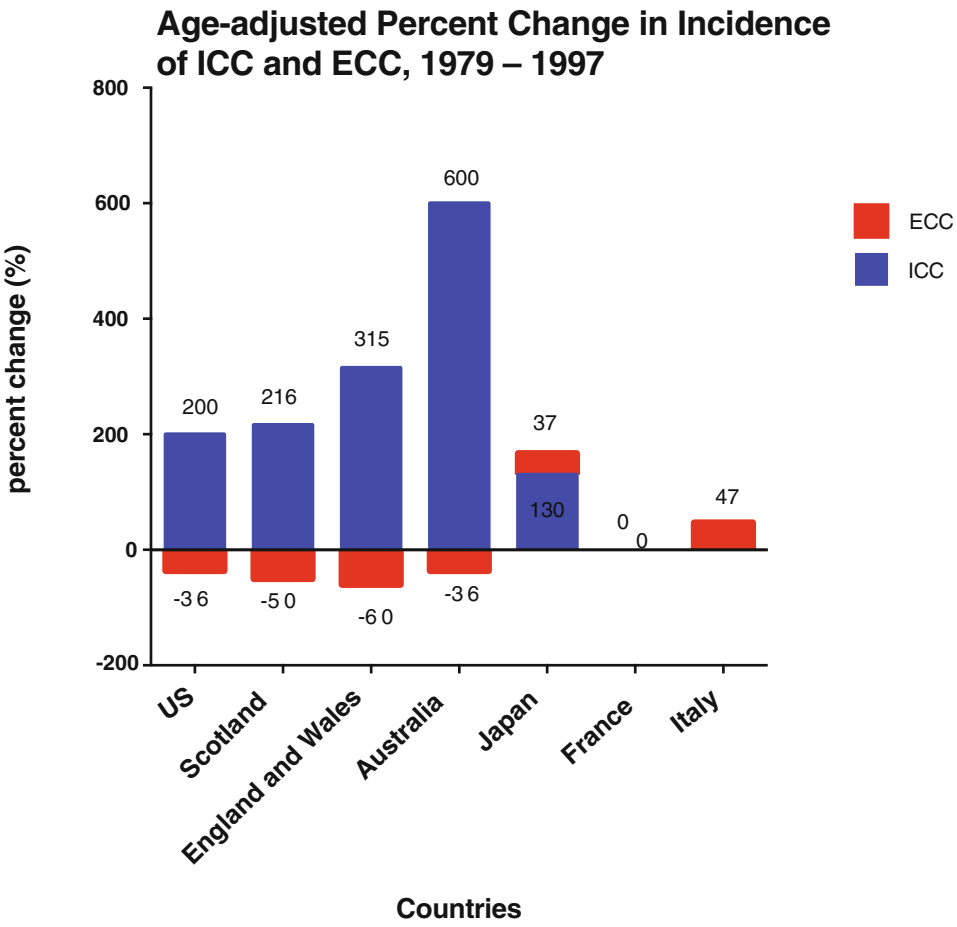



**Fig. 3** Age-adjusted percentage change in incidence rate (per 100,000) of ICC and ECC in Select Countries, 1979–1997 [41]. Percent change is shown on *y*-axis and select countries on the *x*-axis. Numerical values for absolute percentage change are shown with the corresponding column

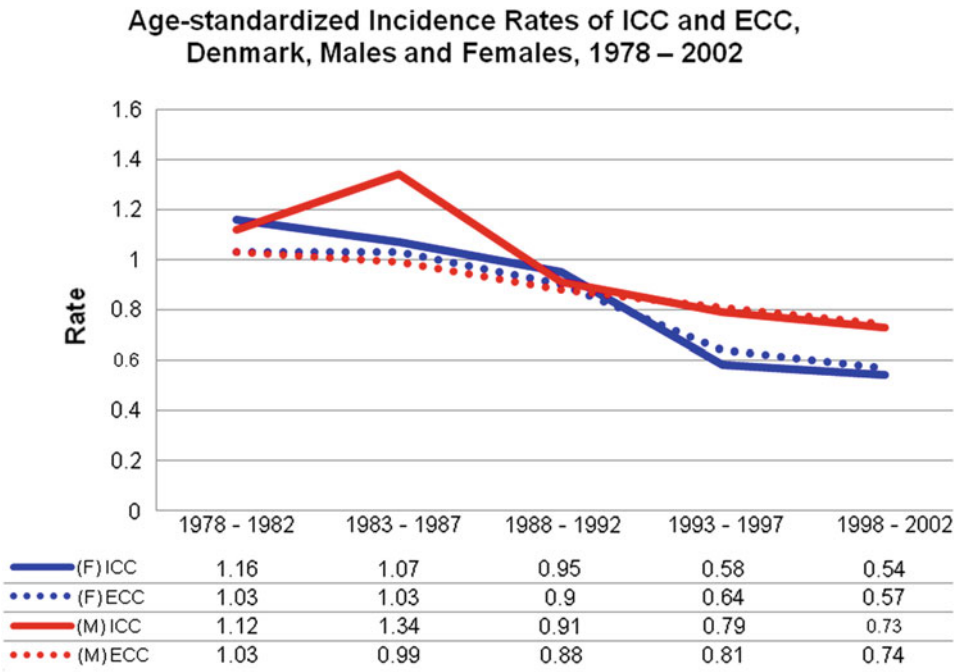



**Fig. 4** Age-standardized (US 2000) incidence rates (per 100,000) of intrahepatic cholangiocarcinoma (*ICC*) and extrahepatic cholangiocarcinoma (*ECC*) for Denmark, Males (*M*) and Females (*F*), 1978–2002 [44]. Incidence rate is shown on the *y*-axis and grouped time intervals in years are shown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

**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 %), northeast (15.8 %), and central (3.8 %) regions of Thailand [54, 61]. *O. viverinni* 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. viverinni* 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].

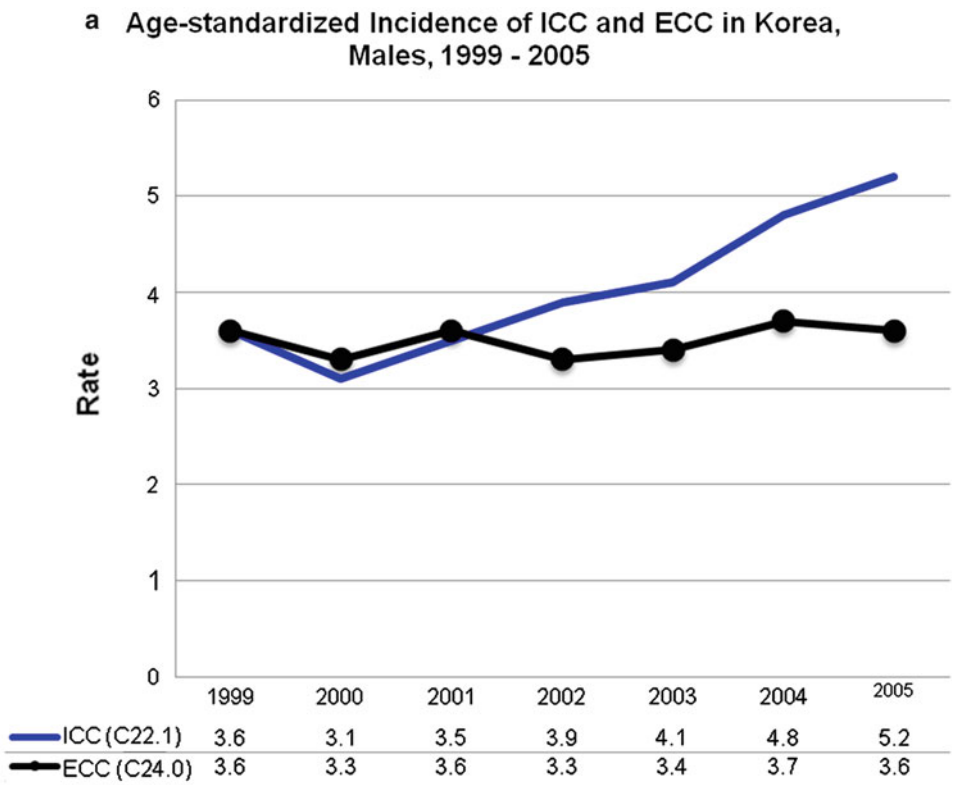
For the rest of Thailand, data in 2002 demonstrated an incidence of 71.4 per 100,000 for ICC in males and 31.6 per 100,000 in females. Corresponding rates of ECC in Korea are dramatically less at 0.4 per 100,000 in males and 0.1 per 100,000 in females [66] (Fig. 5a, b). Recent results documenting the 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].



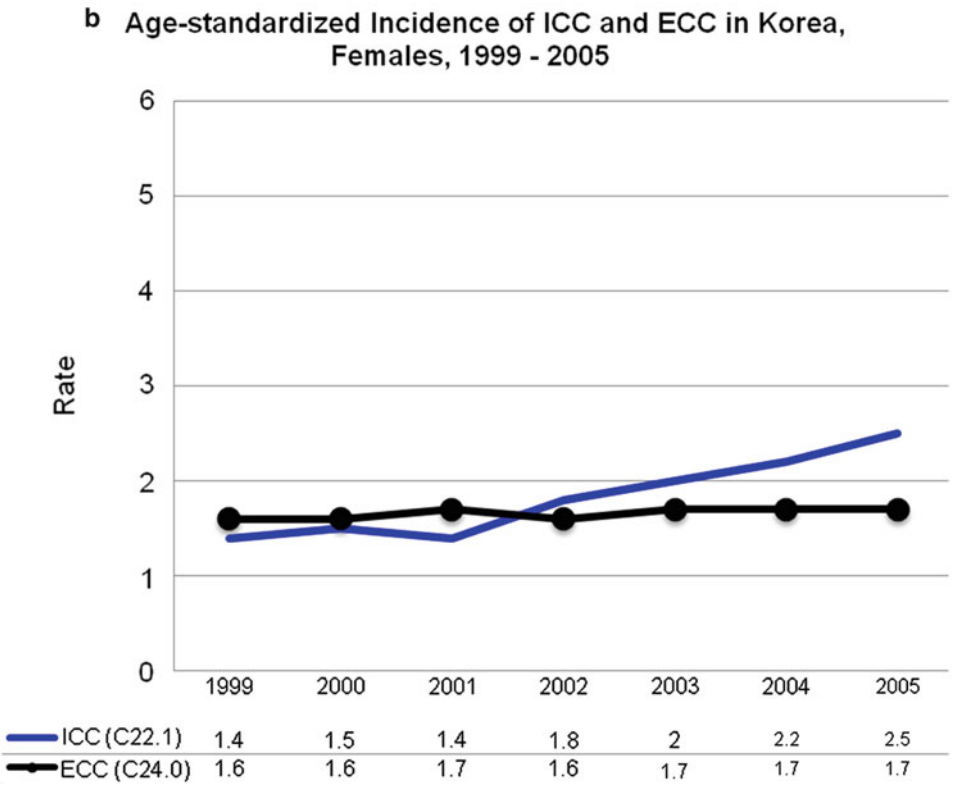




**Fig. 5** Age-standardized incidence rates (*ASIR*) of intrahepatic cholangiocarcinoma (C22.1) and extrahepatic cholangiocarcinoma (C24.0) in Korean, **a** males and **b** females. Rates shown on *x*-axis are per 100,000 person-years. Overall rate for ICC is 4.1 males, 1.8 females with a +7.9 % and +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 $\geq$ 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*

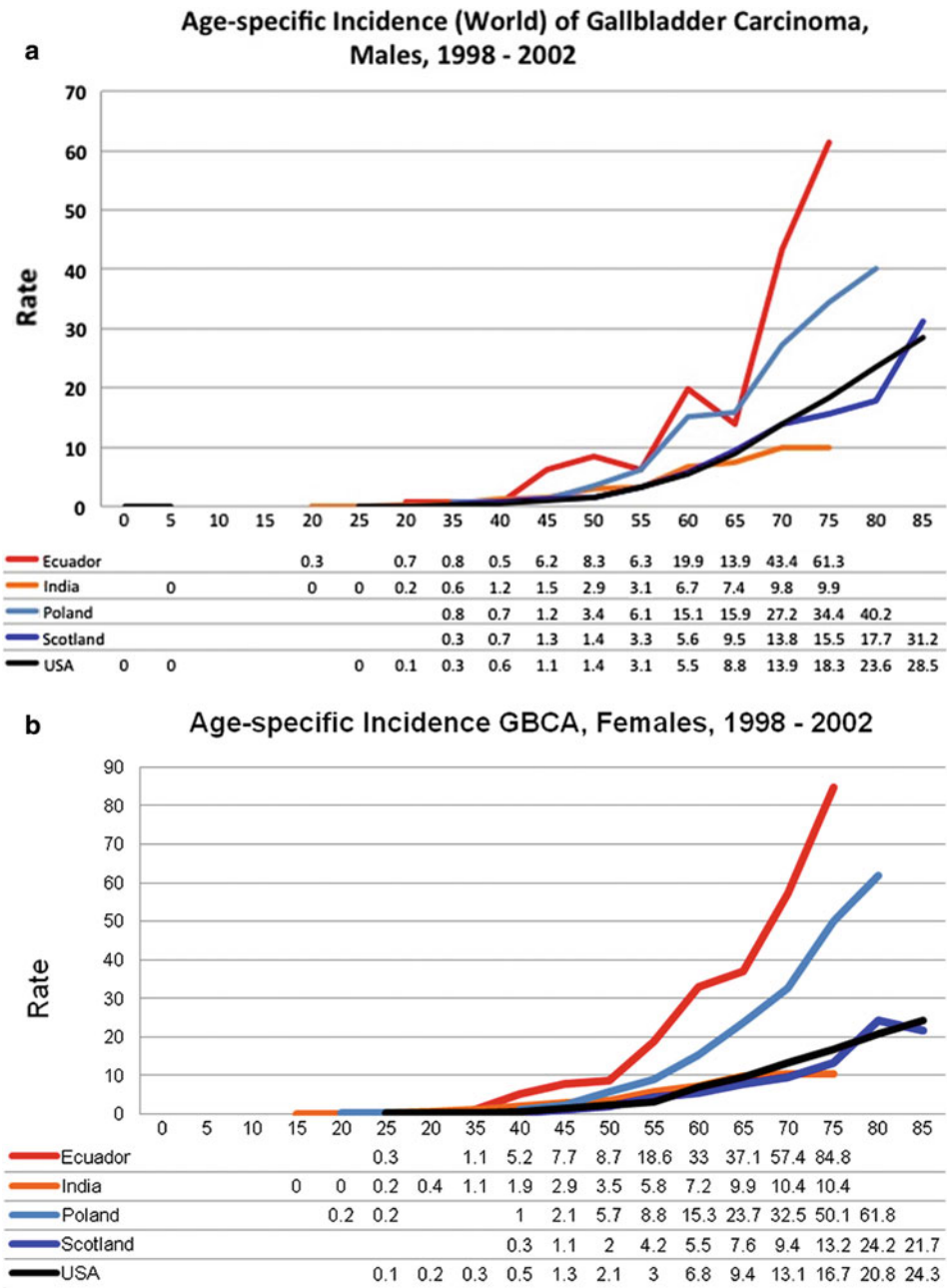



**Fig. 6** Age-specific incidence world rates (*ASRW*) per 100,000 of GBCA in, **a** males and **b** females, 1992–2009 [195]. Rates shown are per 100,000 person-years on the *y*-axis. Patient age in years are shown on the *x*-axis

*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 long-standing 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].

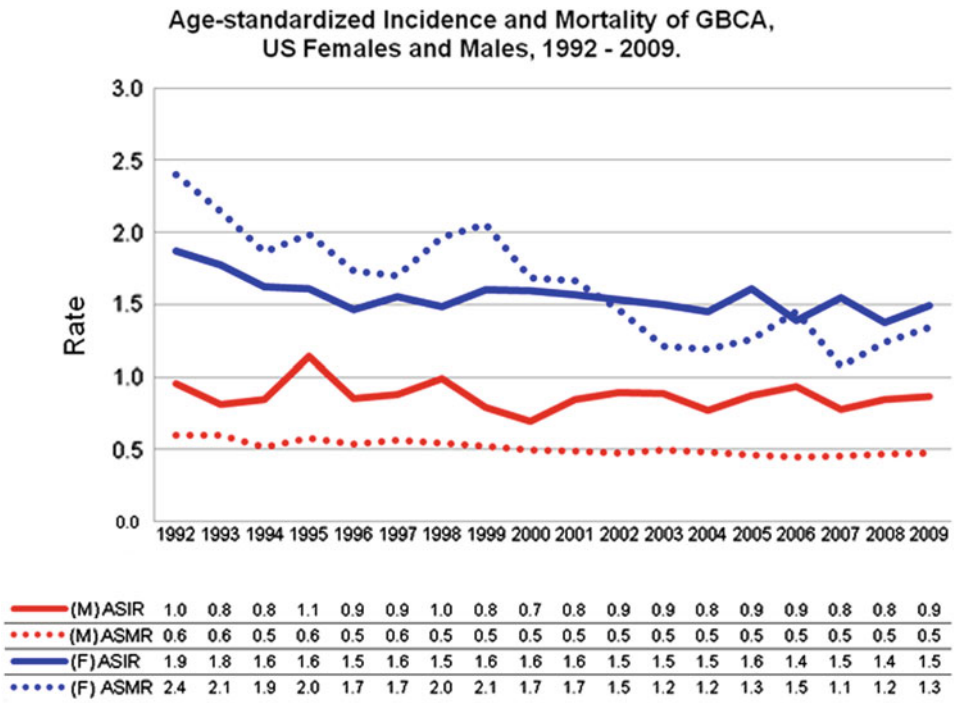



**Fig. 7** Age-standardized (US 2000 Standard Population) incidence rates (per 100,000) of GBCA in the United States. Incidence rate is shown on the *y*-axis and grouped time intervals in years are shown on the *x*-axis with the graphed incidence data shown below in tabular format. *ASIR* age-standardized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

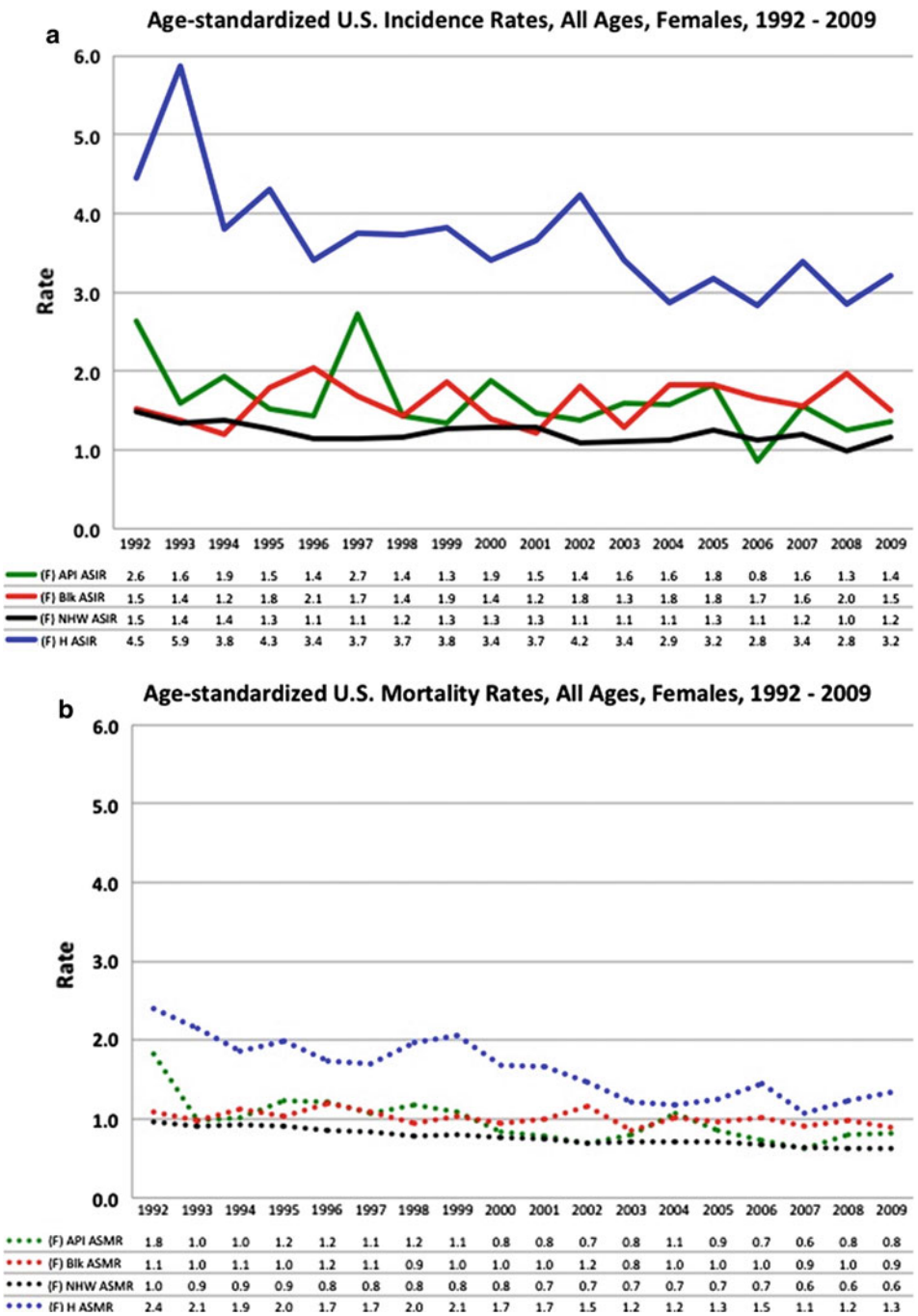



**Fig. 8** Age-standardized (US Census 2000) **a** incidence and **b** mortality of gallbladder carcinoma in the United States, all females, 1992–2009. Rates shown are per 100,000 person-years. ASIR, age-standardized incidence ratio. *ASMR* age-standardized mortality ratio. *F* females. *API* Asian/Pacific 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 multicontinent, 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

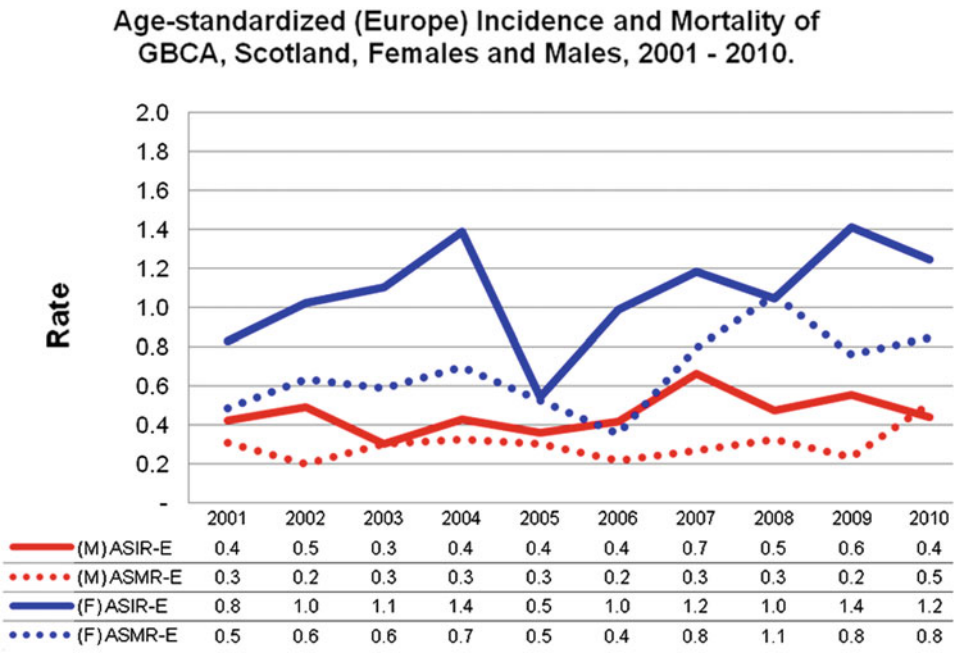



**Fig. 9** Age-standardized (Europe) incidence and mortality of gallbladder carcinoma in Scotland, males and females, 2001–2010. Rates shown are per 100,000 person-years. *ASIR-E* age-standardized incidence ratio, Europe. *ASMR-E* age-standardized mortality ratio, Europe. *M* males. *F* females. *Source*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

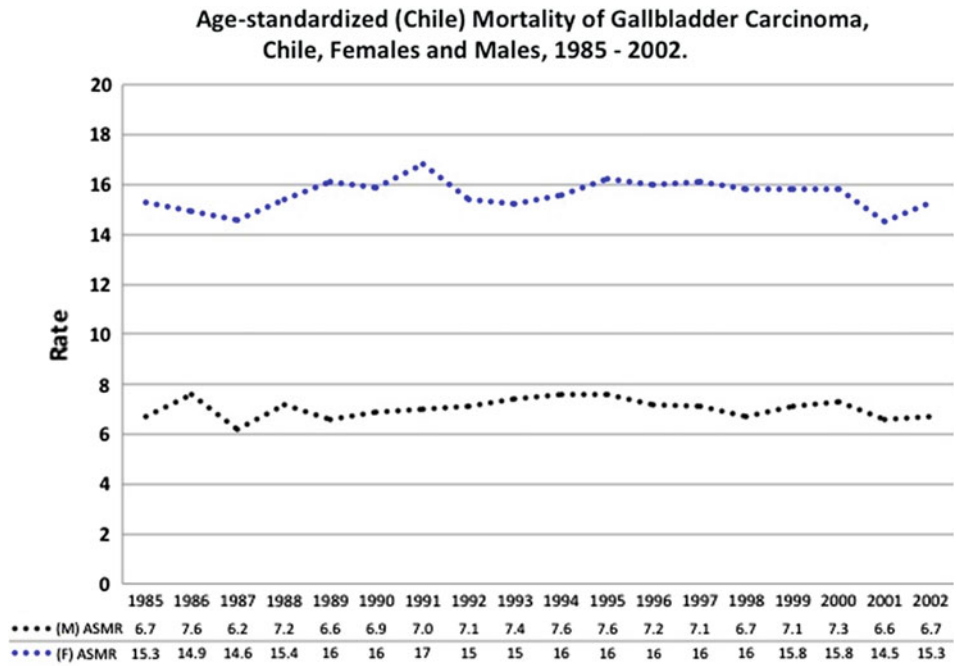



**Fig. 10** Age-standardized (Chile) mortality rates (per 100,000) of GBCA, Chile, both sexes, 1985–2002 [268]. *ASMR* age-standardized mortality rate. *M* males. *F* females

(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

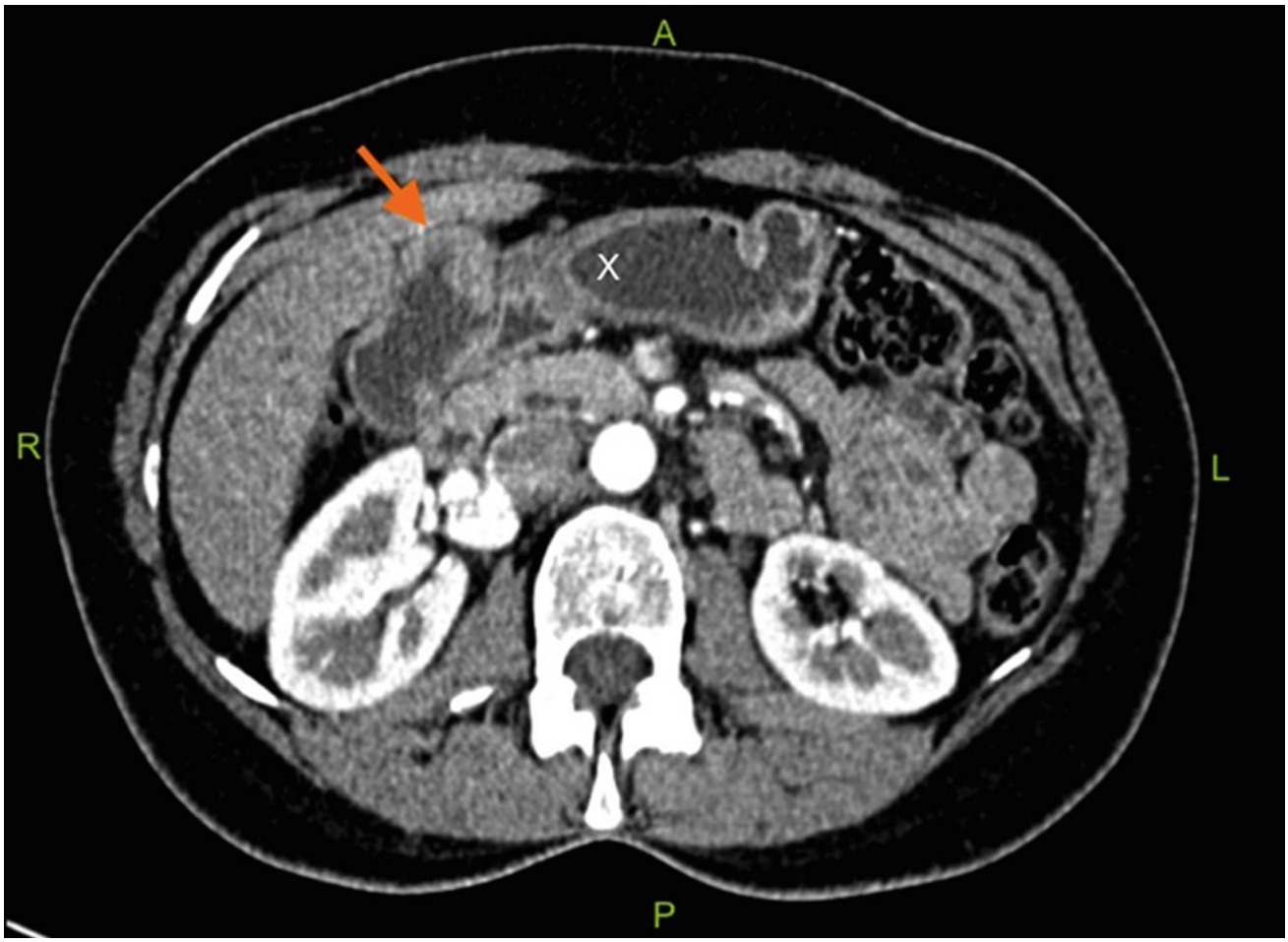



**Fig. 11** **a** Ultrasound image of polypoid lesion of the gallbladder. 2.7 cm exophytic, mucosal enhancing polypoid lesion in fundus of gallbladder. pT2 Nx GBCA with lymphatic and vascular invasion at histologic analysis of cholecystectomy specimen. **b** Axial contrast-enhanced CT image of gallbladder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

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# Role of Growth Factor Signaling Pathways in Biliary Tract Cancer

Kaoru Kiguchi and John DiGiovanni

## Contents




**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

K. Kiguchi (✉) · J. DiGiovanni
Dell Pediatric Research Institute, College of Pharmacy, The University of Texas at Austin, 1400 Barbara Jordan Blvd., Austin, TX 78723, USA
e-mail: kkiguchi@austin.utexas.edu

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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/$PGE_2$ 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 (TGFα), 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 $E_2$ ($PGE_2$). This suggests that the COX enzymes and the prostanoids 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].

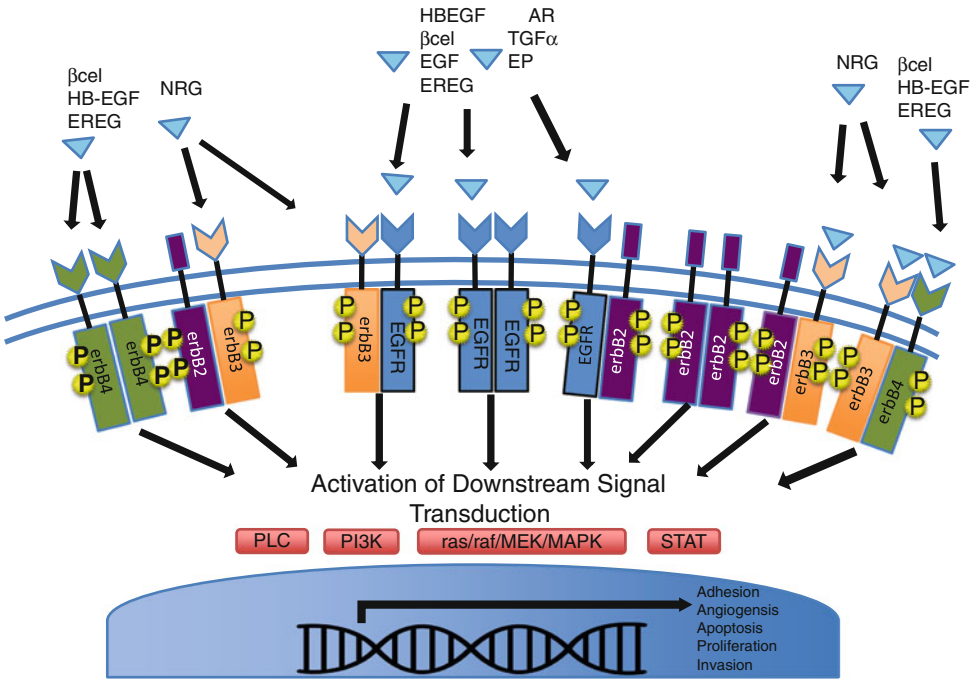


**Fig. 1** ErbB family signaling system. Cross talk between erbB2/EGFR and other erbB receptor tyrosine kinase members and downstream signaling which leads to cell proliferation, survival, and migration (see text in detail)

## 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 Cγ, 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

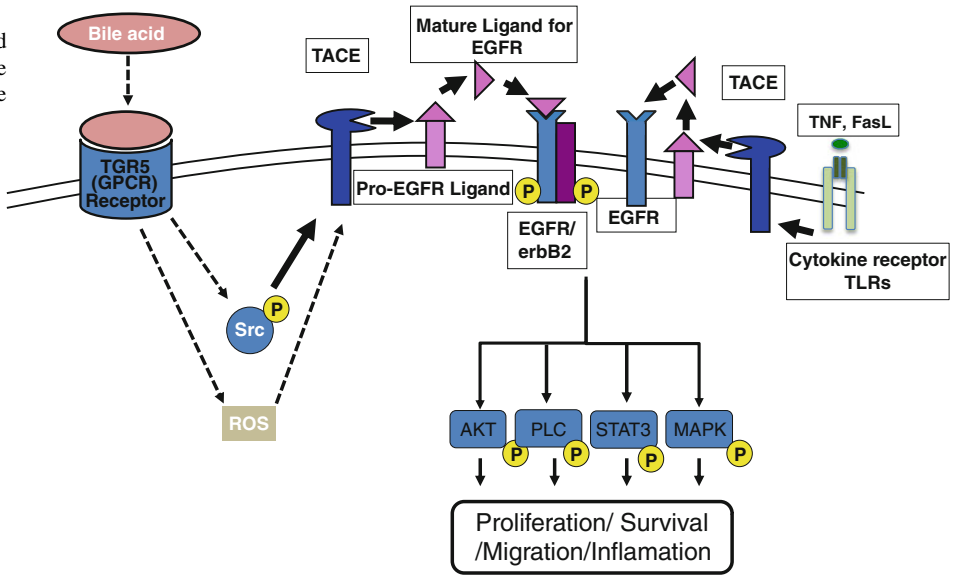


**Fig. 2** ErbB2/EGFR transactivation through TLRs, GPCR, and TACE and cross talk between src which leads to the development of biliary tract cancer (see text in detail)

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 TNF-α 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

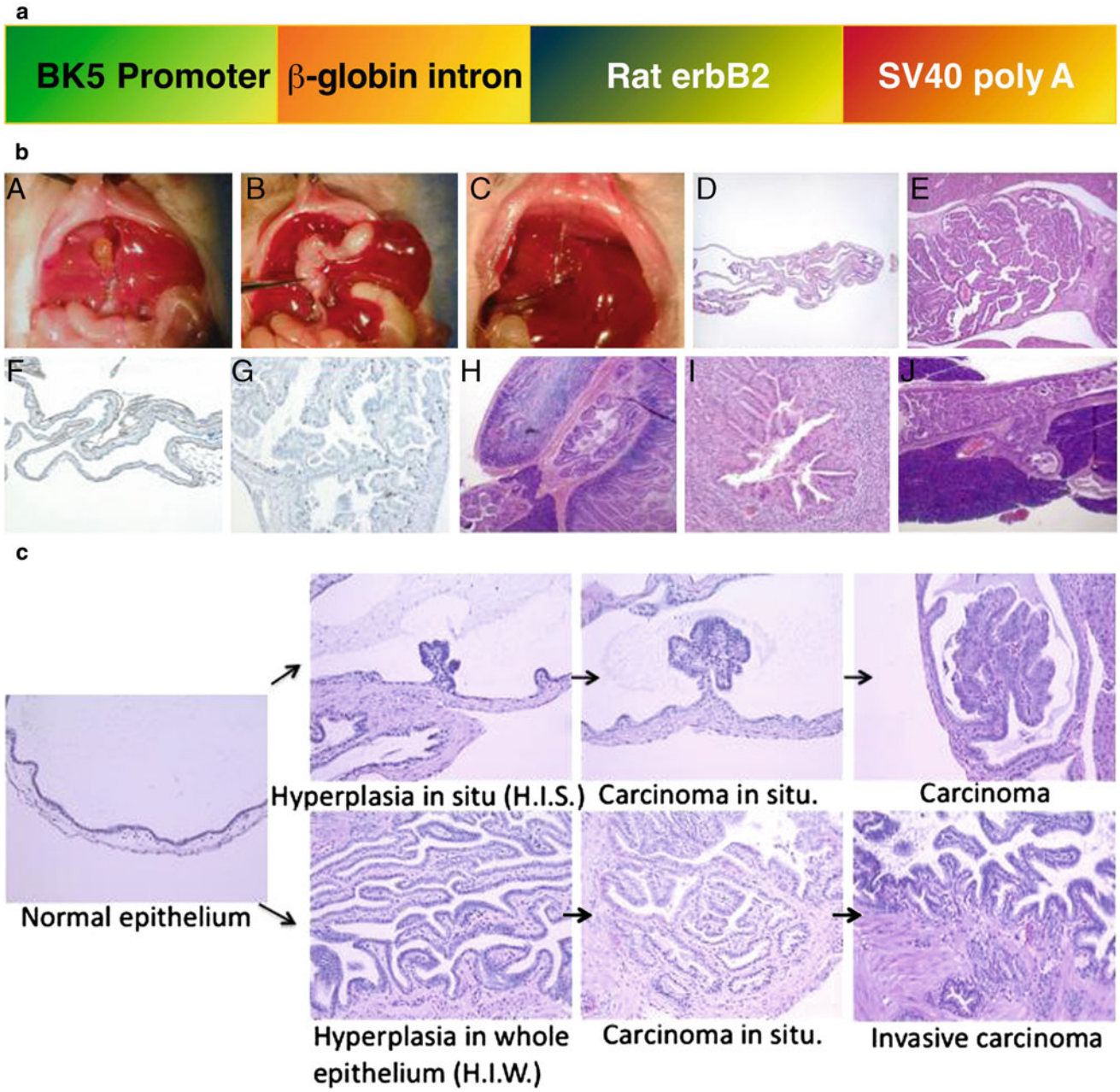


**Fig. 3** **a** The DNA construct used to generate BK5.erbB2 mice. **b** Gross appearance and histological evaluations of BTC in BK5.erbB2 mice. *A* Gallbladder of wild-type mouse, *B* BK5.erbB2 mouse at 3 months of age, *C* anomalous fasciculus form of gallbladder in the early stage of gallbladder 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.

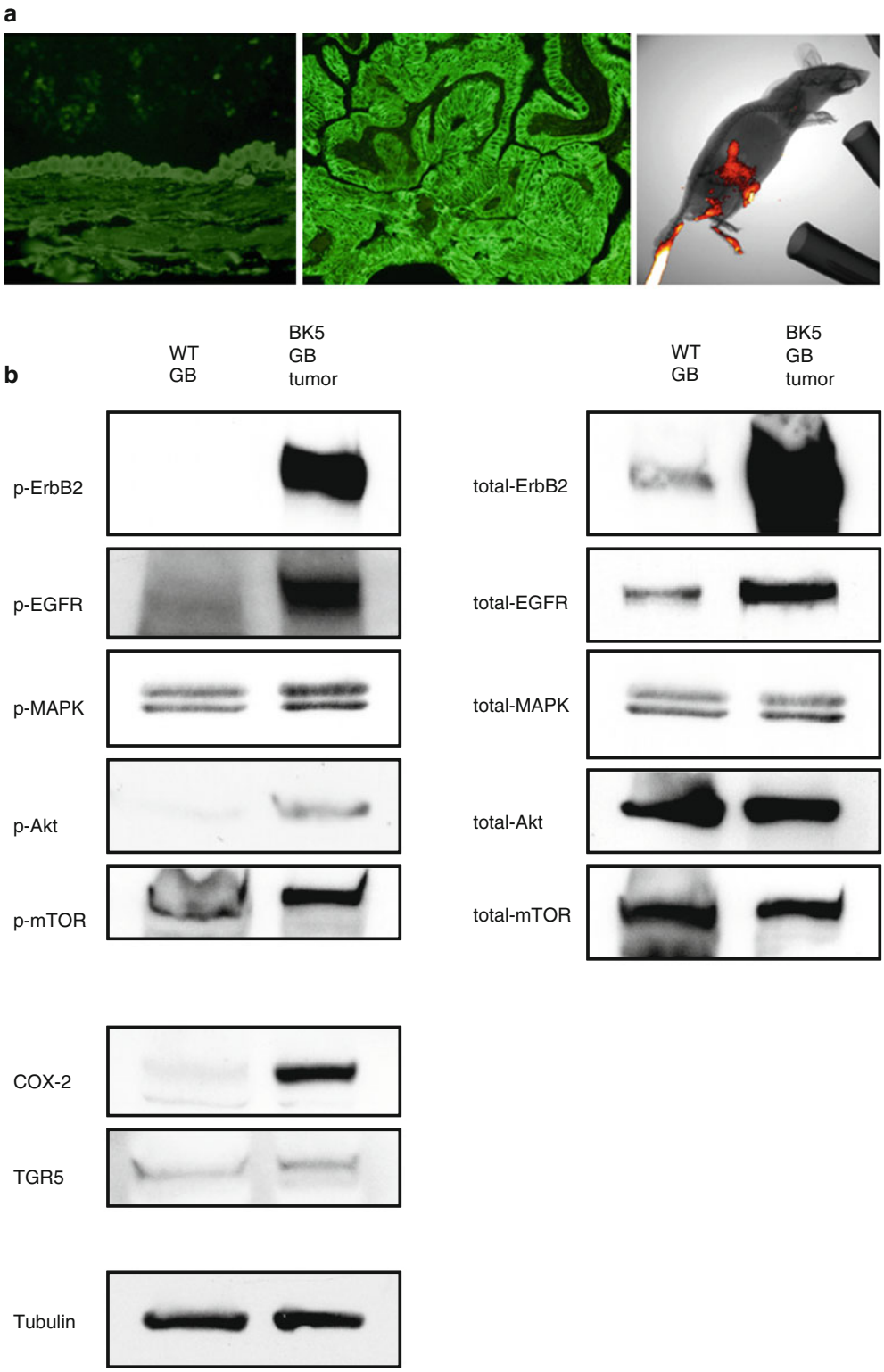


**Fig. 4** **a** Expression of ErbB2: immunostaining for erbB2 in gallbladder from wild-type mouse (*left*) and BK5.erbB2 mouse (*middle*) of 3-month-old mouse. Image of GBC detected by Kodak in-vivo Imaging System FX-Pro, 48 h after EGF-labeled probe injection (*right*, *arrow*). High-intensity area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

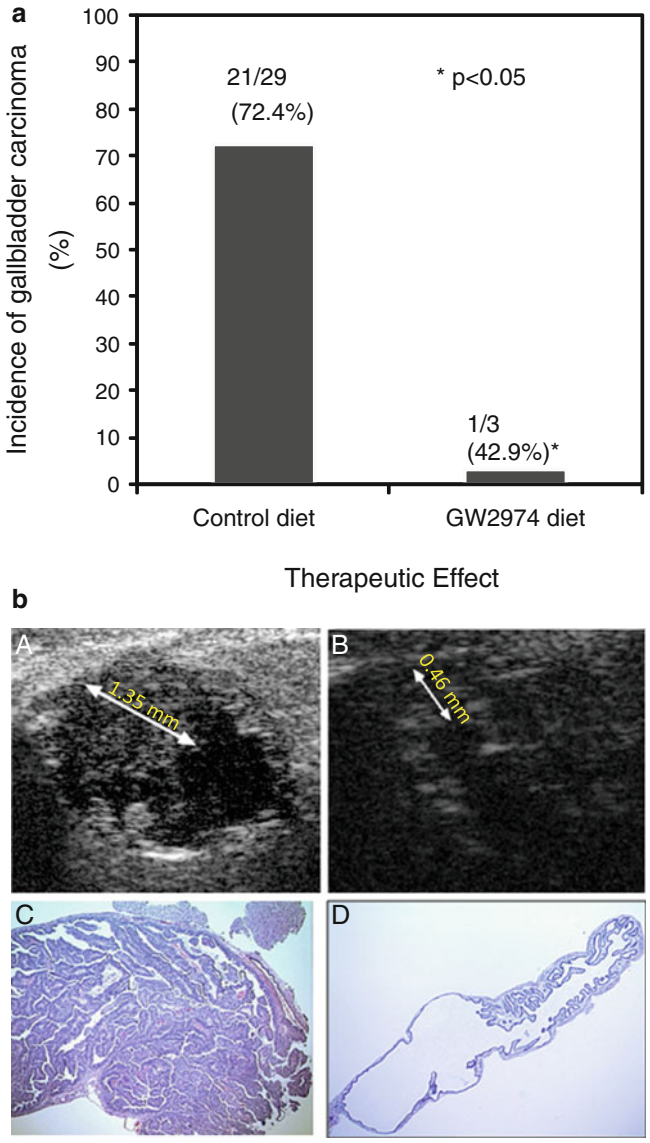


**Fig. 5** Therapeutic effect of tyrosine kinase inhibitor, GW2974, and HDAC inhibitor, PCI-24781, on the incidence of GBC in BK5.erbB2 mice. **a** Incidence of GBC in BK5.erbB2 mice. Two month-old BK5.erbB2 mice were treated with AIN76 control diet or AIN76 diet containing 200 ppm GW2974 for one month.*, $P < 0.01$. **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 preclinical 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

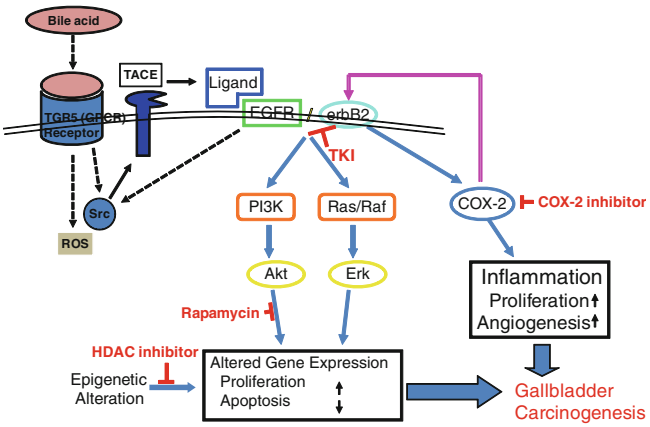


**Fig. 6** Proposed pathway in which erbB2/EGFR, COX-2, bile acid, and src may play a role during the BTC carcinogenesis. ErbB2 overexpression/activation may accelerate the transactivation cascade of during the development of BTC. Figure is adopted from Kiguchi et al. [75]

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.

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# Pathology of Gallbladder Cancer

Vikram Deshpande and Andrea Primiani

## Contents



V. Deshpande (✉)
Department of Pathology, Massachusetts General Hospital, Boston, USA
e-mail: vdeshpande@partners.org

A. Primiani
Harvard Medical School, Massachusetts General Hospital, Boston, USA

**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 neoplasmas, 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.

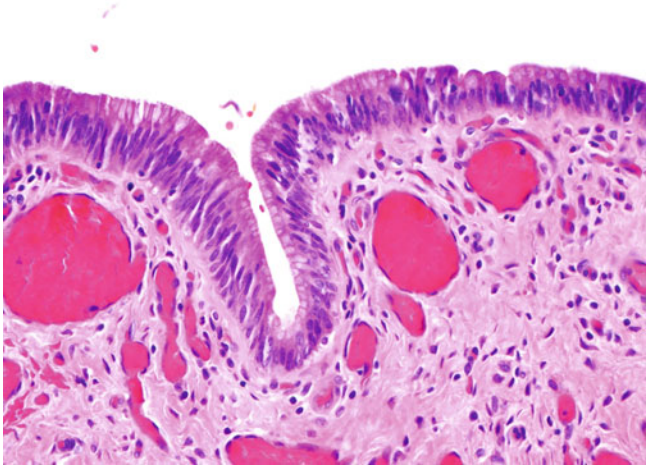

**Fig. 1** Gallbladder mucosa showing low-grade dysplasia. The epithelium shows mild nuclear atypia, nuclear stratification, and hyperchromasia. However, the polarity of the cells is maintained, thus arguing against the diagnosis of high-grade dysplasia

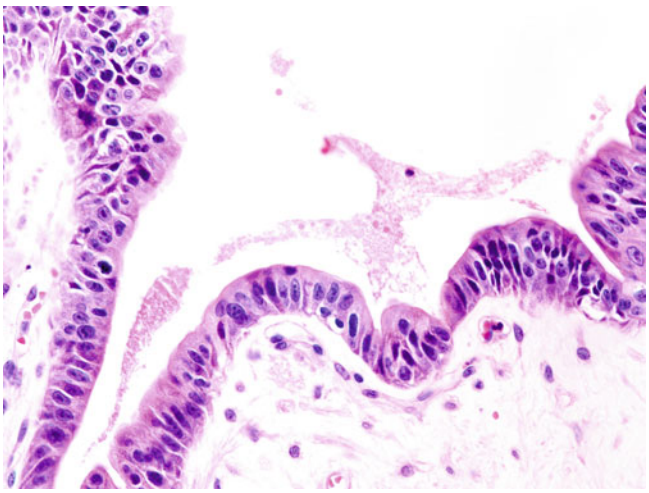

**Fig. 2** A high-powered view of the lining epithelium of the gallbladder. The image shows high-grade dysplasia. Note the marked nuclear atypia, occasional prominent nucleoli, and mitotic figures, as well as the loss in cells polarity

#### 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, polypoidal 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

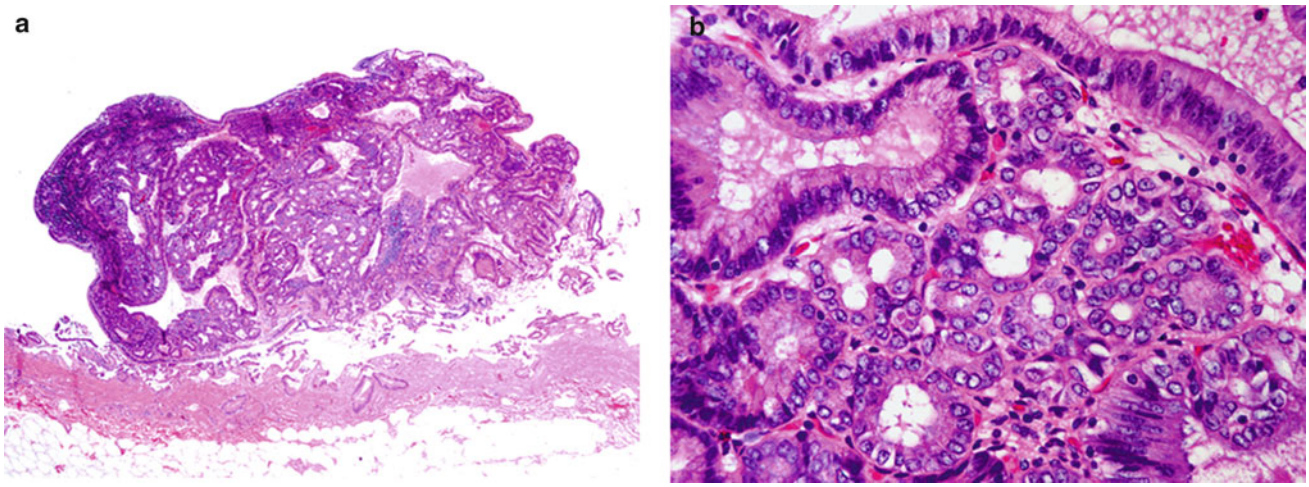


**Fig. 3** **a** Low-power view of a gallbladder adenoma. The adenoma is composed of predominantly small glands, although occasional larger glandular structures are also present. Attachment to the underlying gallbladder wall is not identified in this image. **b** High-powered view of a gallbladder adenoma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

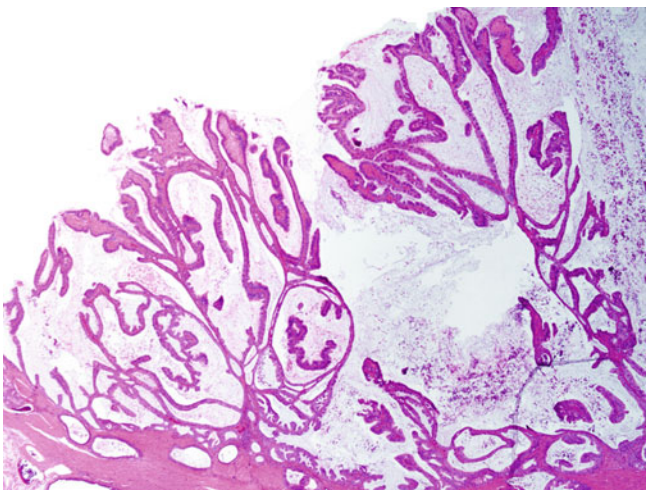

**Fig. 4** Intracystic papillary neoplasm of the gallbladder

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 premalignant lesion. The prognosis of patients with polypoidal 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 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].

**Table 1** Neoplasms of the gallbladder [16]

| Epithelial tumors | Mesenchymal tumors |
|---|---|
| *Benign* | *Benign* |
| Cholesterol polyp | Granular cell tumor |
| Adenomyoma | Leiomyoma |
| *Premalignant lesions* | *Malignant* |
| Adenoma (tubular, papillary, or tubulopapillary type) | Rhabdomyosarcoma |
| Biliary intraepithelial neoplasia (BilIN) | Leiomyosarcoma |
| Intracystic papillary neoplasm | *Lymphoma* |
| Mucinous cystic neoplasm | *Metastases* |
| *Malignant* | |
| 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 | |
| *Neuroendocrine neoplasms* | |
| 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 | |

### 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

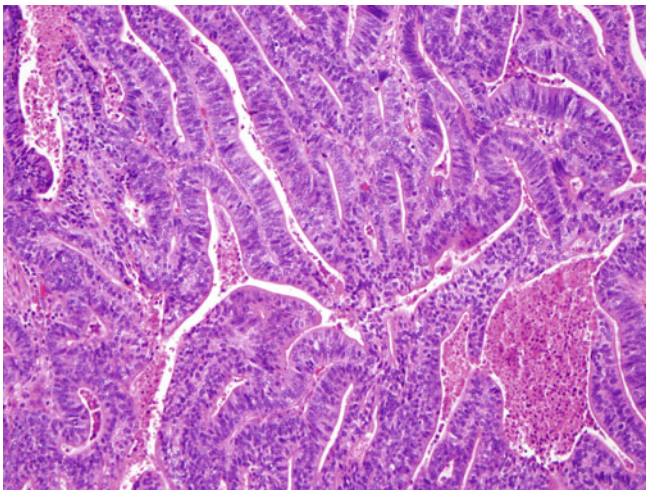

**Fig. 5** Invasive adenocarcinoma of the gallbladder

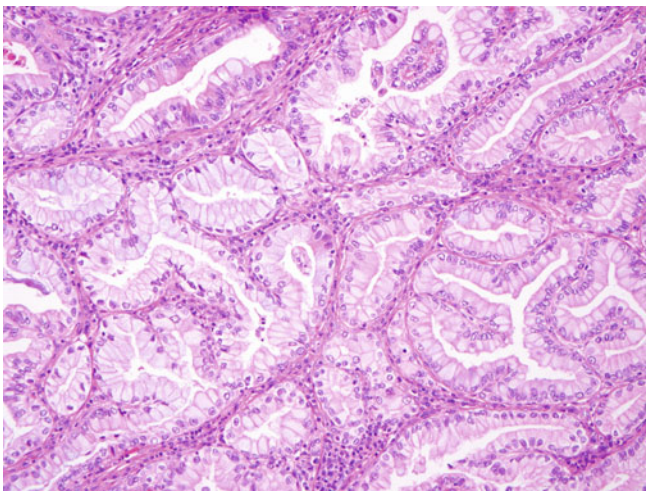

**Fig. 6** Invasive gallbladder carcinoma with a gastric phenotype

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 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.

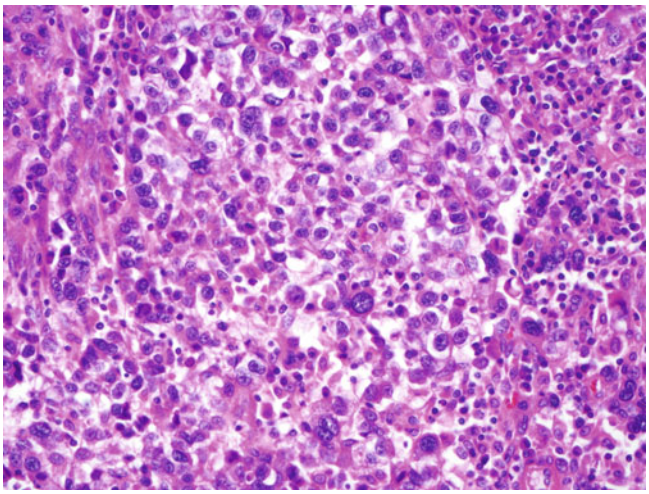

**Fig. 7** Poorly differentiated invasive adenocarcinoma of the gallbladder. The tumor lacks evidence of glandular differentiation

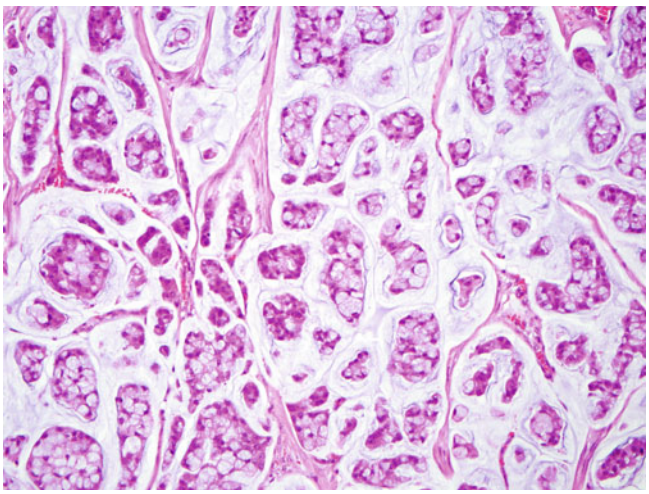

**Fig. 8** Mucinous adenocarcinoma of the gallbladder. Note the abundant extracellular mucin within which cohesiveness of neoplastic cells is seen

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.

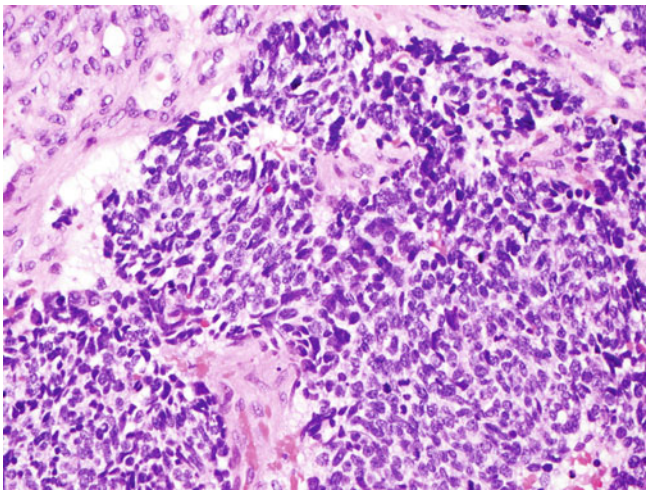

**Fig. 9** Neuroendocrine carcinoma with small cell features. The tumor cells are small with hyperchromatic nuclei and nuclear molding. This tumor closely resembles a small cell carcinoma of the lung

### 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 tumors 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]

| | |
|---|---|
| *Primary tumor* (*T*) | |
| 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 |
| *Regional lymph nodes* (*N*) | |
| 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 |
| *Distant metastasis* (*M*) | |
| M0 | No distant metastasis |
| M1 | Distant metastasis |

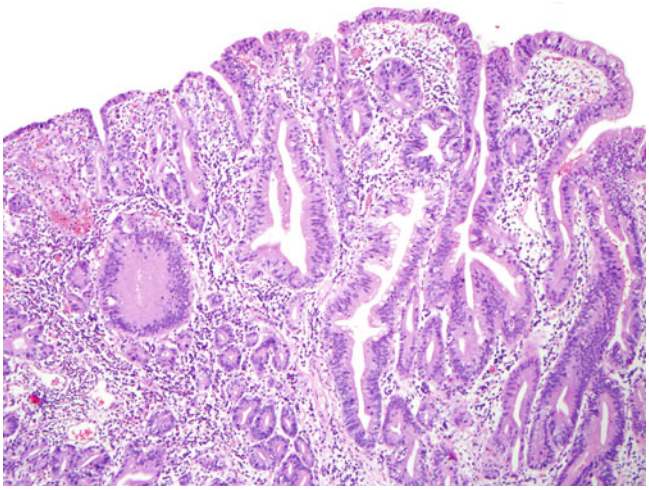

**Fig. 10** The gallbladder mucosa shown in this image is composed of glands lined by high-grade nuclear atypia. In this example of early gallbladder carcinoma, the marked architectural atypia suggests invasion of the lamina propria

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 pT is 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].

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# Histopathology and Molecular Pathogenesis of Cholangiocarcinoma

Laura Rubbia-Brandt and Benoit Terris

## Contents




**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.



L. Rubbia-Brandt (✉)
Division of Clinical Pathology, Faculty of Medicine of Geneva, Geneva University Hospital, 1211 Geneva, Switzerland
e-mail: laura.rubbia-brandt@hcuge.ch

B. Terris
Service de pathologie, Hôpital Cochin Assistance Publique-Hôpitaux de Paris, Université Paris Descartes, 750014 Paris, France


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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 boarders 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.

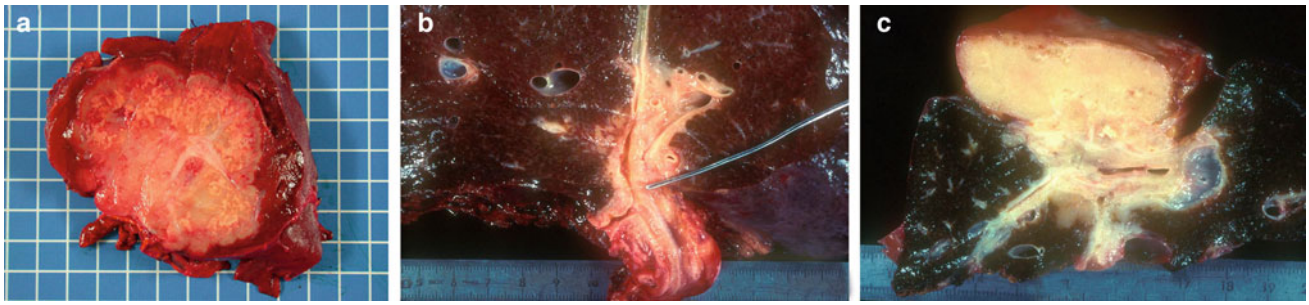


**Fig. 1** Different macroscopical types of cholangiocarcinomas. **a** the mass forming type. **b** the periductal infiltrating type. **c** the mixed type

### 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 |
|---|---|
| *Adenocarcinoma* | *Adenocarcinoma conventional type* |
| Well differentiated | Well differentiated |
| Moderately differentiated | Moderately differentiated |
| Poorly differentiated | Poorly differentiated |
| | *Adenocarcinoma unconventional type* |
| | Trabecular |
| | Perihilar |
| | Ductular |
| *Rare variants* | *Rare variants* |
| 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 |

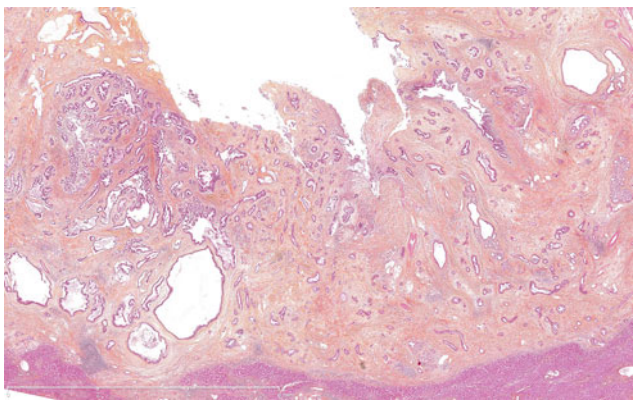

**Fig. 2** Microscopical pattern of a well differentiated CC composed of tubular glands and abundant desmoplastic stroma along a large intrahepatic bile duct

1. Trabecular subtype, made of polygonal eosinophilic tumoral cells arranged in thick, occasionally anastomozing 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).

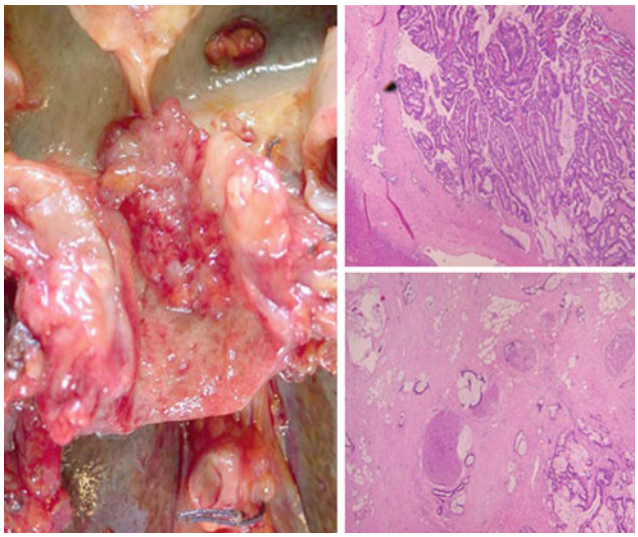

**Fig. 3** **a** Macroscopical polipoïd types of extrahepatic bile duct carcinoma. **b** Microscopical papillary pattern of a moderate differentiated carcinoma, with **c** invasive component

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:

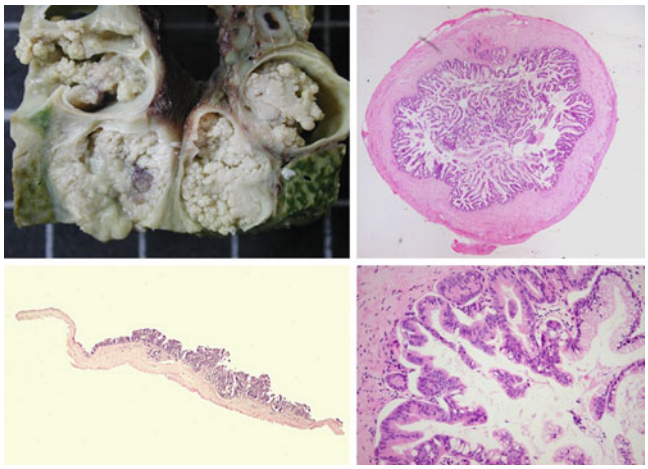

**Fig. 4** Macroscopic and microscopical pattern intraductal papillary neoplasm (IPNB)

(1) a flat intraepithelial bilirary 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 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-$\beta$, IL-8, and TGF-$\alpha$ 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 cholangicarcinoma 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 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.

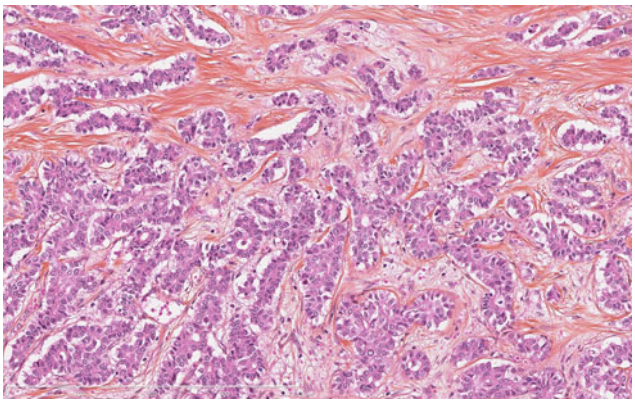

**Fig. 5** Microscopical pattern of combined hepatocellular cholangiocarcinoma

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 stoma 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.

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# Novel Biomarkers for Cholangiocarcinoma

Ross C. Smith

## Contents




**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.


R. C. Smith (✉)
Department of Surgery, University of Sydney, Sydney, NSW, Australia
e-mail: ross.smith@sydney.edu.au



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

**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 |

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 chloangiocarcinoma 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/$\beta$-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-$\beta$, 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 ampullopancreatic 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 | *P53* 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 pancreatico-biliary 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 phosopoarray 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, cysteinylation, 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.

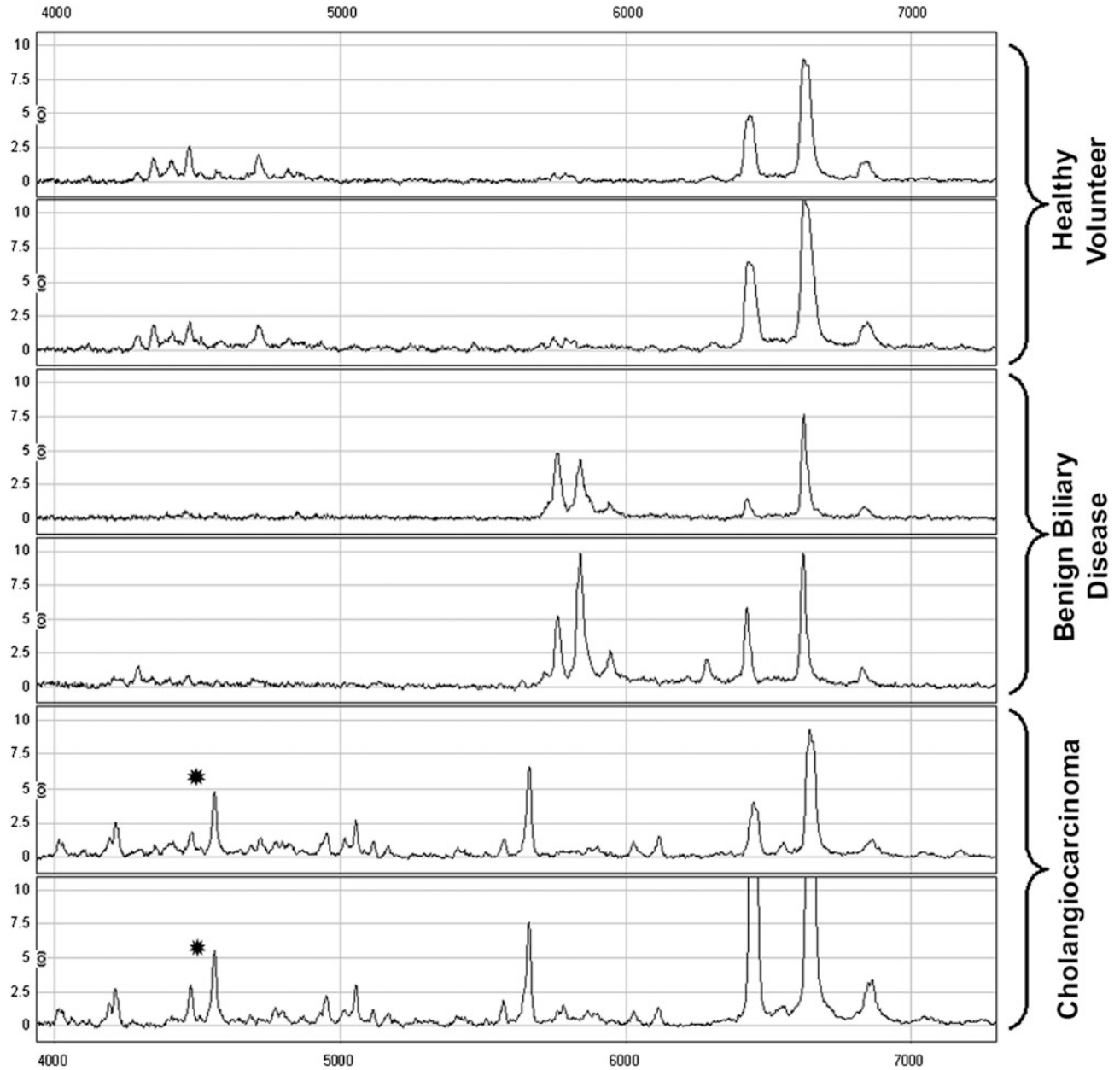


**Fig. 1** The printout of the protein mass profile for a small segment of the SELDI MS curve. Results are from the spectrum of two subjects from each of the groups: healthy controls, benign biliary disease, and cholangiocarcinoma. The *asterisk marks* the m/z 4462 peak. Modified from Scarlett et al. [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 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

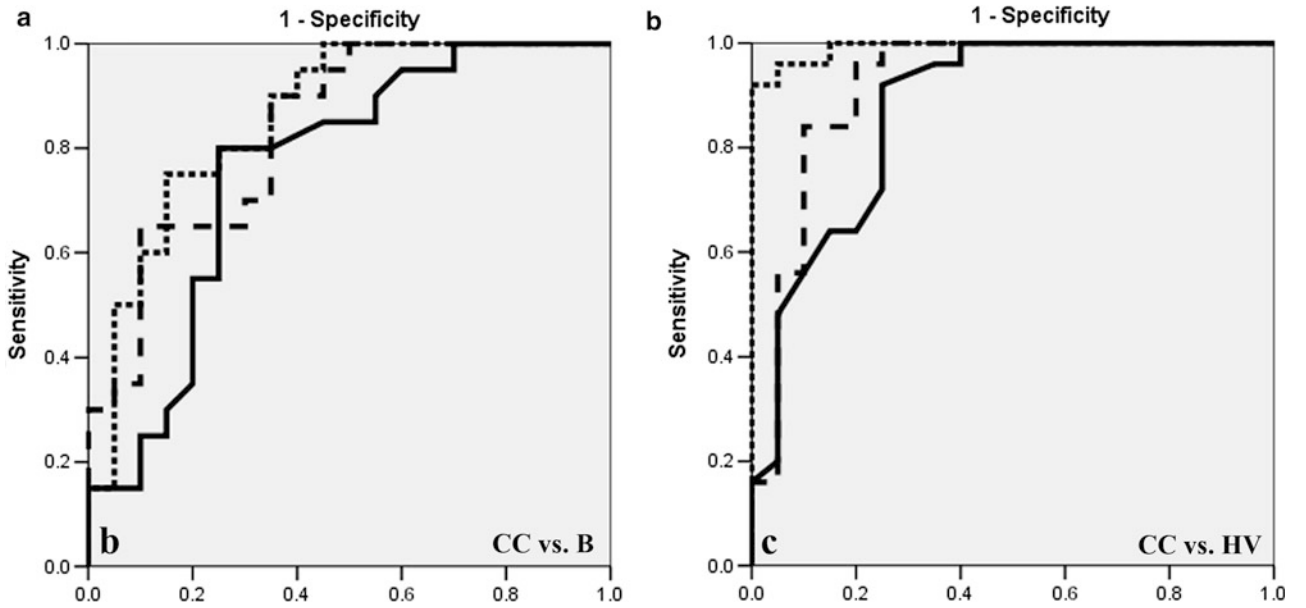


**Fig. 2** ROC curves for the serum results from the following: **a** cholangiocarcinoma versus benign disease. *Solid line* marker m/z 4462, *dashed line* 2-marker panel, and *dotted line* CEA added to the panel and **b** cholangiocarcinoma versus healthy volunteers. *Solid line* marker m/z 11535, *dashed line* 3-marker panel, *dotted line* CEA, and CA19-9 added to the panel. Modified from Scarlett et al. [34]

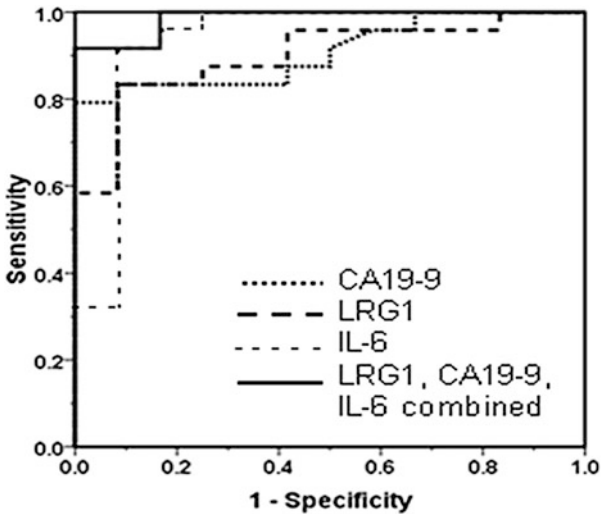


**Fig. 3** ROC curves for CA19-9, LRG1, and IL-6 and for CA19-9, LRG1, and IL-6 combined (AUC 0.98) in discriminating cholangiocarcinoma from benign biliary disease

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

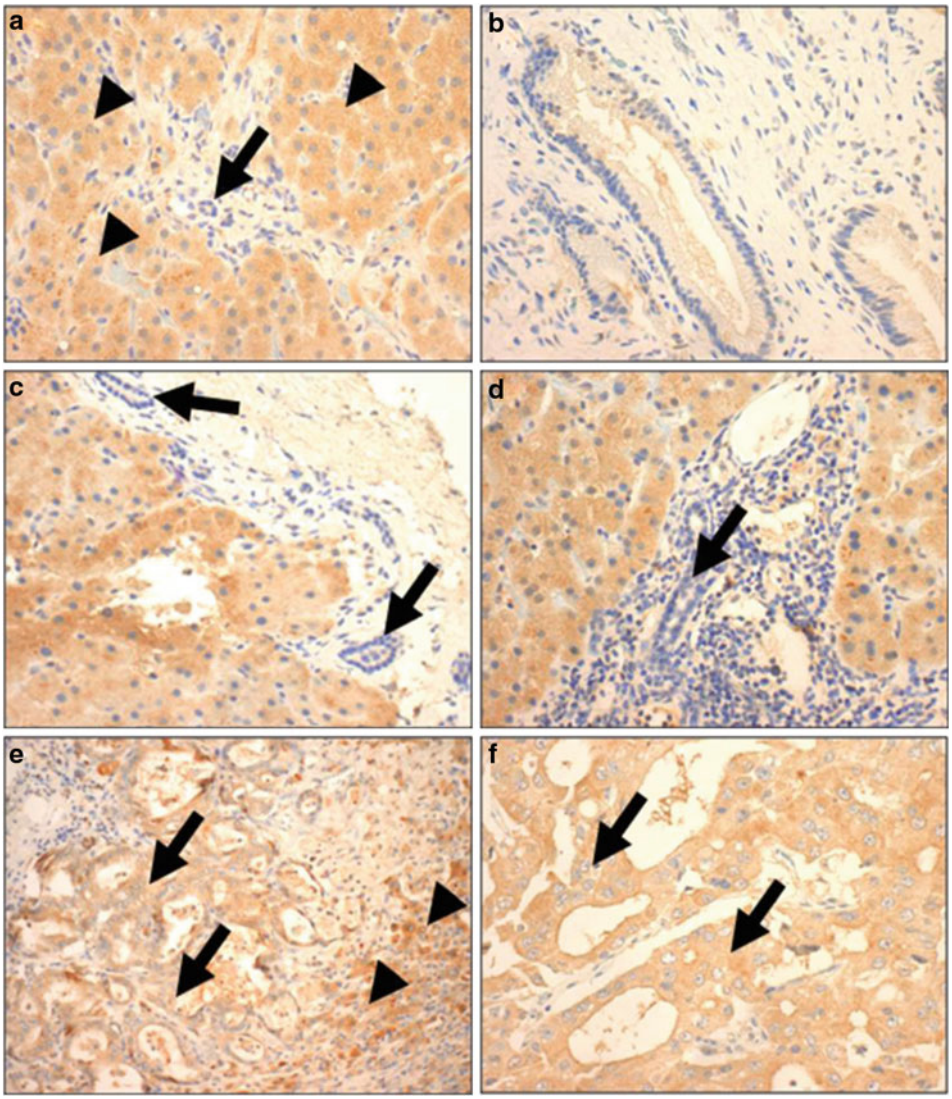


**Fig. 4** LRG1 immunohistochemistry analysis demonstrating **a** moderate expression of LRG1 in normal liver (*arrowheads*) with absent expression in biliary epithelium (*arrow*) (original magnification 400×); **b** absent expression of LRG1 in normal biliary epithelium from gallbladder (original magnification 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 LRG (*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.

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# Pathologic Staging

Taofic Mounajjed and Tsung-Teh Wu

## Contents




**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

T. Mounajjed · T.-T. Wu (✉)
Division of Anatomic Pathology, Mayo Clinic, Hilton 11, 200 1st St. SW, Rochester, MN 55905, USA
e-mail: wu.tsungteh@mayo.edu

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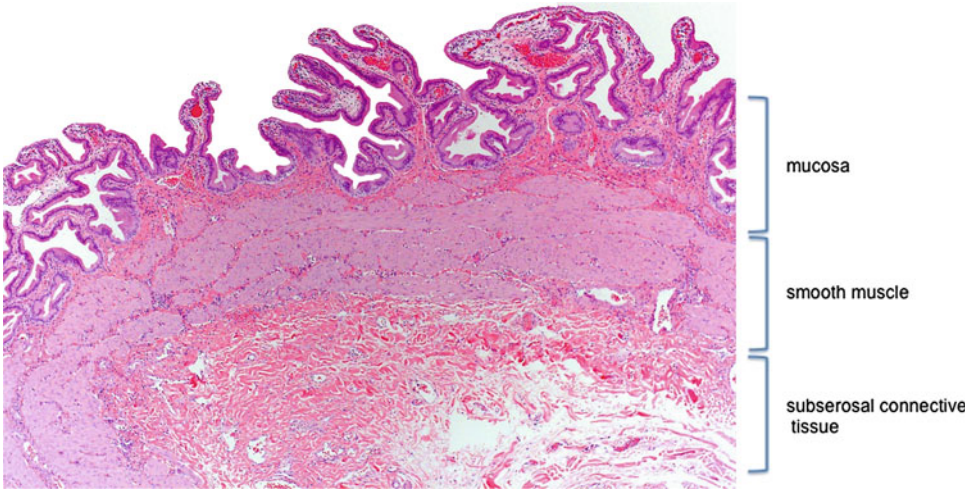


**Fig. 1** Normal gallbladder histology. The mucosa consists of a single layer of tall columnar epithelial cells and underlying lamina propria. Underneath the mucosa are a smooth muscle layer and a perimuscular connective tissue layer. The serosa is not shown in this figure

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

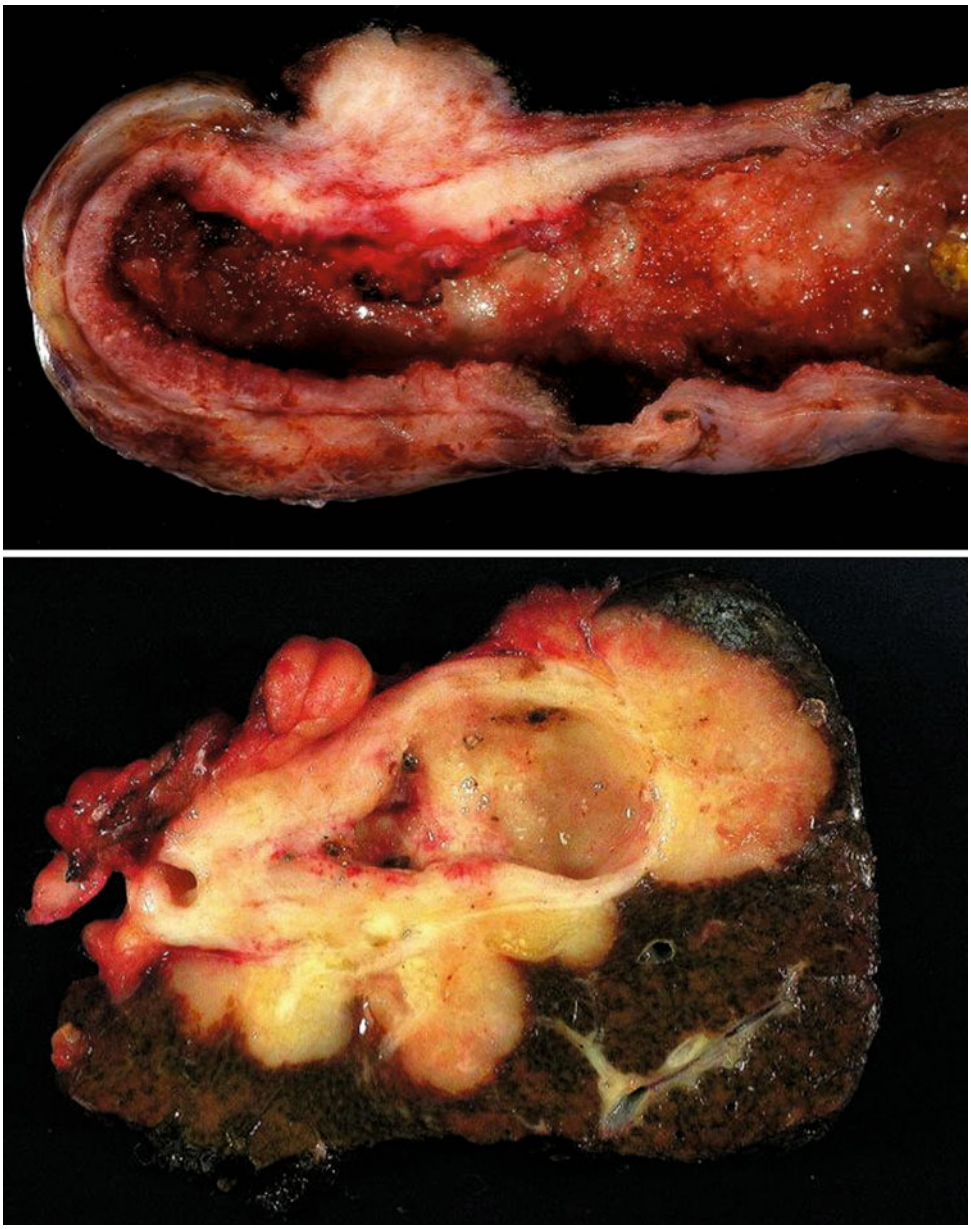

**Fig. 2** Gross features of gallbladder carcinoma. *Upper* carcinoma involving the gallbladder fundus and body and forming a transmural mass with diffuse wall infiltration. *Lower* gallbladder carcinoma infiltrating surrounding liver

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

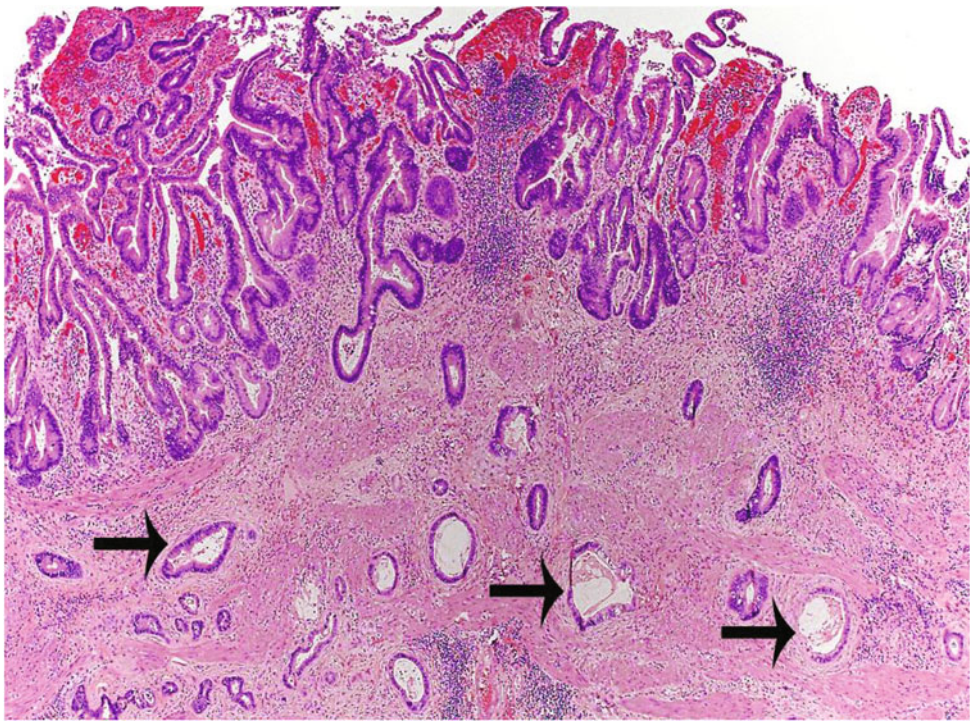

**Fig. 3** Histologic features of gallbladder carcinoma. Invasive adenocarcinoma is characterized by infiltrative growth of variably sized and irregularly shaped glands (*arrows*), with desmoplastic response

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 1 | 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

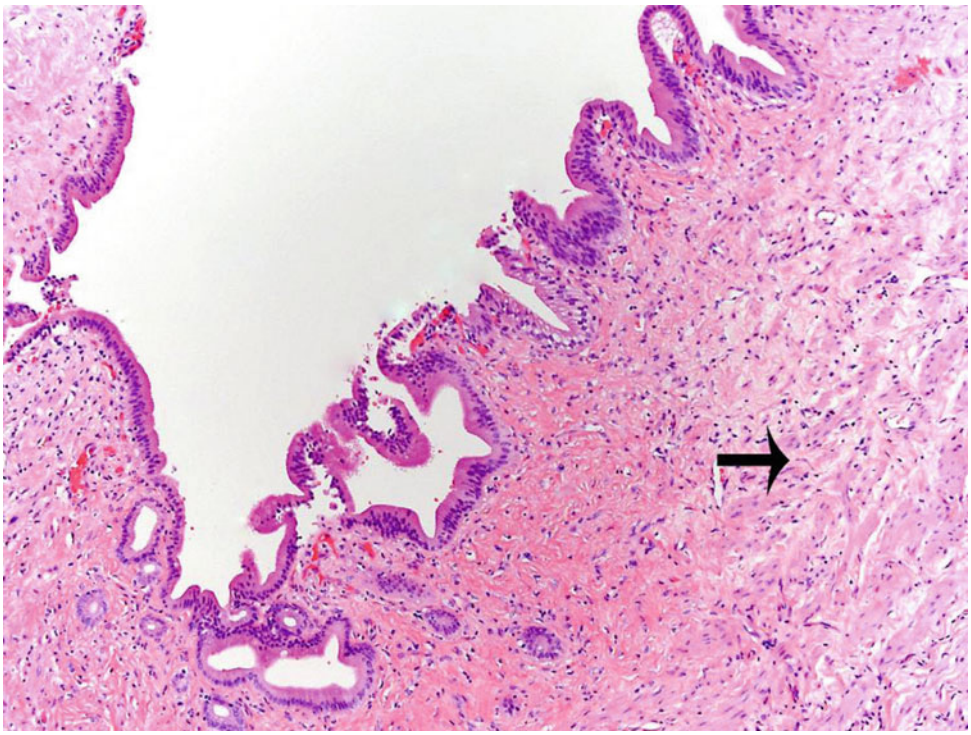

**Fig. 4** Normal extrahepatic bile duct histology. The mucosa consists of columnar epithelium with small longitudinal folds and a subepithelial layer containing elastic and collagen fibers. Scattered small bundles of smooth muscle (*arrow*) underlie the mucosa

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,

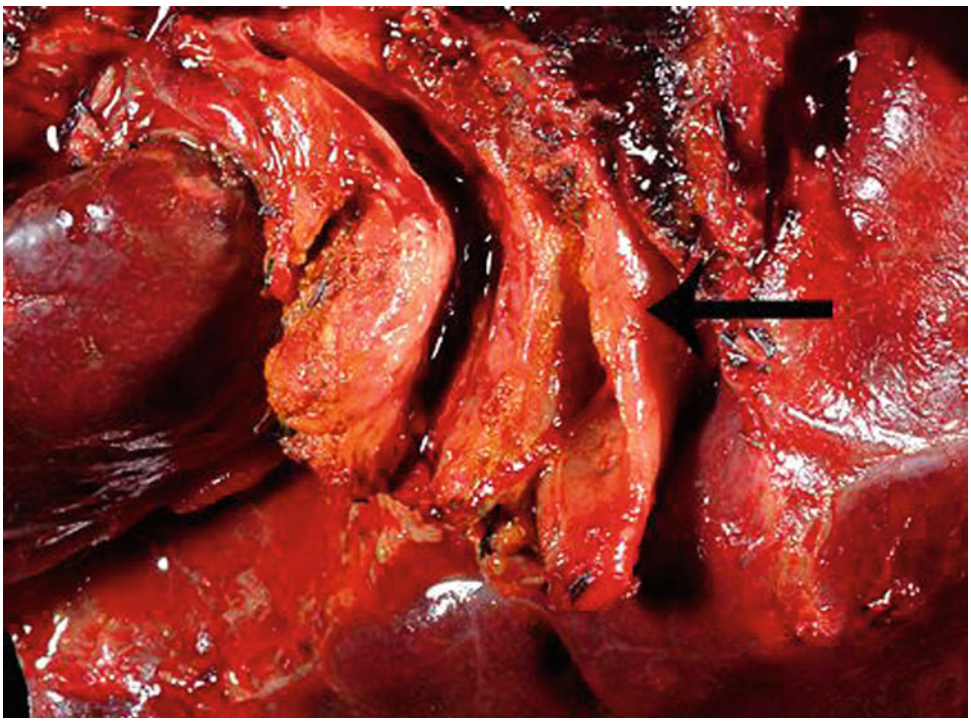

**Fig. 5** Gross features of perihilar bile duct carcinoma. Perihilar carcinoma characterized by diffuse thickening of the extrahepatic bile ducts

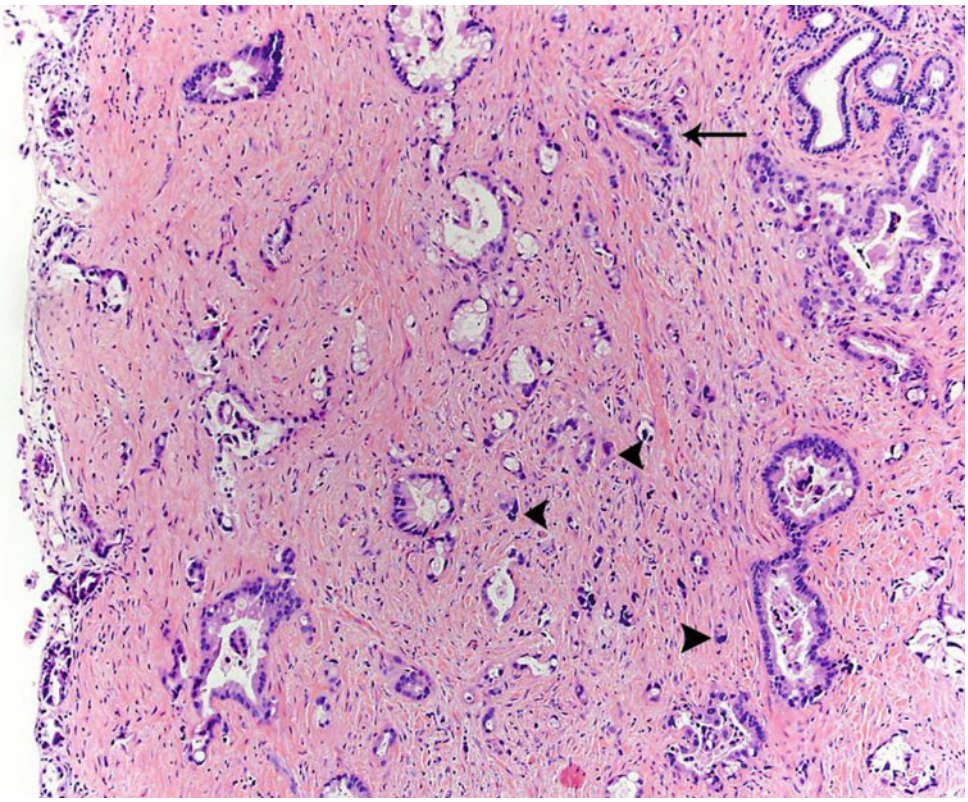

**Fig. 6** Histologic features of bile duct carcinoma. Invasive adenocarcinoma is characterized by infiltrative growth of variably sized and irregularly shaped glands (*arrow*) as well as single cells (*arrowheads*), with desmoplastic response

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 stating, 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]

| | |
|---|---|
| *Primary tumor* (*T*) | |
| 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 |
| *Regional lymph nodes* (*N*) | |
| 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 |
| *Distant metastasis* (*M*) | |
| 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

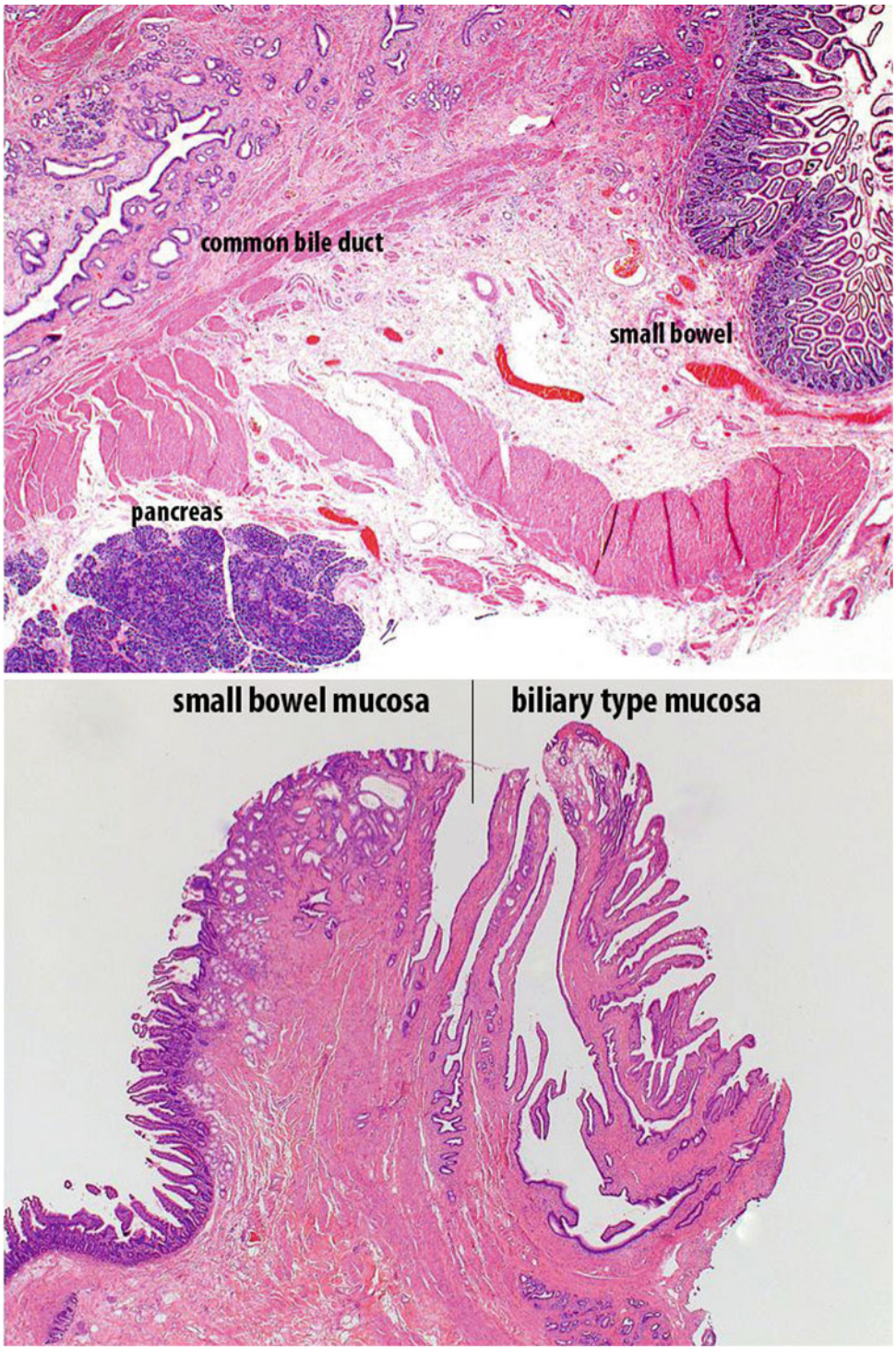


**Fig. 7** Normal histology of the ampulla of Vater. A histologic section from the ampullary region shows confluence of small bowel mucosa and common bile duct wall; the pancreas is also present in this section (*top*). The bile duct epithelial lining becomes papillary with long narrow fronds projecting 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.

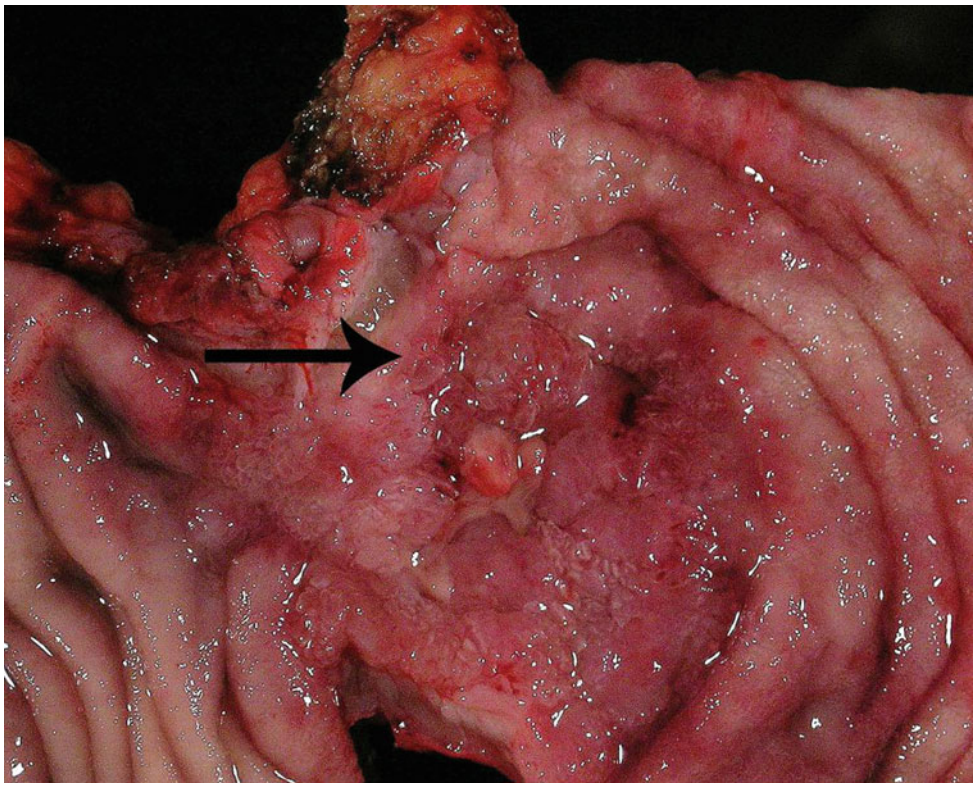

**Fig. 8** Gross features of ampullary carcinoma. Ampullary carcinoma (*arrow*) forming an ulcerated mass (mixed ulcerated type)

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

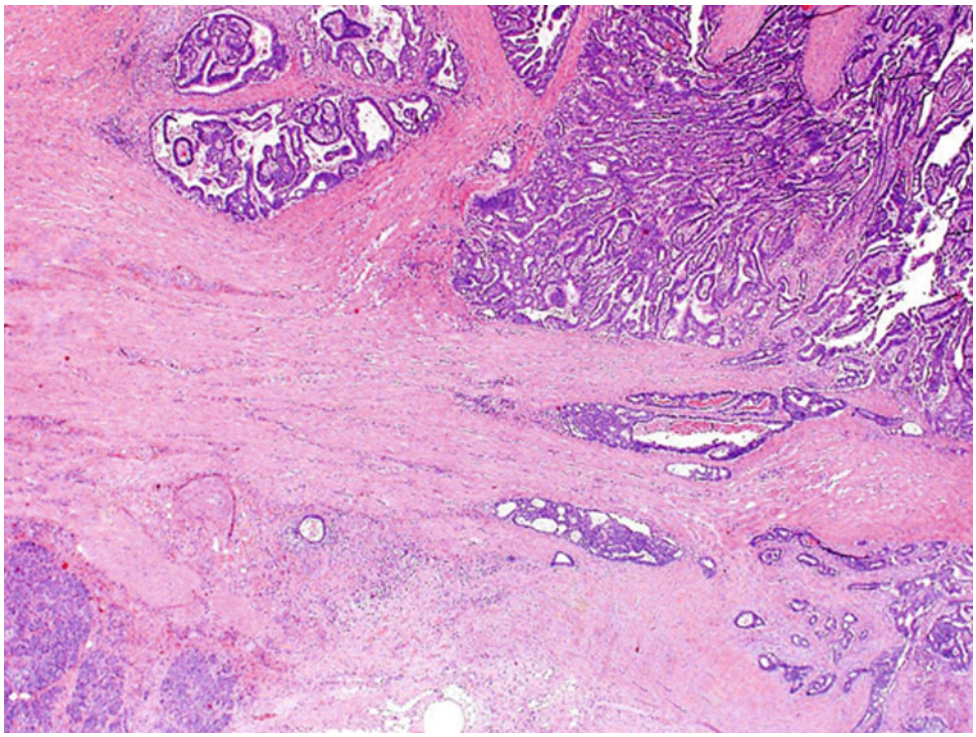

**Fig. 9** Histologic features of ampullary carcinoma. Invasive adenocarcinoma characterized by complex infiltrating glandular growth invading the wall of the duodenum

**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 |

**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 |

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.

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].

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# Molecular Profiling

Domenico Alvaro and Vincenzo Cardinale

## Contents



**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].

D. Alvaro (✉) · V. Cardinale
Division of Gastroenterology,
Department of Medico-Surgical Sciences and Biotechnologies,
Pontino, Fondazione Eleonora Lorillard Spencer Cenci,
Sapienza University of Rome, Viale dell'Università 37, 00185 Rome, Italy
e-mail: domenico.alvaro@uniroma1.it

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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 *P53* mutations (%) | Notes |
|---|---|---|---|---|---|
| Jonas et al. [23] | Germany | 12 | Perihilar | 2 (16.6) | *P53* exons 5–8 evaluated |
| Sturm et al. [22] | USA | 27 | Perihilar | 7 (26.31) | *P53* exons 5–8 evaluated |
| Petmitr et al. [21] | Thailand | 20 | IH-CCA | 1 (5) | *P53* exons 5–8 evaluated |
| Kang et al. [24] | Korea | 40 | IH-CCA | 14 (35.7) | *P53* exons 5–8 evaluated |
| Furubo et al. [25] | Japan | 15 | IH-CCA (peripheral) and perihilar | 3[a] (20) | *P53* exons 5–8 evaluated |
| Kamikawa et al. [19] | Japan | 22 | IH-CCA | 9 (41.6) | *P53* exons 5–8 evaluated; thorotrast exposed patients |
| Della Torre et al. [26] | Italy) | 13 | Not specified | 2 (15.3) | *P53* exons 5–8 evaluated |
| Tullo et al. [27] | Europe | 29 | Perihilar | 7 (24) | *P53* 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) | *P53* exons 5–8 evaluated |
| Khan et al. [18] | UK | 31 | IH-CCA | 24 (76) | Complete *P53* mutational signatures |
| | | | | | Three new frameshift mutations and two new intron mutations discovered |
| Liu et al. [29] | China | 36 | Not specified | 22 (62) | *P53* exons 5–8 evaluated |
| Kiba et al. [20][c] | Thailand | 26 | IH-CCA | 9 (35.7) | *P53* exons 5–8 evaluated |
| | | | | | 2 patients with *KRAS* mutations, none carrying both *P53* and *KRAS* mutations |
| Kiba et al. [20][c] | Japan | 12 | IH-CCA | 4 (33.3) | *P53* exons 5–8 evaluated<br>7 patients with *KRAS* mutations, none carrying both *P53* and *KRAS* mutations |
| Imai et al. [30][c] | Japan | 7 | IH-CCA[b] | 2 (28.5) | *P53* exons 5–8 evaluated |
| Itoi et al. [31][c] | Japan | 12 | Not specified | 4 (33.3) | 6 patients with *KRAS* mutations, none carrying both *P53* and *KRAS* mutations |
| | | | | | *KRAS* and *P53* 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 courts 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-alpha that, in turn, was stimulated via IkappaB kinase-dependent nuclear factor-kappaB (NF-kappaB) 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 *KRAS* 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 *KRAS* mutations also exhibited methylated *P16*; 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 *KRAS* mutation |
| Xu et al. [39] | China | 13 | Not specified | 5 (38.2) | 2 patients (5.9 %) harbored both *KRAS* and PIK3CA mutations |
| Isa et al. [41] | Japan | 23 | IH-CCA (peripheral) and perihilar | 9 (39.1) | Patients with *KRAS* mutations worst survival rates; *KRAS* 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 *KRAS* 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) | *P53* exons 5–8 also evaluated; 9 patients (35.7 %) with *P53* mutations |
| Kiba et al. [20][b] | Japan | 12 | IH-CCA | 7 (58.4) | *P53* exons 5–8 also evaluated and the overall number of patients with *P53* mutations was 4 (33.3 %) |
| Ohashi et al. [36][b] | Japan | 21 | IH-CCA | 10 (48) | *P53* exons 5–8 also evaluated; 2 patients (7.1 %) with *P53* mutations; *KRAS* 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) | *P53* exons 5–8 also evaluated; 2 patients (28.5 %) with *P53* mutations |
| Itoi et al. [31][b] | Japan | 12 | Not specified | 6 (50) | *P53* exons 5–8 also evaluated; 4 patients (33.3 %) with *P53* mutations |
| | | | | | *KRAS* 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 *SOCS3*. The methylation of *SOCS3* 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 viaIL-6-mediated activation of the Erk1/2/Rsk1/C/EBPb 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-alpha, 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 PCS 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 (*GNAZ*, *MDK*), transcription (*FOXM1*, *HOXB7*, *DRIL1*), DNA synthesis (*TOP2A*, *TOP2B*, *NAV2*, *BUB1B*, *CKS2*), antiapoptosis (*BIRC5*, *S100P*), angiogenesis (*ECGF1*), cytoskeleton (*FSCN1*, *PRC1*, *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*, *DLC1*, *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-fluorouracile, 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 *P53* mutations (%) | Overall number of patients with *KRAS* mutations (%) | Notes |
|---|---|---|---|---|---|
| Yokoyama et al. [100] | Japan | 22 | 13 (58) | ND | *P53* exons 5–8 evaluated |
| Yokoyama et al. [100] | Chile | 20 | 12 (60) | ND | *P53* exons 5–8 evaluated |
| Nigam et al. [101] | North India | 22 | 2 (9.1) | ND | *P53* exons 5–8 evaluated |
| Itoi et al. [31] | Japan | 7 | 3 (42.9) | 4 (57) | *P53* exons 5–8 evaluated; none patient with both *P53* and *KRAS* mutations |
| | | | | | *KRAS* and *P53* abnormalities not detected in non-neoplastic tissues |
| Kim et al. [102] | South Korea | 15 | 3 (20.0) | 5 (35.7) | *P53* exons 5–8 evaluated; none patient with both *P53* and *KRAS* mutations |
| | | | | | *P53* and *KRAS* mutations were not found in five dysplasias around cancers and three adenomas; 30.7 % of GBC patients carried also *P16* mutations |
| Nagahashi et al. [103] | Japan | 22 | 11 (50) | 0 (0) | *P53* exons 5–8 evaluated; None patient with both *P53* and *KRAS* mutations |
| | | | | | Dysplastic epithelia obtained from gallstone patients demonstrated less frequent *P53* mutations (11 %) |
| Nagahashi et al. [103] | Hungary | 18 | 6 (33.3) | 1 (5.5) | *P53* exons 5–8 evaluated; none patient caring both *P53* and *KRAS* mutations |
| | | | | | Dysplastic epithelia obtained from gallstone patients demonstrated less frequently *P53* 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 *KRAS* 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 *KRAS* 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 *KRAS* 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) | *BRAF* mutations observed in 7/21 (33 %) GBC; *KRAS* and *BRAF* 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 pancreatobiliary 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 AUBPD 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.

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

Heljä Oikarinen

## Contents




**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.



H. Oikarinen (✉)
Department of Diagnostic Radiology,
Oulu University Hospital (OYS),
POB 50FI-90029 Oulu, Finland
e-mail: helja.oikarinen@ppshp.fi


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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) pulished by Springer Science and Business Media LLC, http://www.springerlink.com.)

**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.

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].

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

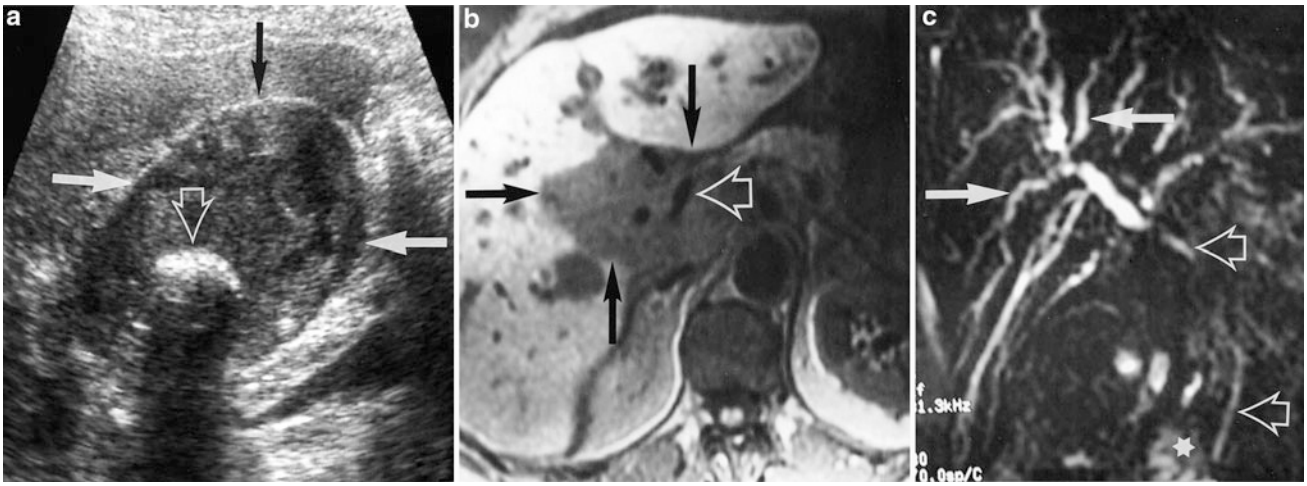


**Fig. 1** Gallbladder carcinoma. **a** Sonography reveals tumorous tissue replacing the gallbladder (*arrows*). A gallstone is also seen (*open arrow*). **b** MRI (T1 fat-saturated gradient echo) shows tumorous tissue even in the hilar area (*arrows*). There are vessels inside the tumorous area (*open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

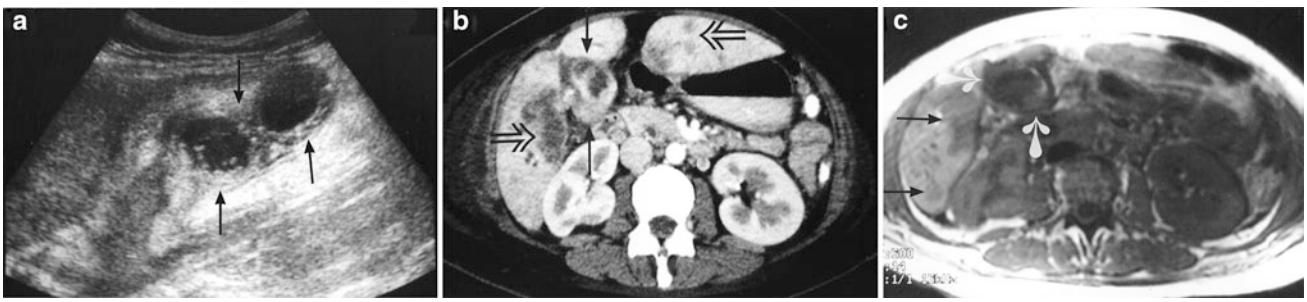


**Fig. 2** Gallbladder carcinoma. **a** The gallbladder is thick-walled and deformed (*arrows*) due to carcinoma (sonography). **b** CT (arterial phase) also reveals a tumorous gallbladder (*arrows*) and metastases of the liver (*open arrows*). **c** The thickened gallbladder wall (*white arrows*) seen 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

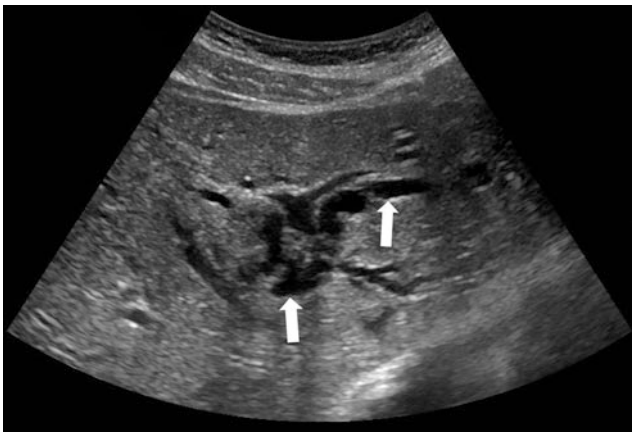

**Fig. 3** Intrahepatic bile duct dilatation (*arrows*) seen at US

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

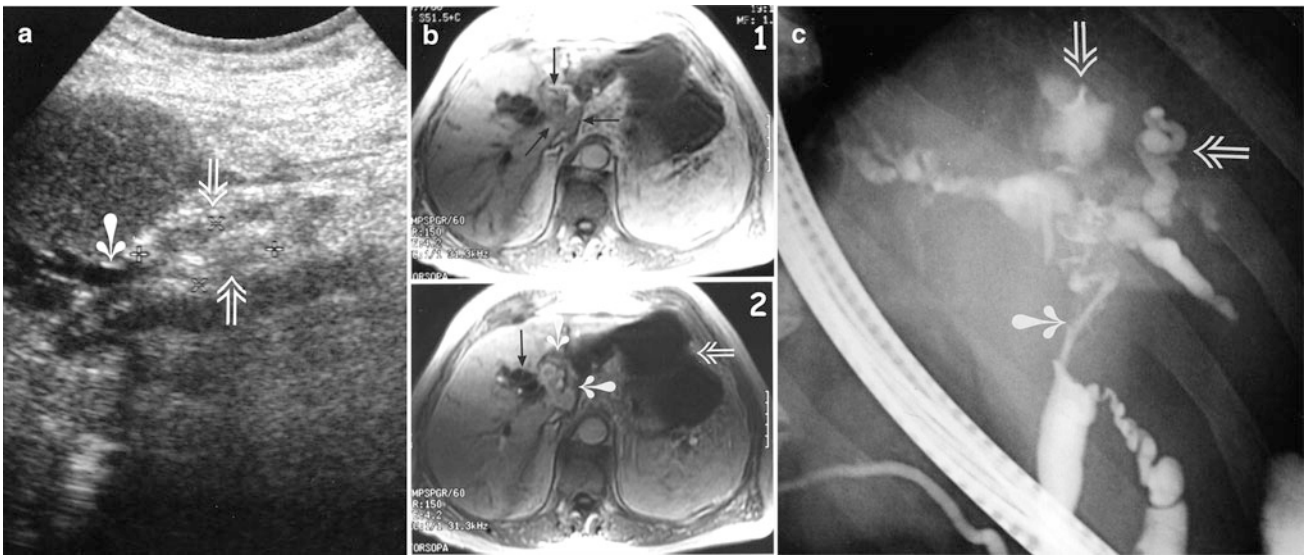


**Fig. 4** Klatskin tumor. **a** Intrahepatic biliary dilatation (*white arrow*) ends in the hilar area, where sonography shows an unclear heterogeneous mass (*open arrows*). **b** MRI (T1 gradient echo) reveals slightly different tissue in the hilar area (*arrows*). *2* Gadolinium-enhanced MRI (T1 gradient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

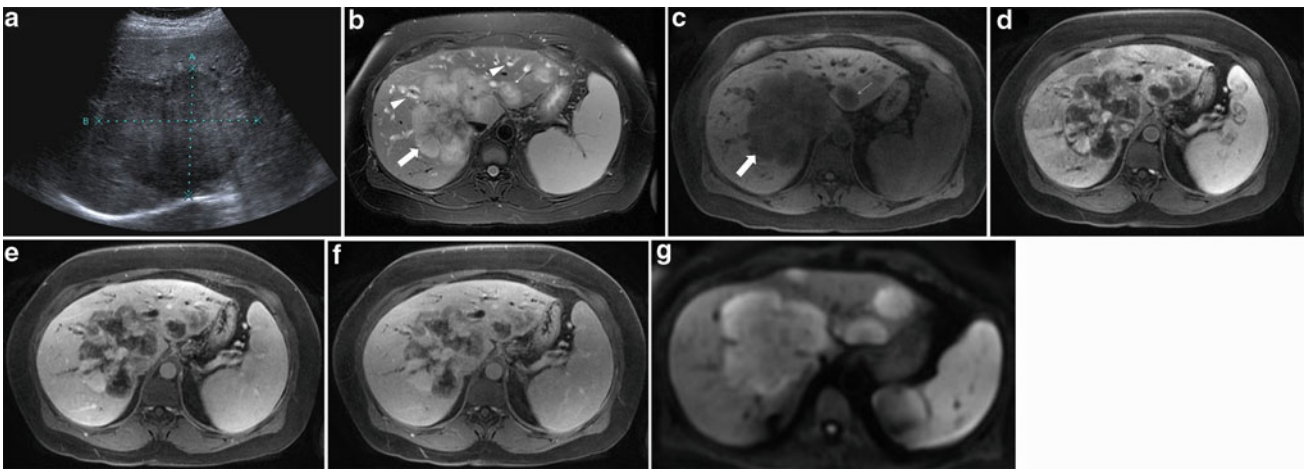


**Fig. 5** Cholangiocarcinoma. **a** A large hypoechoic, heterogenic expansion is seen in the central area of the liver at US. **b** MRI (fat-saturated T2 axial FSE) reveals a large hyperintense, lobulated tumor (*thick arrow*) with intrahepatic metastases (*thin arrow*) and bile duct dilatation (*arrowheads*).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

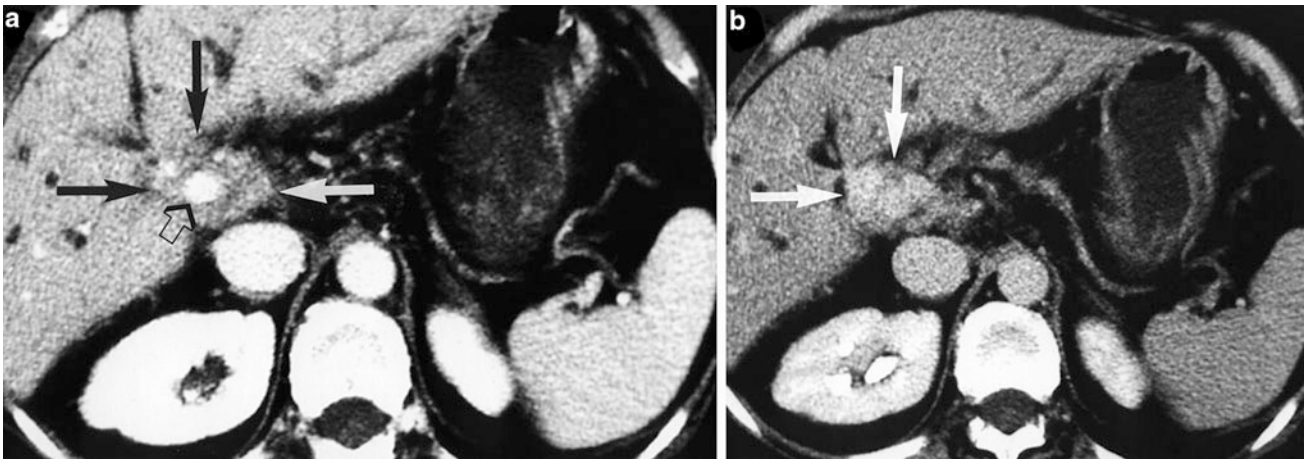


**Fig. 6** Bile duct carcinoma. **a** CT in the venous phase shows a heterogeneous mass (*arrows*) in the hilar area around the portal vein (*open arrow*). The common bile duct is not seen because of obliteration caused by the tumor. **b** In the delayed phase, the mass shows enhancement (*arrows*) [2]

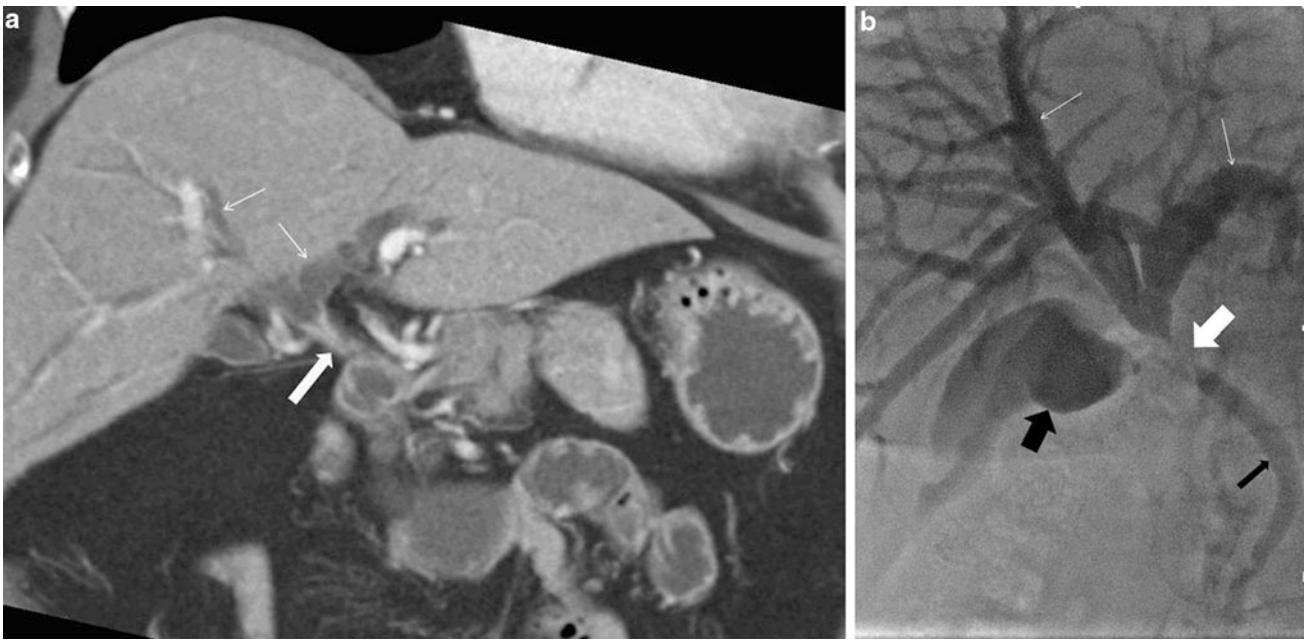


**Fig. 7** Cholangiocarcinoma. **a** Coronal view reconstruction of contrast-enhanced CT shows a stricture with enhanced wall thickening of the common hepatic duct (*thick arrow*) and intrahepatic bile duct dilatation (*thin arrows*). **b** A stricture of the common hepatic duct (*white thick arrow*)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

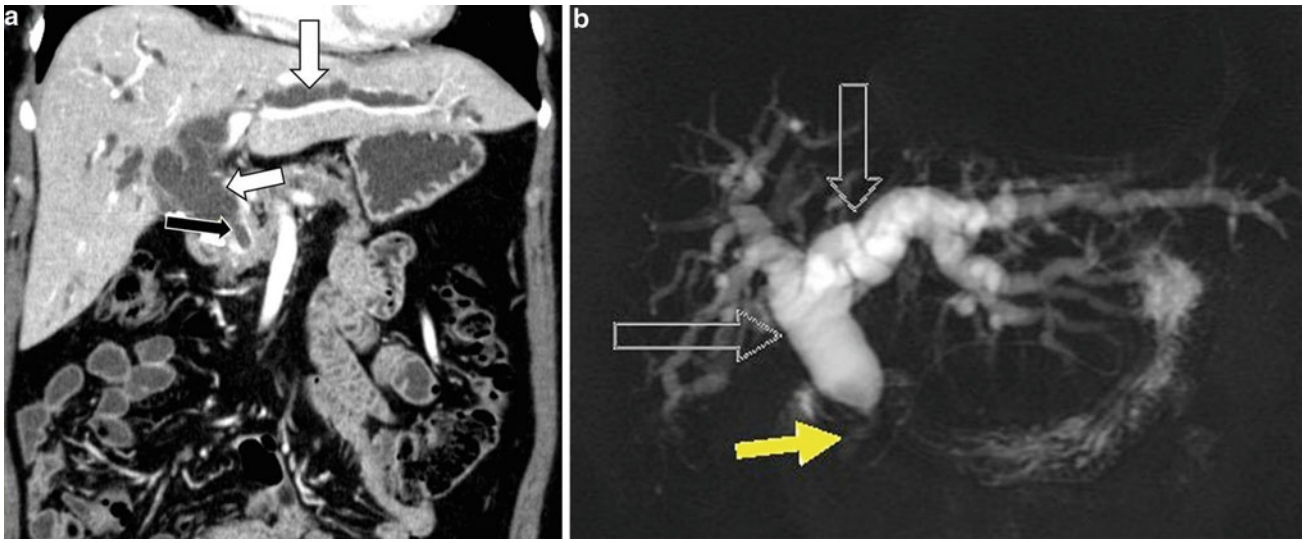


**Fig. 8** Carcinoma of the common bile duct. **a** Coronal view reconstruction of contrast-enhanced CT reveals enhancement of the thickened wall of a 2-cm stricture in the distal common bile duct (*black arrow*) and marked intra- and extrahepatic bile duct dilatation (*white arrows*). **b** MRCP (4-cm-thick slab) visualizes a stricture in the distal common bile duct (*arrow*) and marked intra- and extrahepatic bile duct dilatation (*open arrows*)

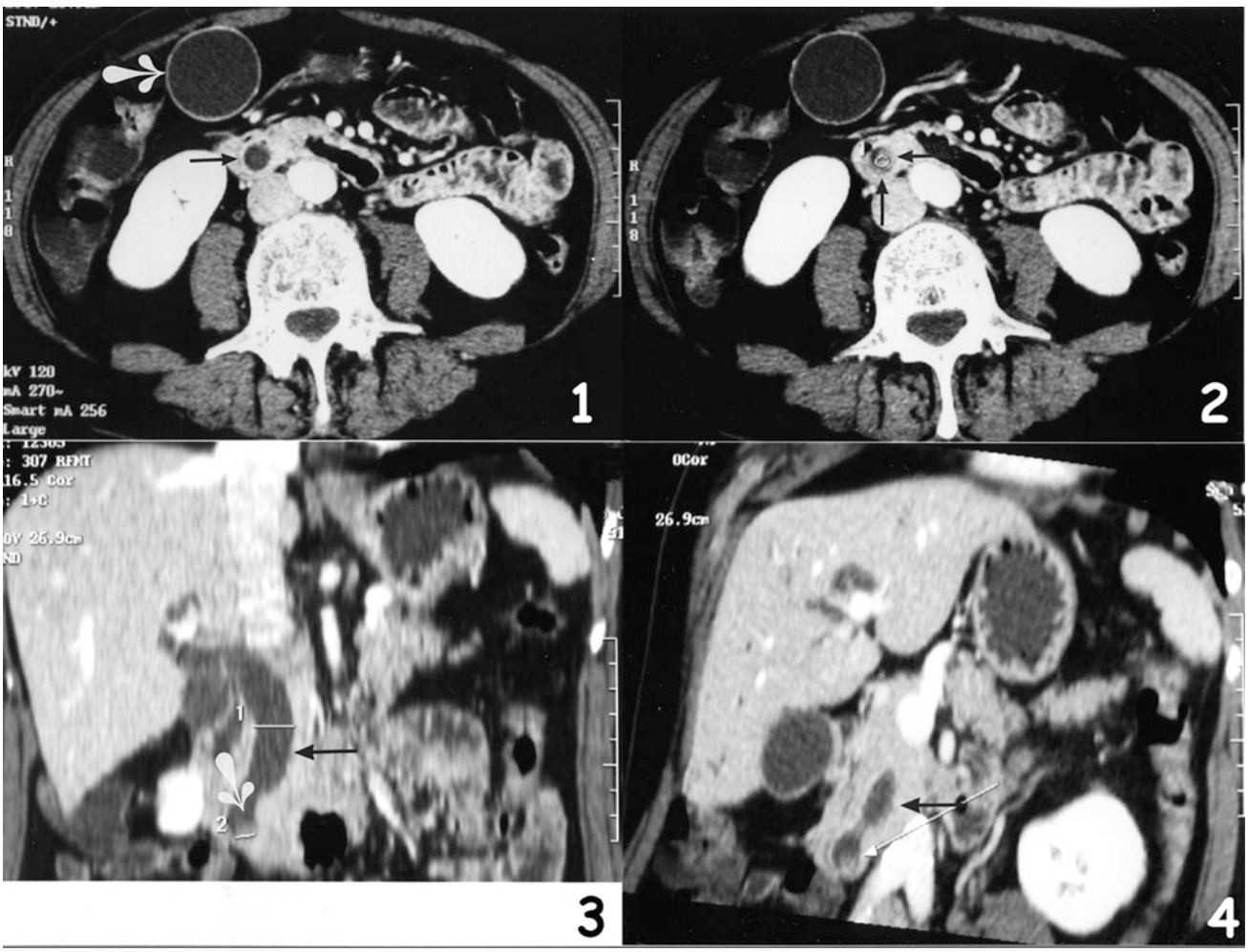


**Fig. 9** Carcinoma of the papilla of Vater. Enhanced CT reveals a dilated gallbladder (*white arrow*) and a common bile duct (*black arrow*) (*1*) with an enhancing small mass in the ampullary area (*arrows*) (*2*). The coronary reconstructions show similar findings: the mass (*white arrow*) and the 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

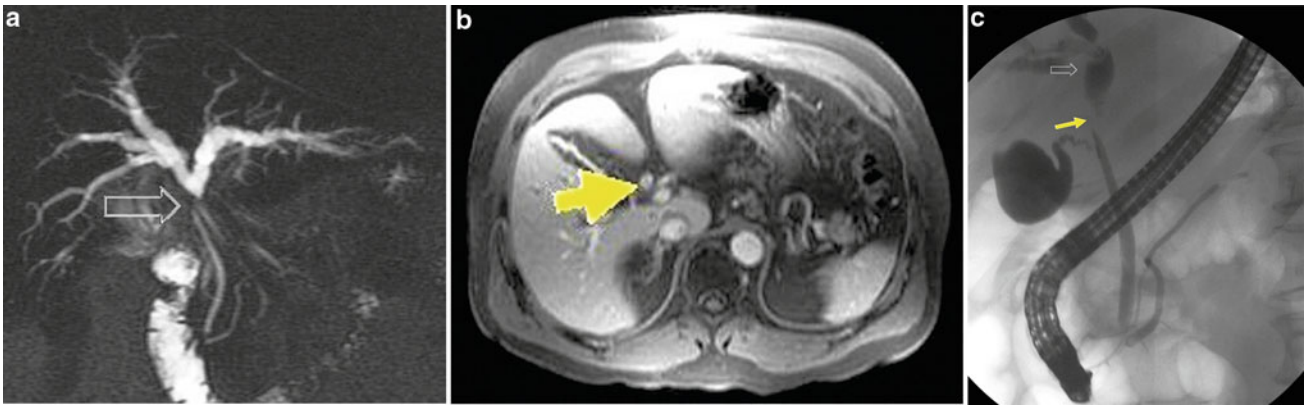


**Fig. 10** Klatskin tumor. **a** MRCP (2-cm-thick slab) shows intrahepatic bile duct dilatation and a short, tight stricture in the common hepatic duct next to the bifurcation (*arrow*). **b** Gadolinium-enhanced MRI (T1 fat-saturated gradient echo) reveals enhancement of the wall thickening of the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

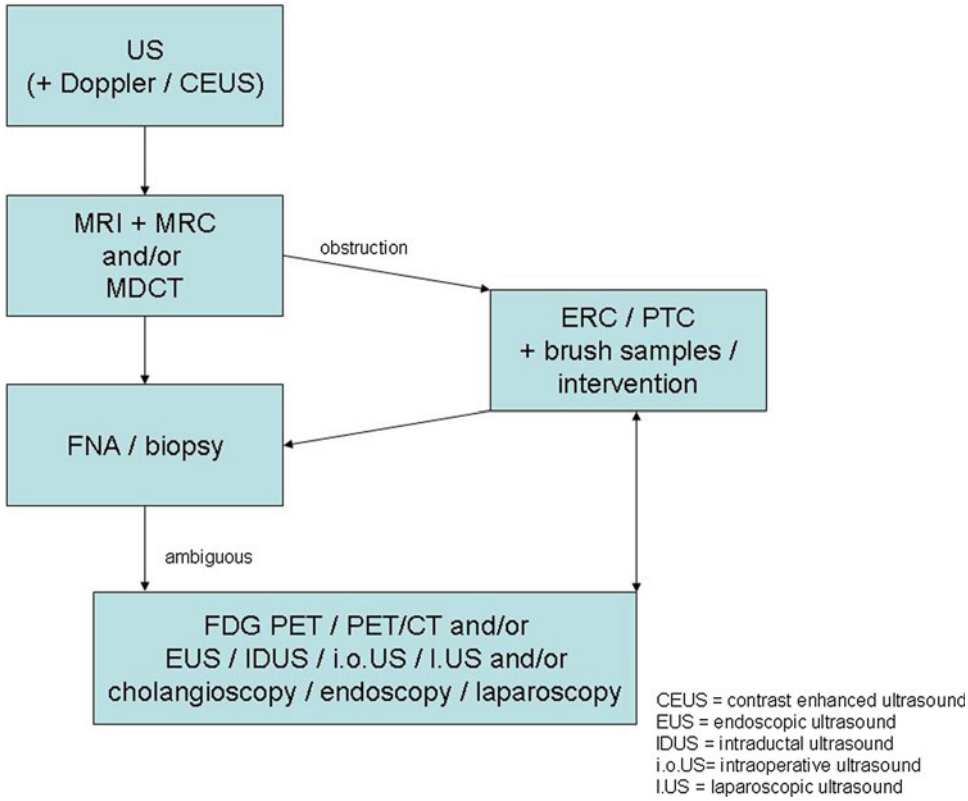


**Fig. 11** Imaging strategies utilized in a typical case of a suspected biliary malignancy

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.

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# Imaging of Hilar Cholangiocarcinoma for Liver Transplantation

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

## Contents




**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

V. M. Zaydfudim · D. M. Nagorney · C. B. Rosen (✉)
Department of Surgery, Mayo Clinic, 200 1st Street SW, Rochester, MN 55905, USA
e-mail: rosen.charles@mayo.edu

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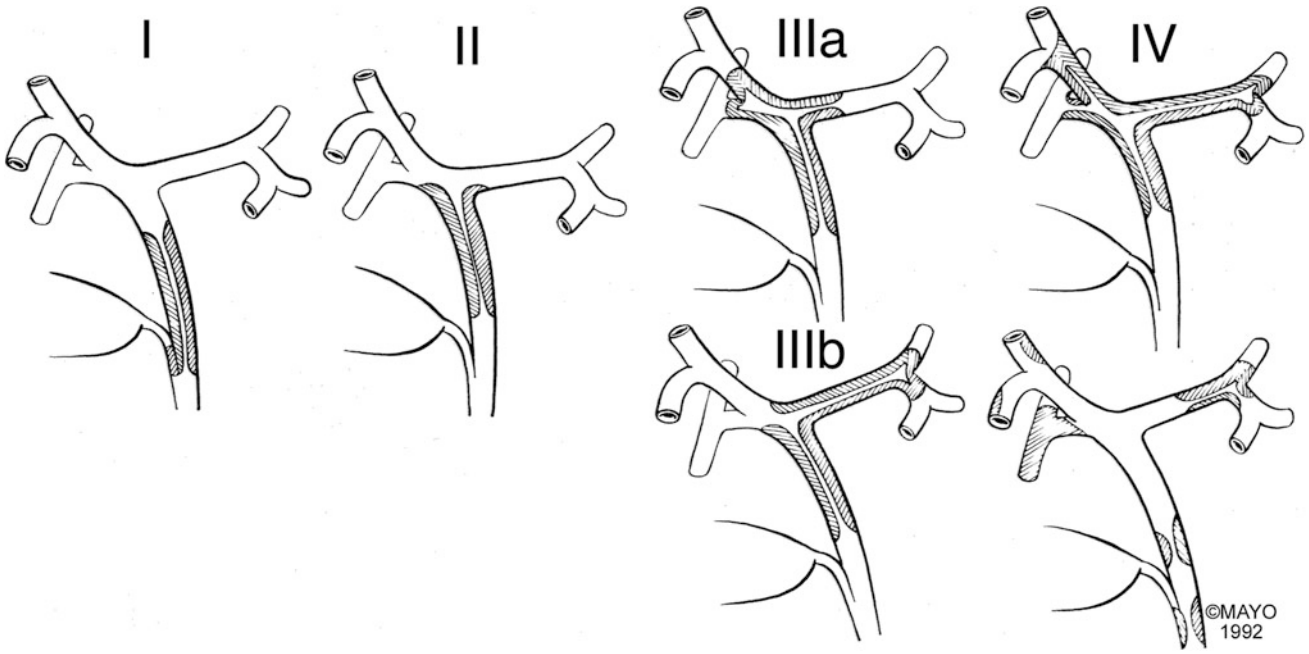


**Fig. 1** Bismuth–Corlette classification of perihilar cholangiocarcinoma: *I* below the bifurcation of right and left hepatic ducts; *II* at the bifurcation of right and left hepatic ducts; *IIIa* at the bifurcation with extension into the right hepatic duct; *IIIb* at the bifurcation with extension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

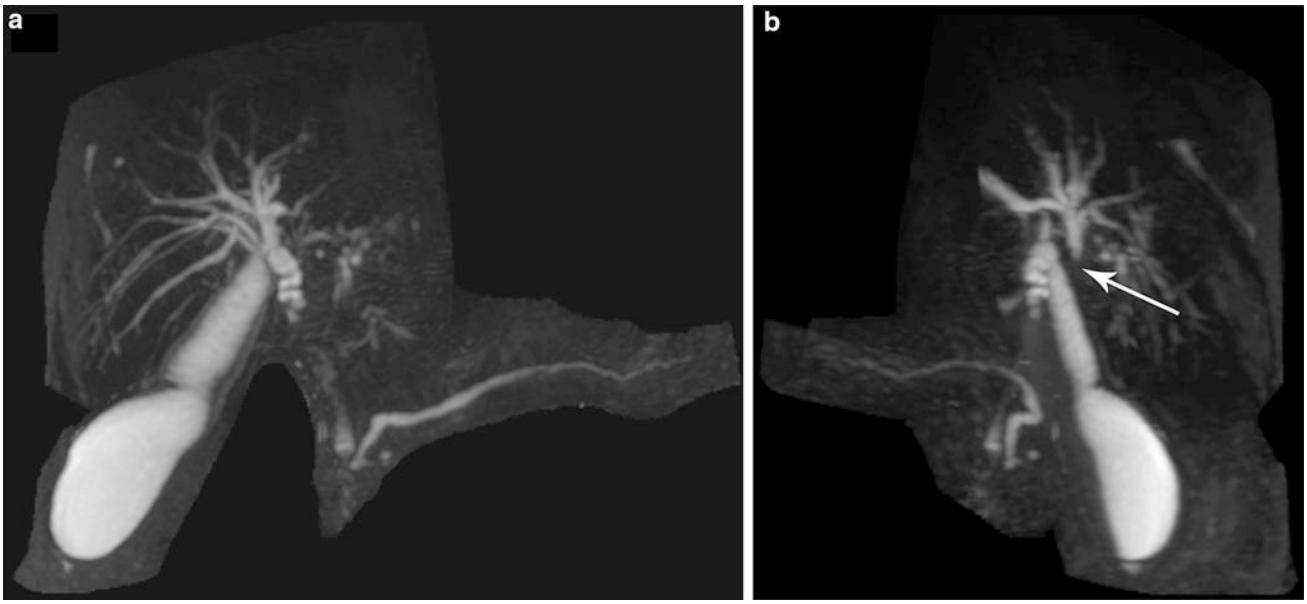


**Fig. 2** Perihilar cholangiocarcinoma with the proximal extent obscured by the cystic duct (Panel **a**). Rotation of the MRCP reconstruction reveals proximal extent at the bifurcation (*arrow*)—a Type II cholangiocarcinoma (Panel **b**)

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].

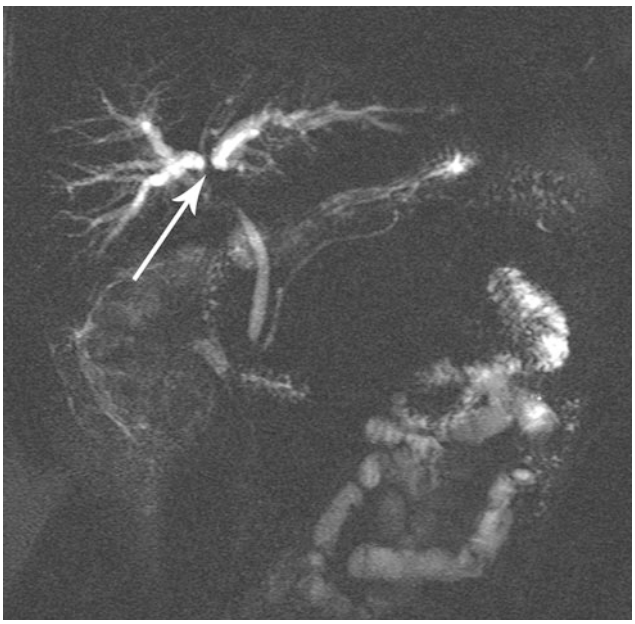

**Fig. 3** MRCP revealing involvement of the ductal bifurcation (*arrow*) by cholangiocarcinoma. Additional rotations of the images and dynamic interpretation in conjunction with cross-sectional imaging are required to distinguish proximal ductal extent (Type IIIA vs. Type IIIB) of the cholangiocarcinoma

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

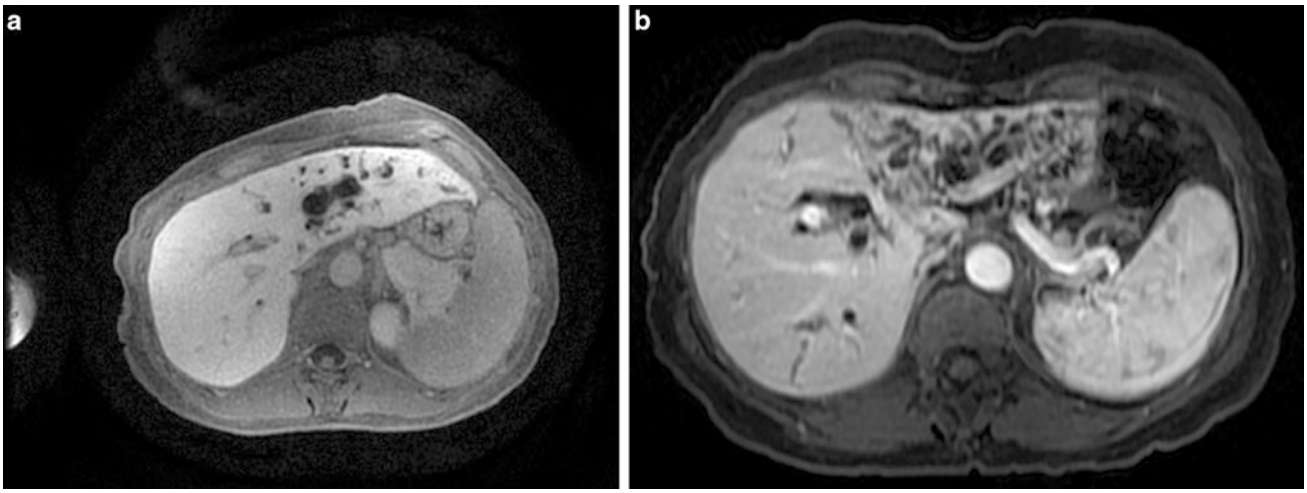


**Fig. 4** Type IIIB hilar cholangiocarcinoma with significant intrahepatic ductal dilatation of the left ductal system (Panel **a**). With time, biliary and/or vascular obstruction leads to development of lobar atrophy (Panel **b**)

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 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].

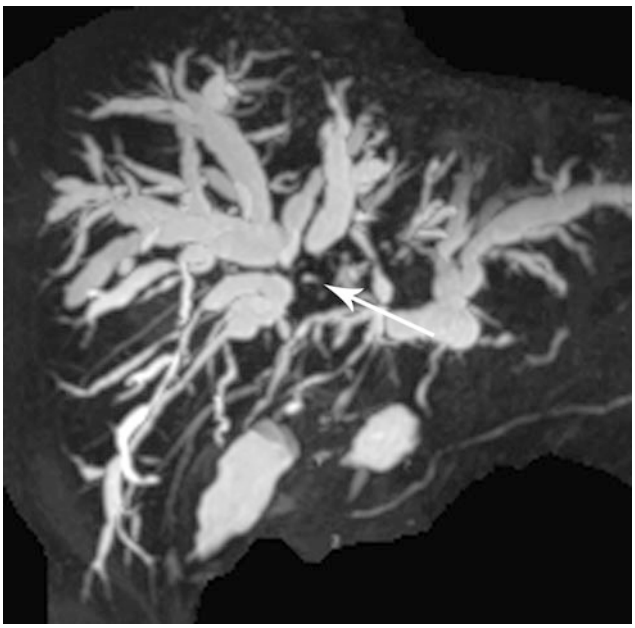

**Fig. 5** Large Type IV hilar cholangiocarcinoma (*arrow*) obstructing biliary drainage from both right and left ductal systems with bilateral ductal dilatation. Tumor extends to secondary ducts in both right and left hepatic lobes

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

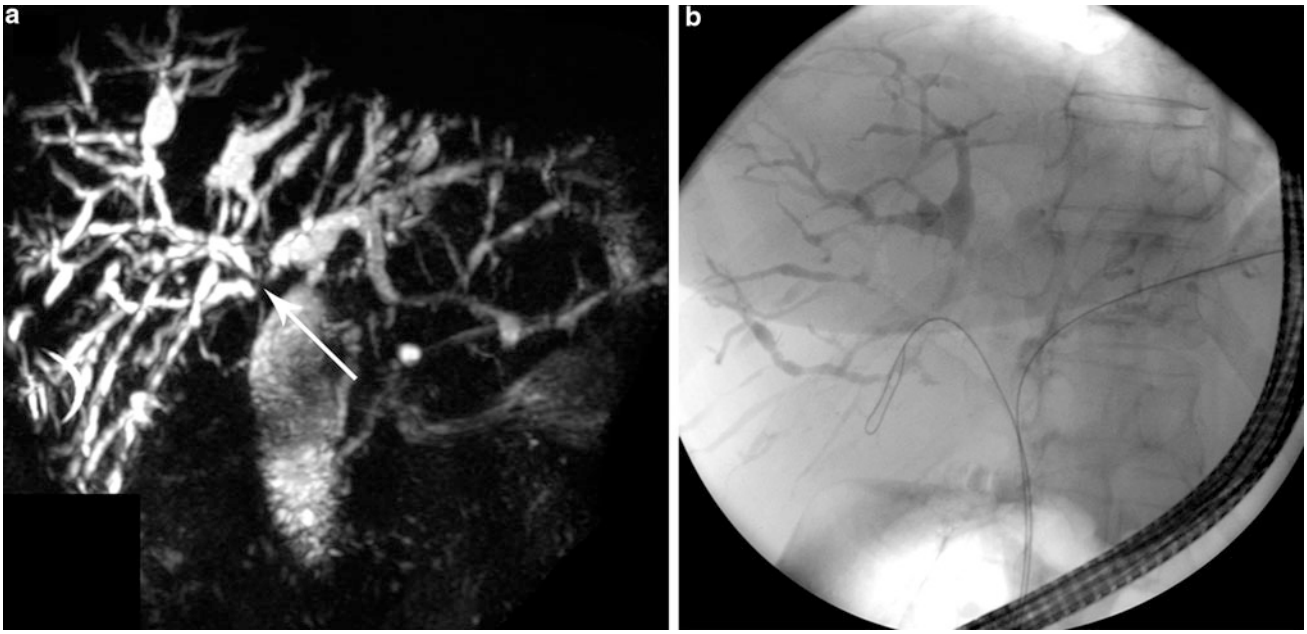


**Fig. 6** MRCP demonstrating a Type IV cholangiocarcinoma (*arrow*) in a patient with PSC with multifocal intrahepatic strictures and dilatations (Panel **a**). A corresponding ERCP demonstrates classic beading of the intrahepatic bile ducts (Panel **b**)

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

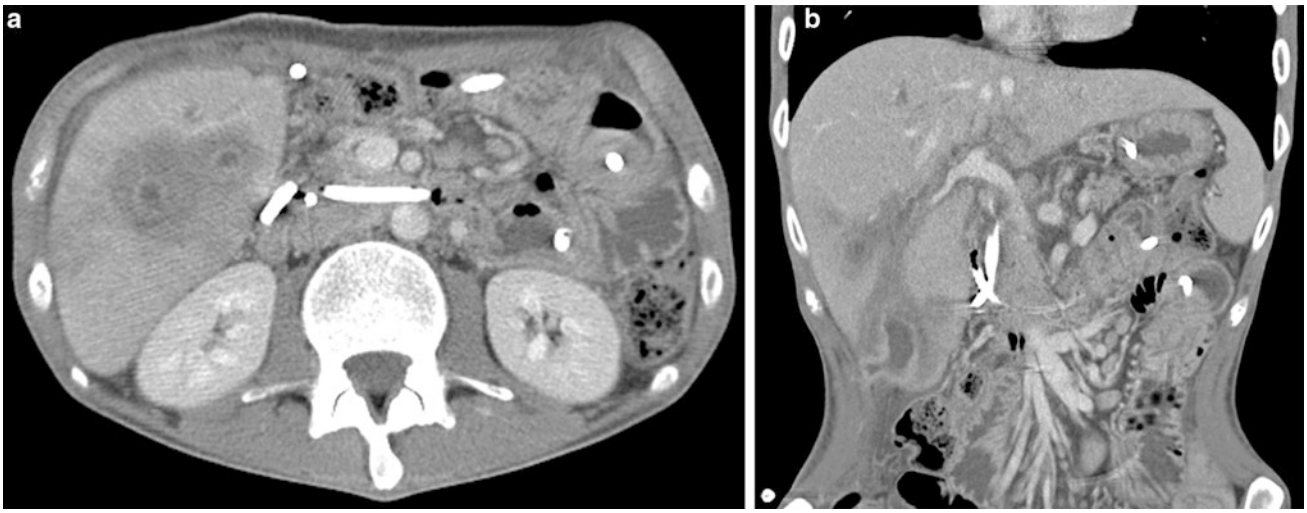


**Fig. 7** Intrahepatic abscess (right lobe)—axial (Panel **a**) and coronal (Panel **b**) reconstructions—in a patient with hilar cholangiocarcinoma and PSC receiving neoadjuvant chemoradiation

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 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.

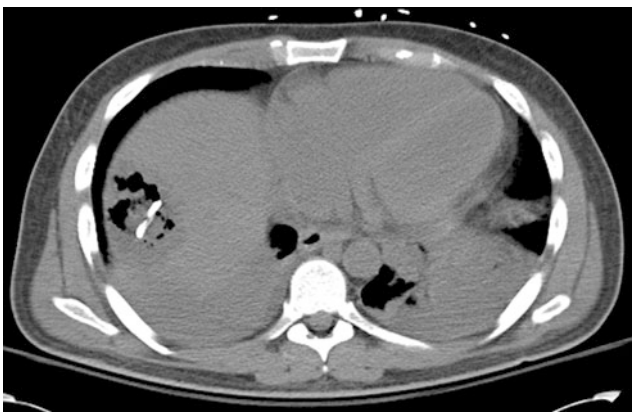

**Fig. 8** Intrahepatic abscess(es) can be successfully managed with percutaneous interventional techniques under radiologic guidance. Pigtail catheter is located within the abscess cavity

## 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 %

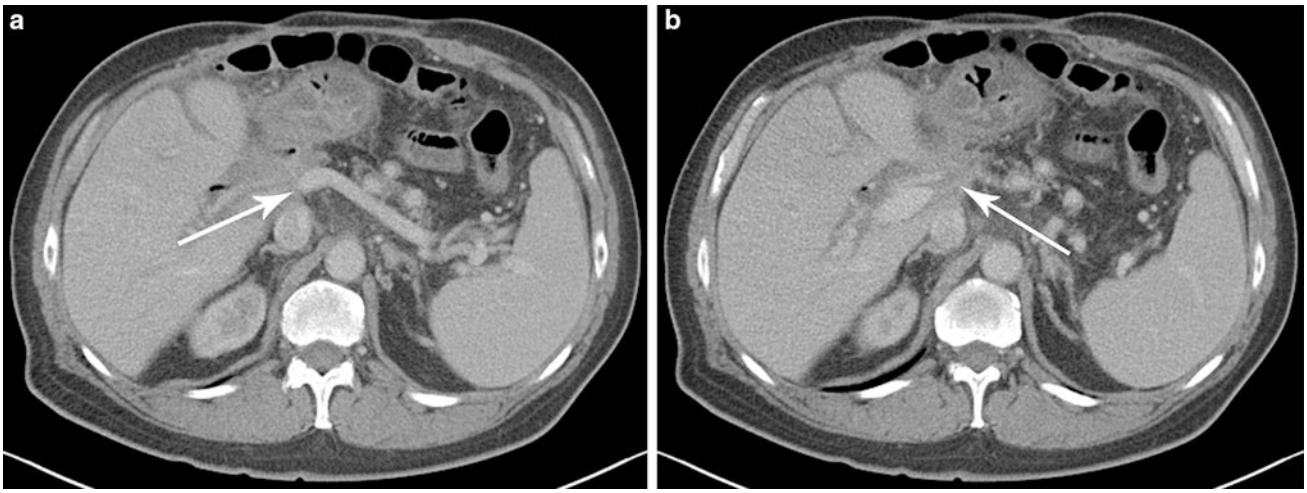


**Fig. 9** Recurrent cholangiocarcinoma *arrow* presenting with hilar venous stenosis at the outflow of the splenomesenteric vein (Panel **a**) into the portal vein (Panel **b**)

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 postoperative 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 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. Retransplantation is necessary for up to 75 % of patients with early HAT, usually due to development of cholangiopathy and hepatic abscesses [30].

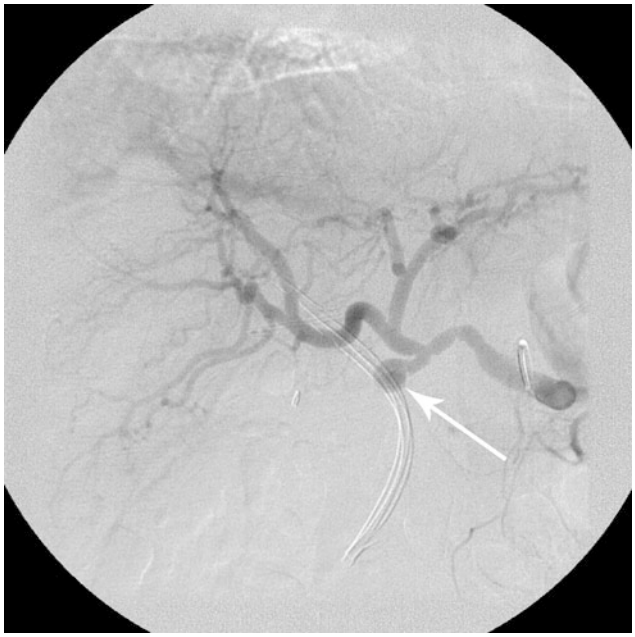

**Fig. 10** Gastroduodenal artery stump pseudoaneurysm (*arrow*) in a patient with stented biliary anastomotic leak

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].

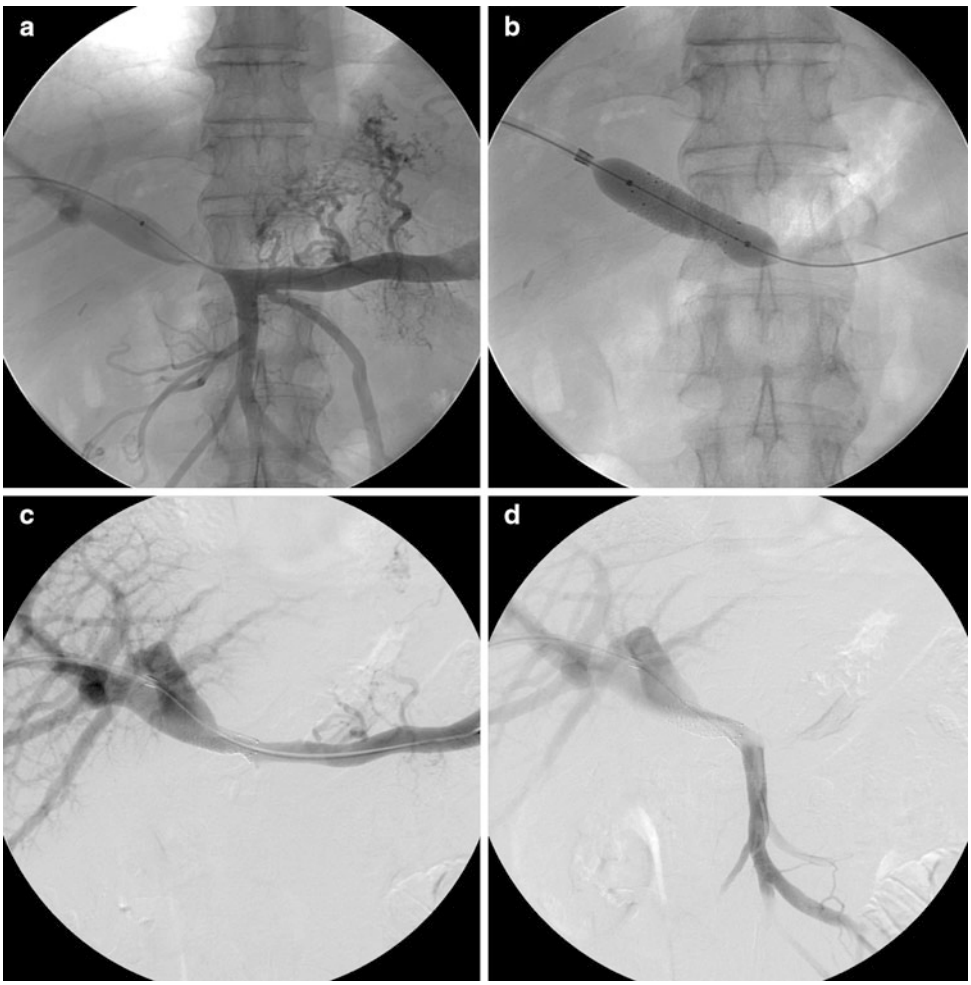


**Fig. 11** Late portal vein stenosis managed with transvenous balloon angioplasty and stent placement (Panels **a** through **d**)

### 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.

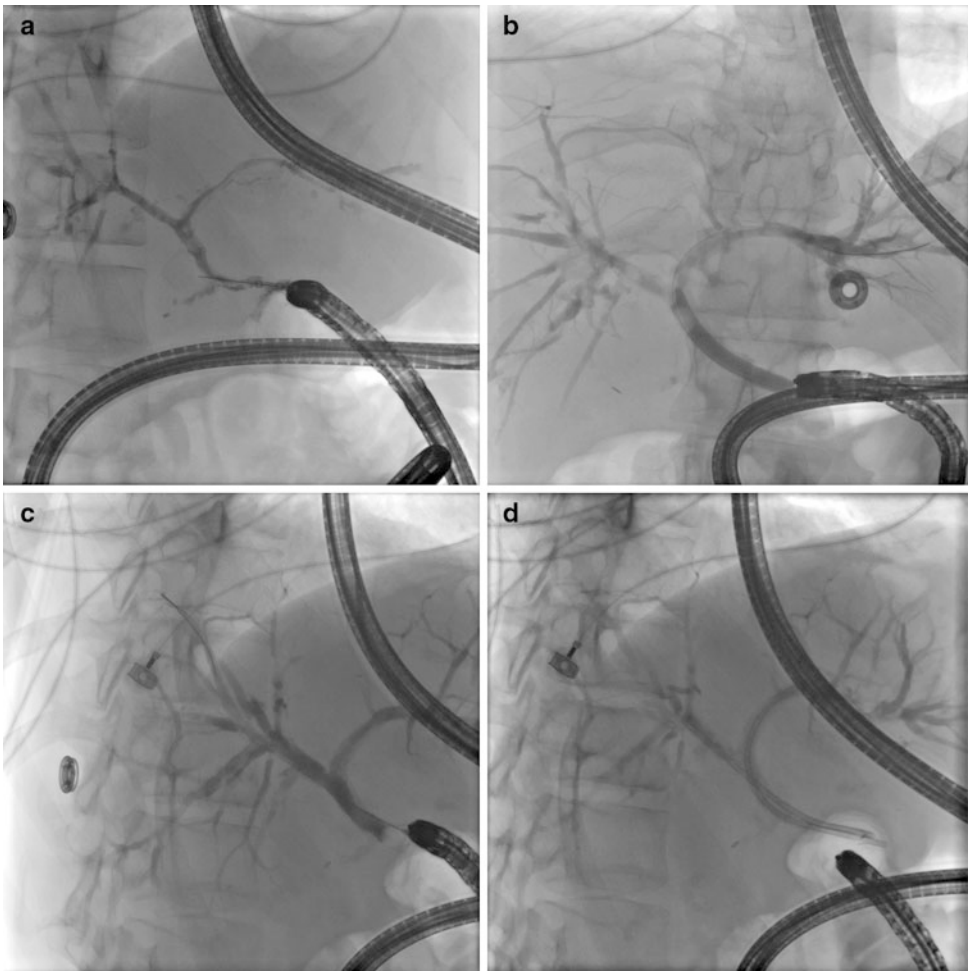


**Fig. 12** Hepaticojejunostomy stricture as well as cholangiopathy in a recipient of donation after cardiac death donor liver. Anastomotic stricture treated endoscopically with balloon dilatation and stent placement (Panels **a** through **d**)

### 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.

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# Magnetic Resonance Imaging (Including MR Cholangiopancreatography)

Susanne Bonekamp, Celia Pamela Corona-Villalobos, Vivek Gowdra Halappa, and Ihab R. Kamel

## Contents



S. Bonekamp · C. P. Corona-Villalobos · V. G. Halappa
I. R. Kamel (✉)
The Russell H. Morgan Department of Radiology and Radiological Sciences, The Johns Hopkins School of Medicine, Baltimore, USA
e-mail: ikamel@jhmi.edu

S. Bonekamp
e-mail: susanne.bonekamp@gmail.com

C. P. Corona-Villalobos
e-mail: ccorona1@jhmi.edu

V. G. Halappa
e-mail: vivekhalappa86@gmail.com

**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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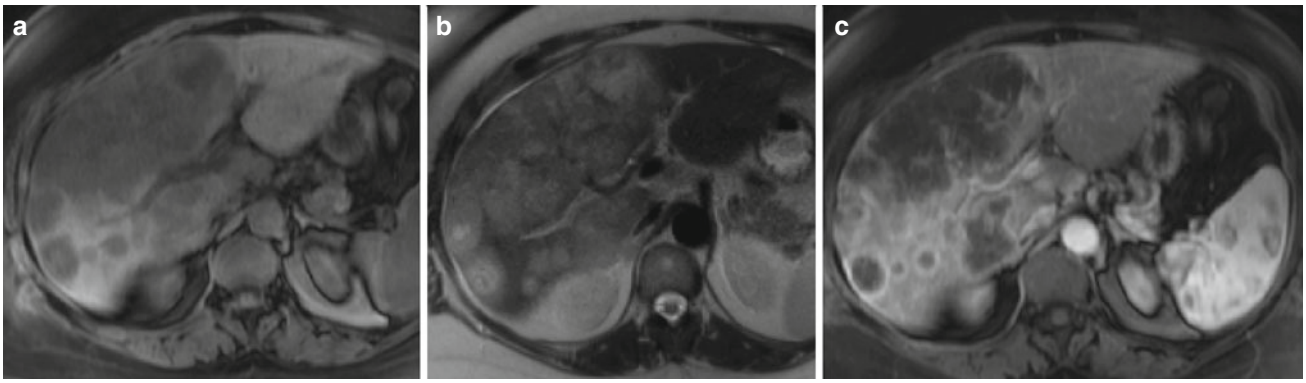


**Fig. 1** **a–b** T1- and T2-weighted images show multiple hepatic masses throughout the liver, with a more confluent area of tumor within the inferior right hepatic lobe consistent with metastatic cholangiocarcinoma. These lesions demonstrate rim enhancement on **c** arterial phase

(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 ethoxybenzyldiethylenetriamine 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

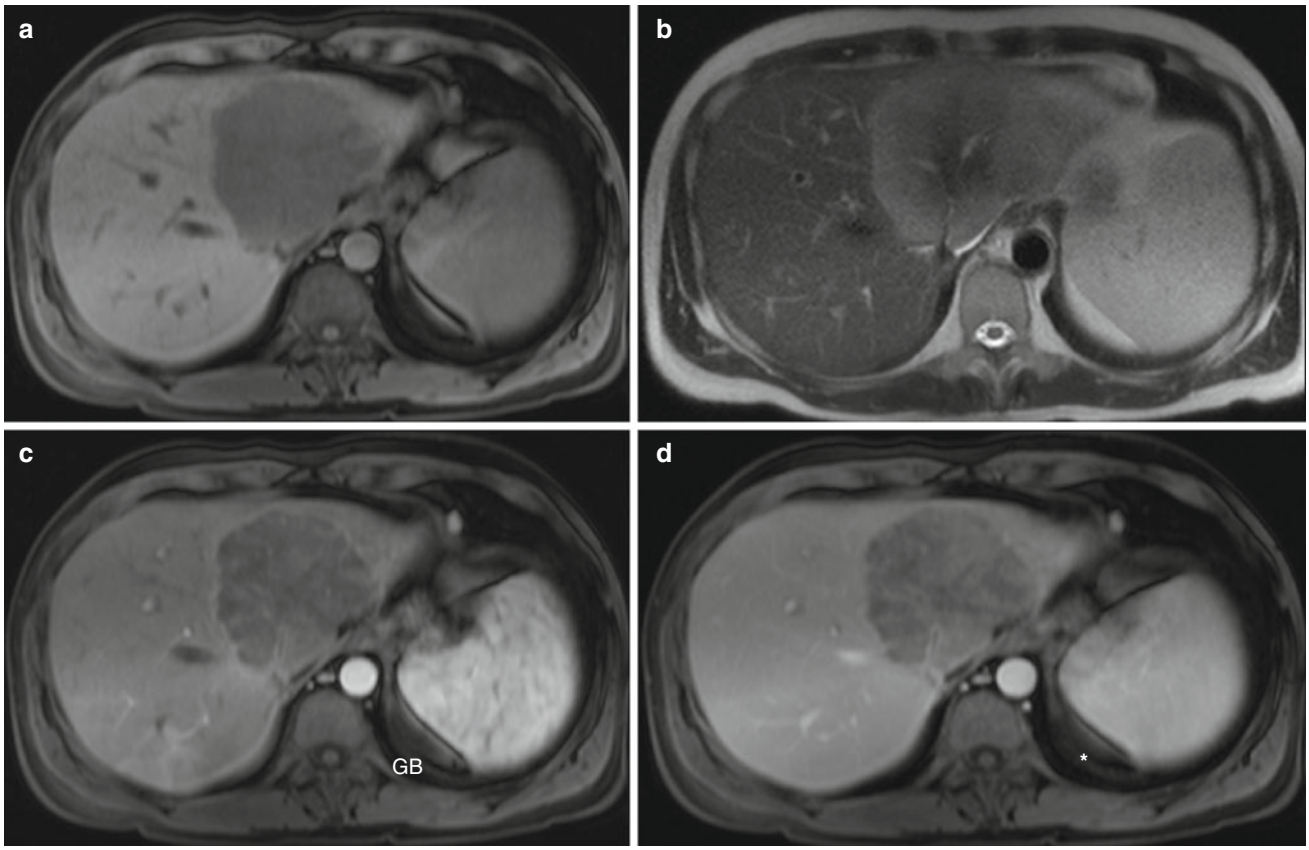


**Fig. 2** A 73-year-old male diagnosed with cholangiocarcinoma. There is a heterogeneous mass of approximately 8 × 5.5 cm to the left of the falciform ligament involving the left and anterior right hepatic lobes. The left and middle hepatic veins are occluded. The mass demonstrates irregular borders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

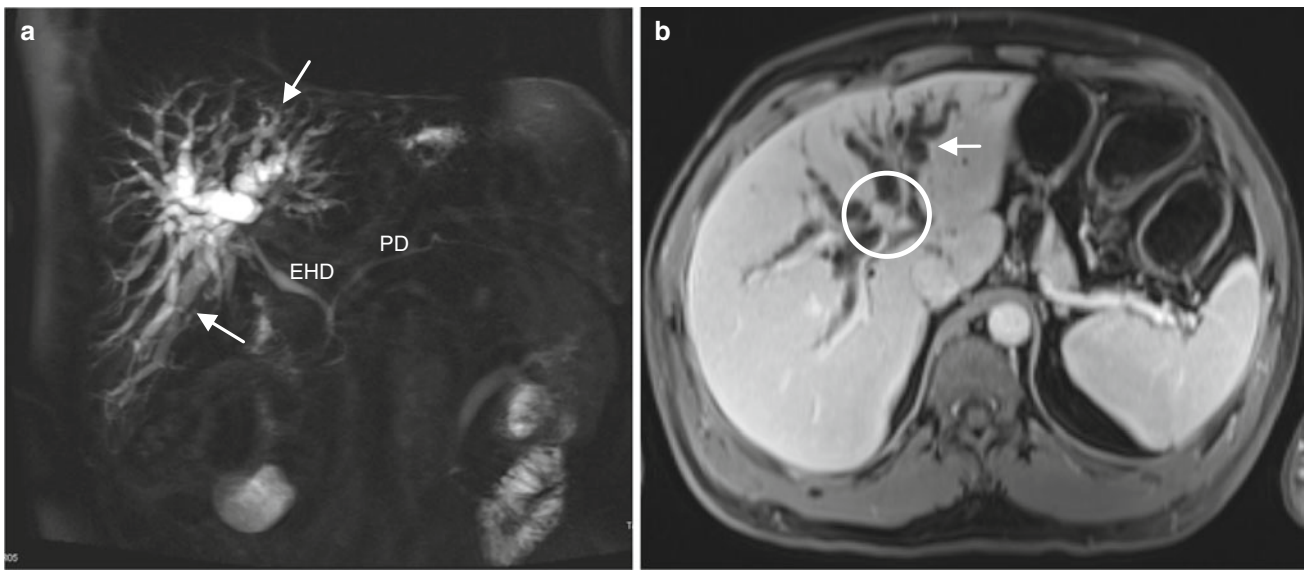


**Fig. 3** **a** MRCP shows marked intrahepatic biliary dilatation or the right and left ducts (*arrows*); the pancreatic duct (*PD*) and the extrahepatic biliary duct (*EHD*) are normal. **b** Delayed contrast-enhanced image depicts an abrupt transition point at the porta hepatis showing a nodular area 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 homogenous 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).

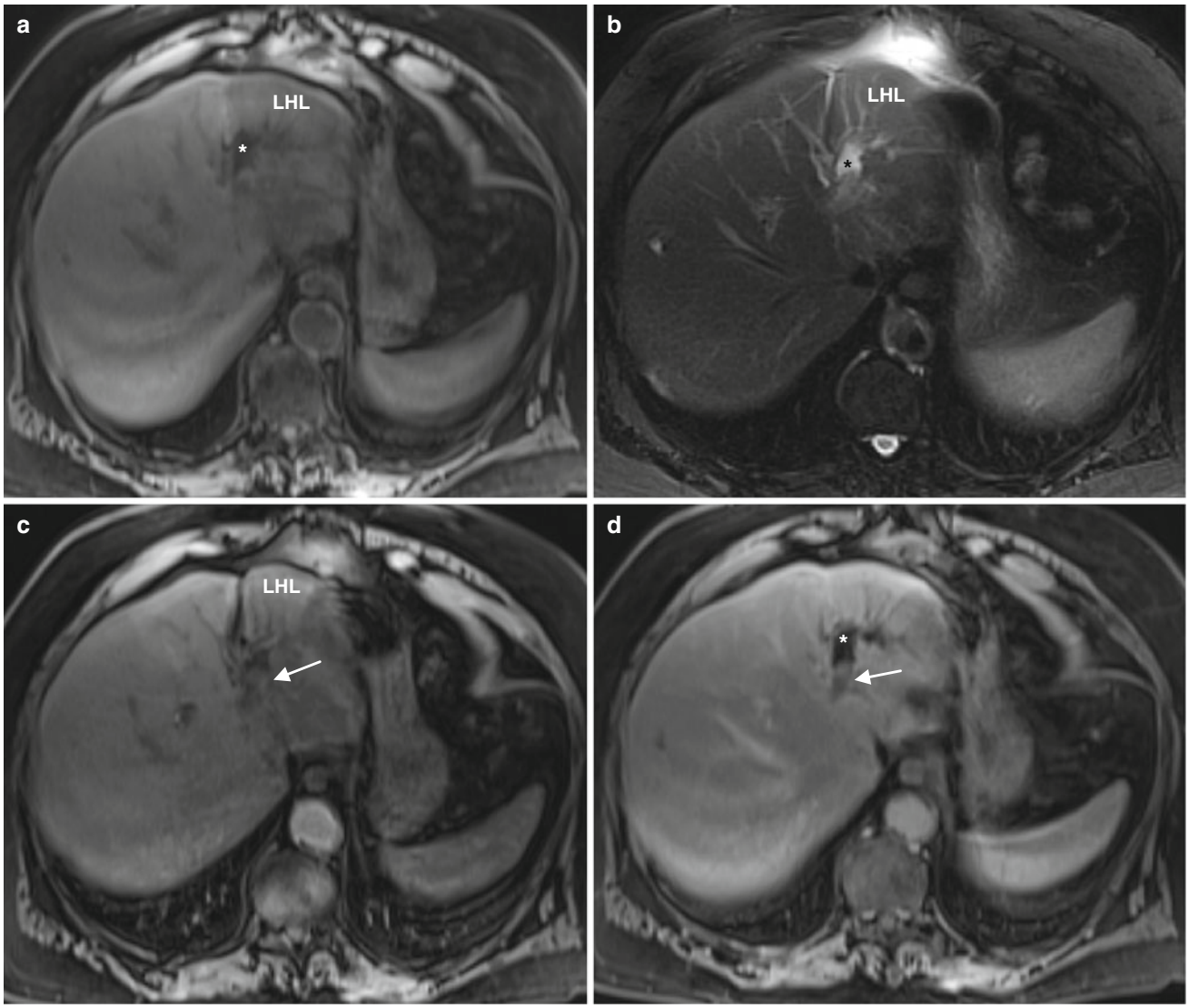


**Fig. 4** **a** T1-weighted and **b** T2-weighted images demonstrate atrophy of the left hepatic lobe (*LHL*) with intrahepatic biliary dilatation (*). The biliary dilatation terminates at the level of an enhancing mass (*arrows*) in the left lobe as shown in the arterial **c** and venous phases **d** 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].

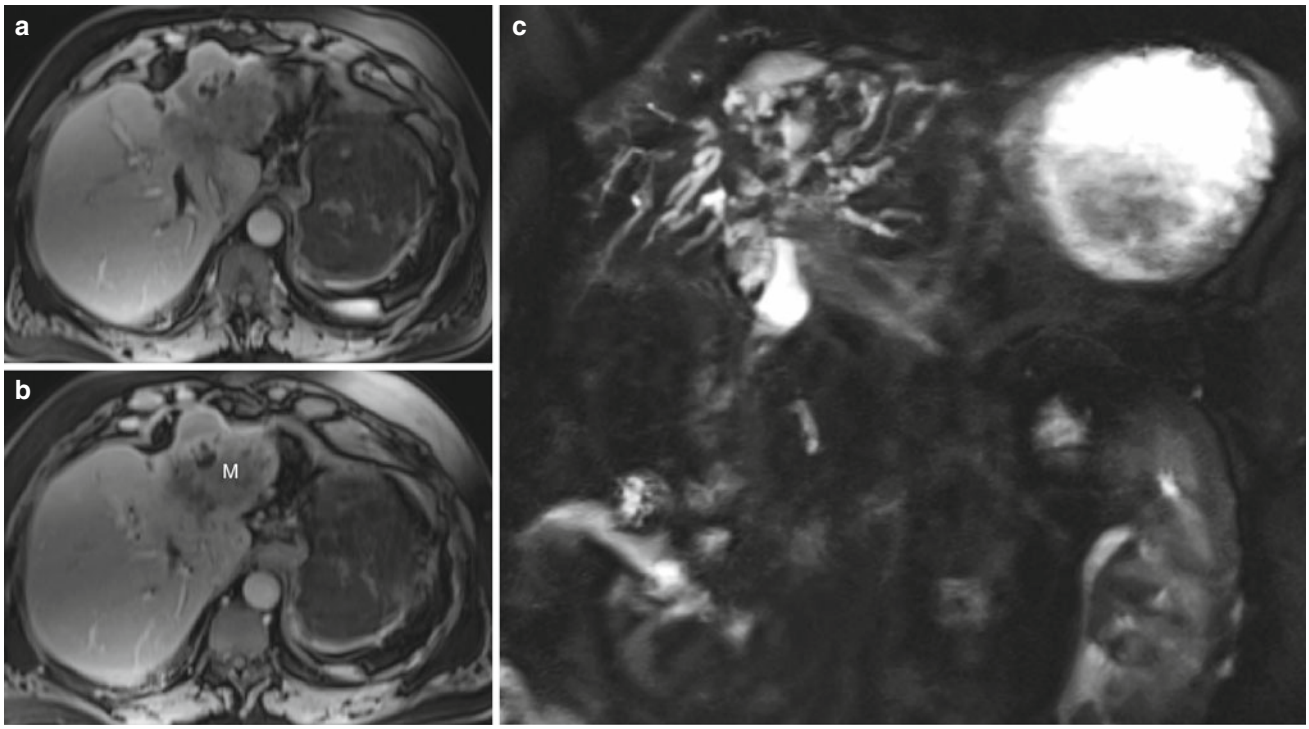


**Fig. 5** There is a hypointense ill-defined 9.4 × 6.2 cm mass within the left hepatic lobe associated with atrophy of the left hepatic lobe. After contrast administration, there is heterogeneously rim enhancement in **a** portal and **b** delayed phases. **c** MRCP shows intrahepatic biliary ductal dilatation in the right and left hepatic lobes

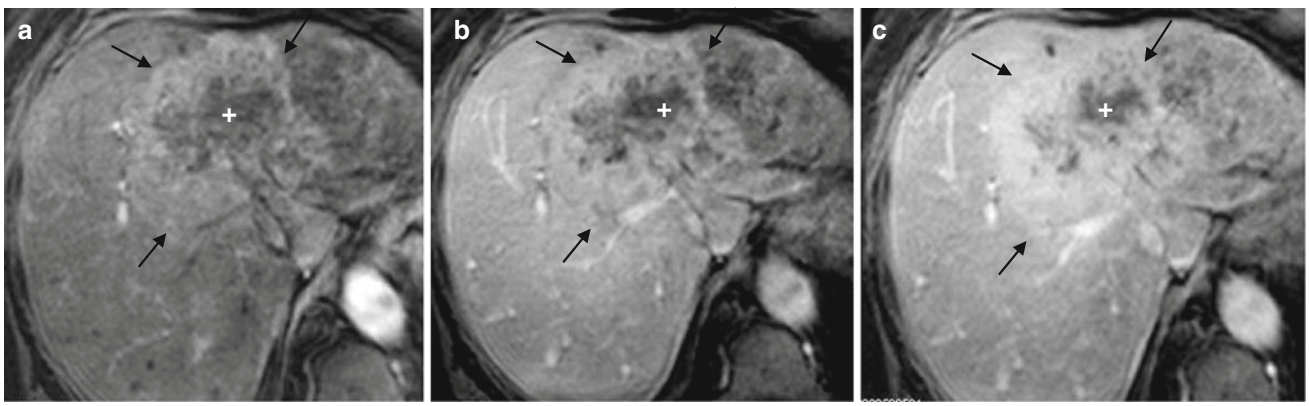


**Fig. 6** A 66-year-old female diagnosed with intrahepatic cholangiocarcinoma. There is a large heterogeneously infiltrating mass involving the right and left hepatic lobes. **a–c** after contrast administration enhanced images showed peripheral enhancement (*arrows*) and central hypointense region 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

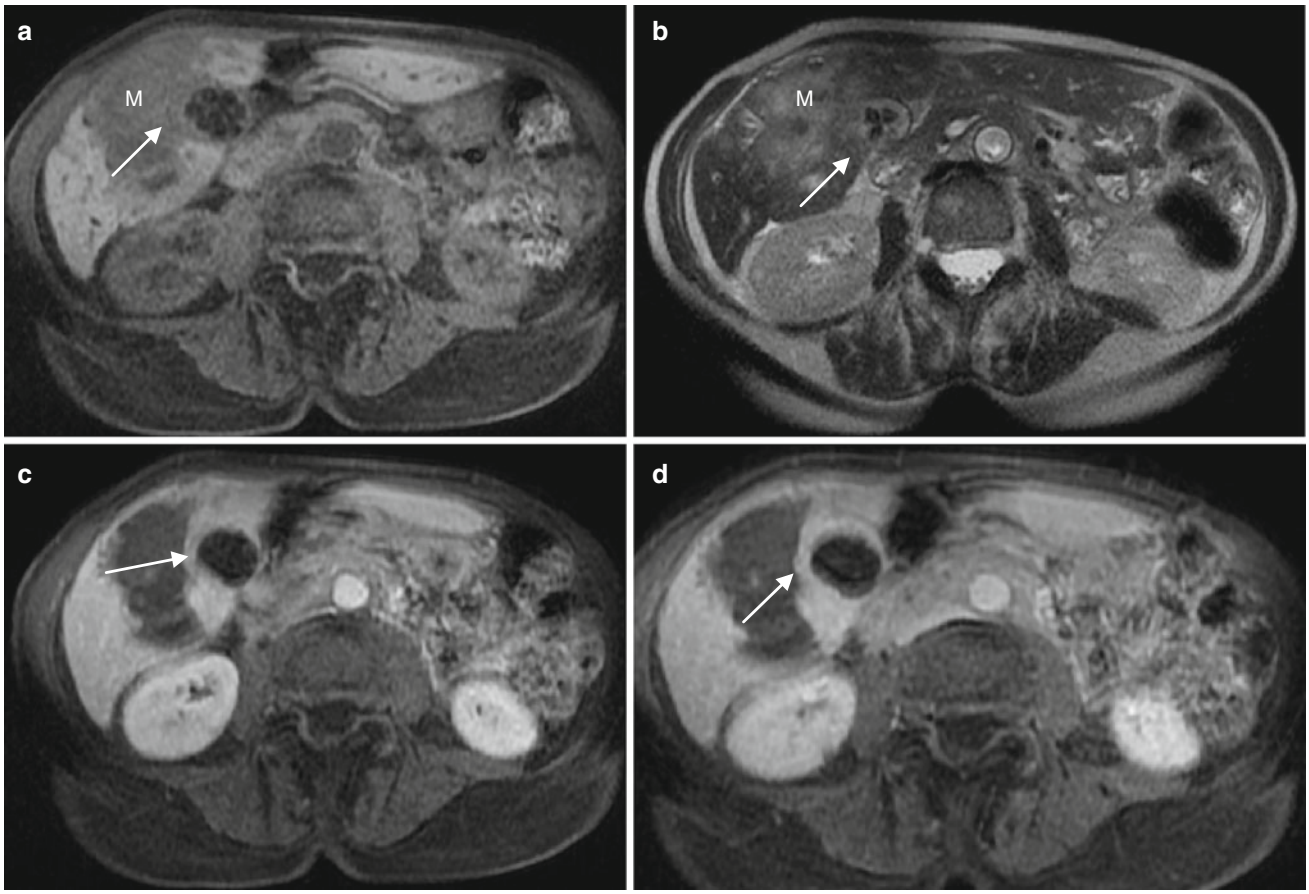


**Fig. 7** A 73-year-old female with history of metastatic cholangiocarcinoma. **a** T1 image shows a dark infiltrative mass with irregular border on the right hepatic lobe. On **b** T2-weighted image, the mass looks heterogeneously hyperintense. After contrast administration, **c** portal and **d** 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

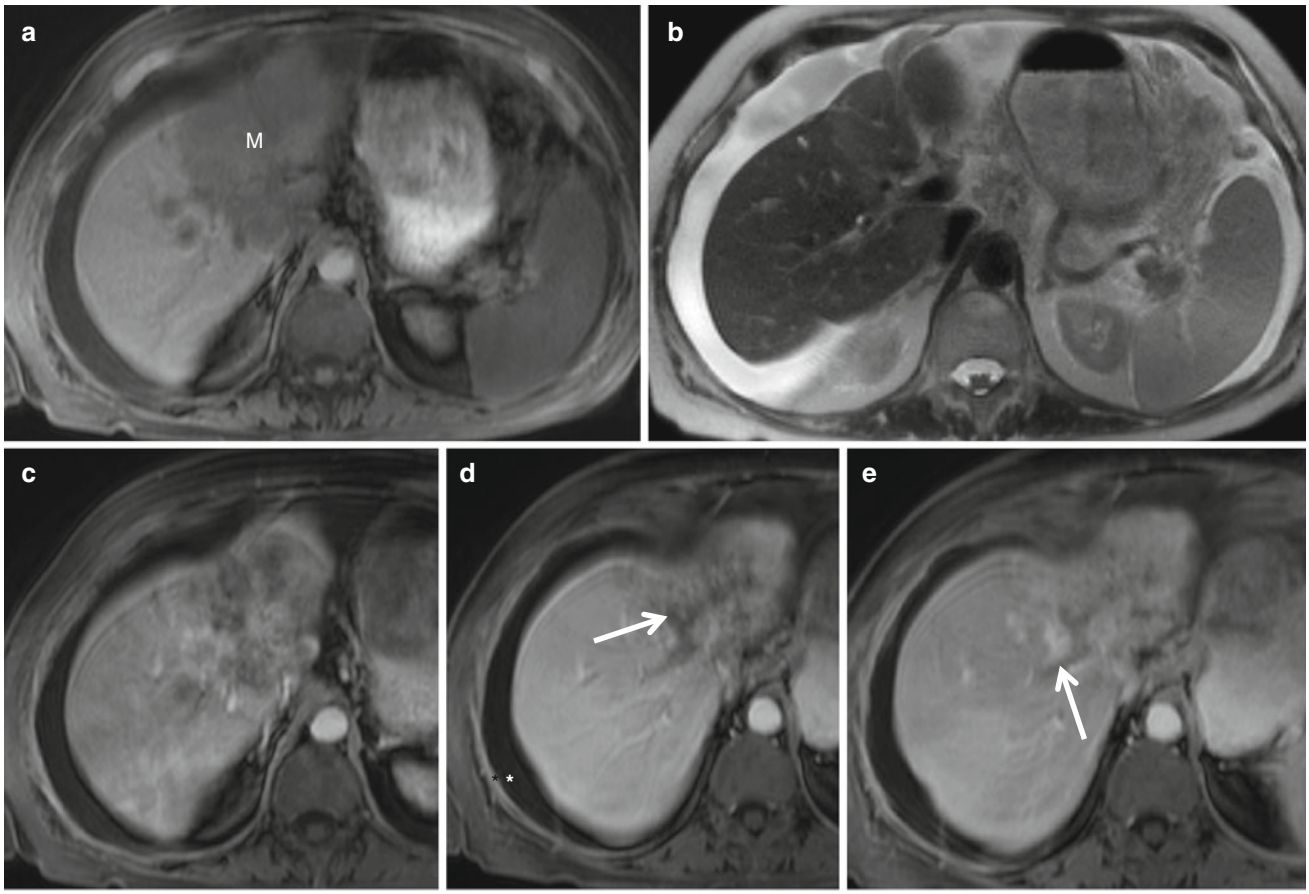


**Fig. 8** Shrinkage of left hepatic lobe is noticed. **a** T1-weighted image shows a hypointense mass (*M*), which nearly replaces the entire left lobe of the liver, and it measures 8 × 7 cm. There is mild intrahepatic biliary duct dilatation (arrows). After contrast administration (**c**–**e**), there is late and heterogeneously enhancement of the mass. There is ascites seen in abdomen (*)

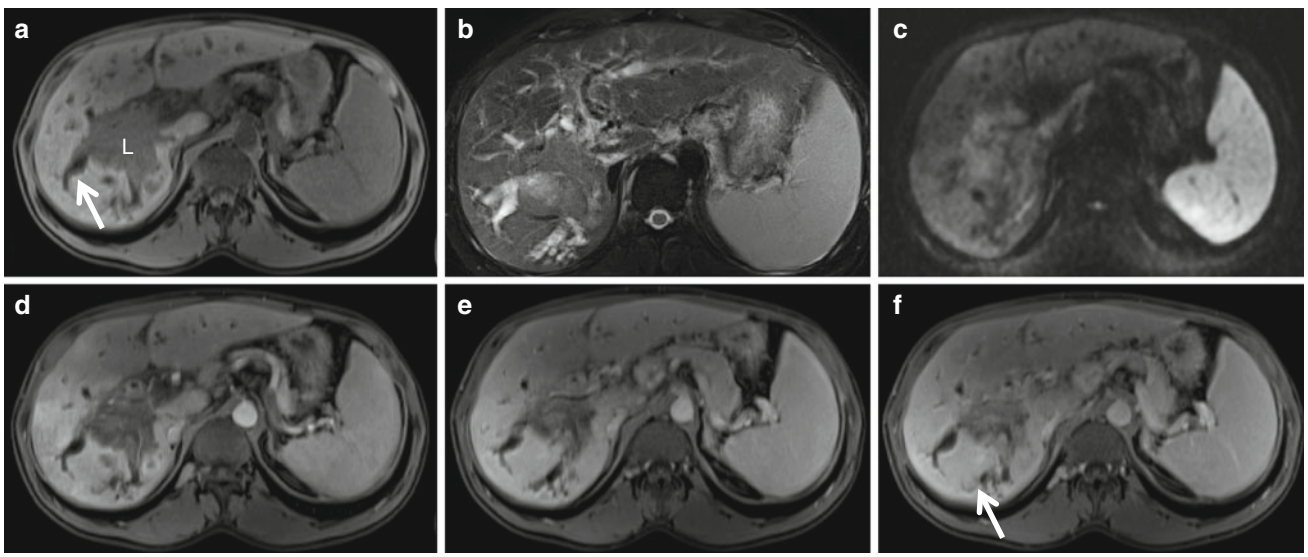


**Fig. 9** A 33-year-old male with diagnosis of cholangiocarcinoma. There is a (**a**) T1 hypointense (**b**) T2, and (**c**) diffusion hyperintense infiltrative lesion (*L*) with (**d**–**f**) delayed enhancement near to the porta hepatis extending to the right hepatic lobe. There is severe intrahepatic 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 washout 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 Periampullary Tumors

Periampullary tumors are neoplasms that arise within 2 cm of the major duodenal papilla and include pancreatic head carcinoma, intrapancreatic bile duct carcinoma, and periampullary 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]. Periampullary tumors appear as low signal intensity masses in the region of the ampulla on T1-weighted fat-suppressed MRI. Most

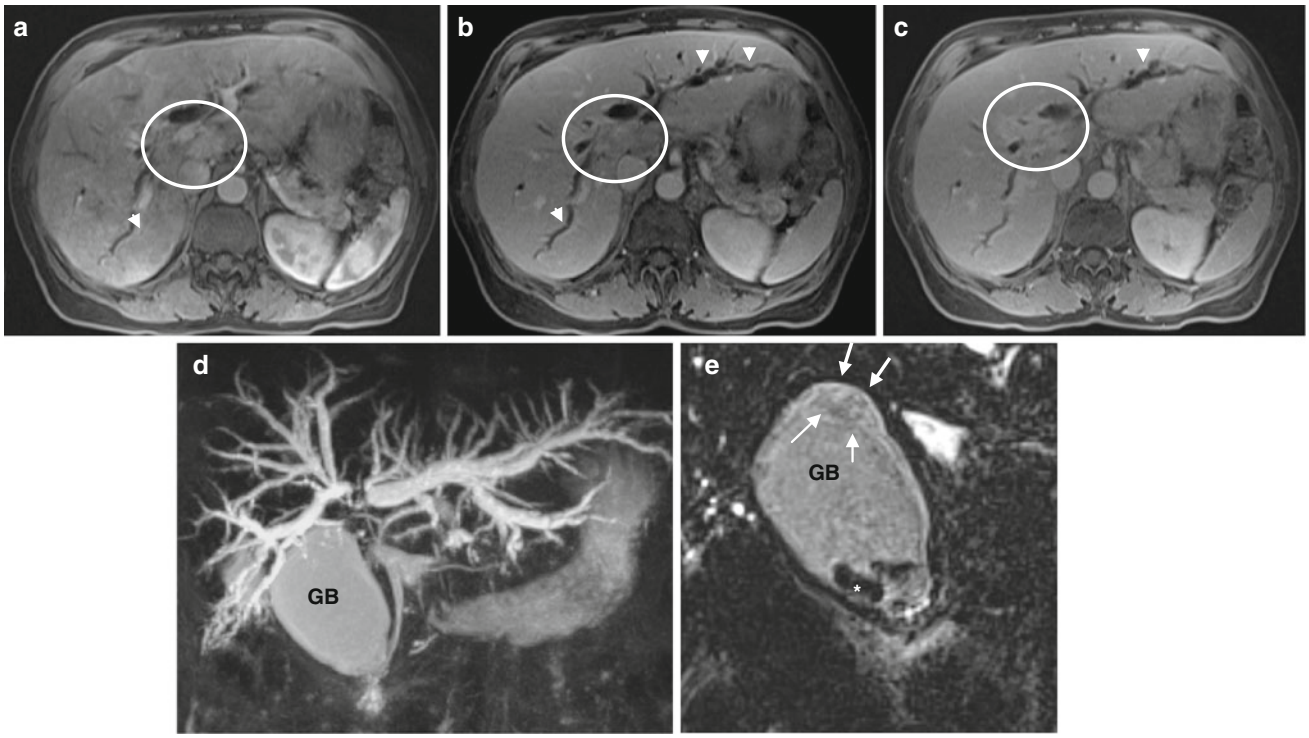


**Fig. 10** A 69-year-old male with diagnosis of metastatic gallbladder adenocarcinoma. Contrast-enhanced MRI demonstrates (**a**–**c**) extensive intrahepatic ductal dilatation (*arrowheads*) with abrupt truncation at the hilar region related to an infiltrating mass measuring 4.2 × 2.6 cm (*circle*). 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 pronounce at the fundus (*arrows*) and presence of choleithiasis (*)

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]. AJPBJ 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

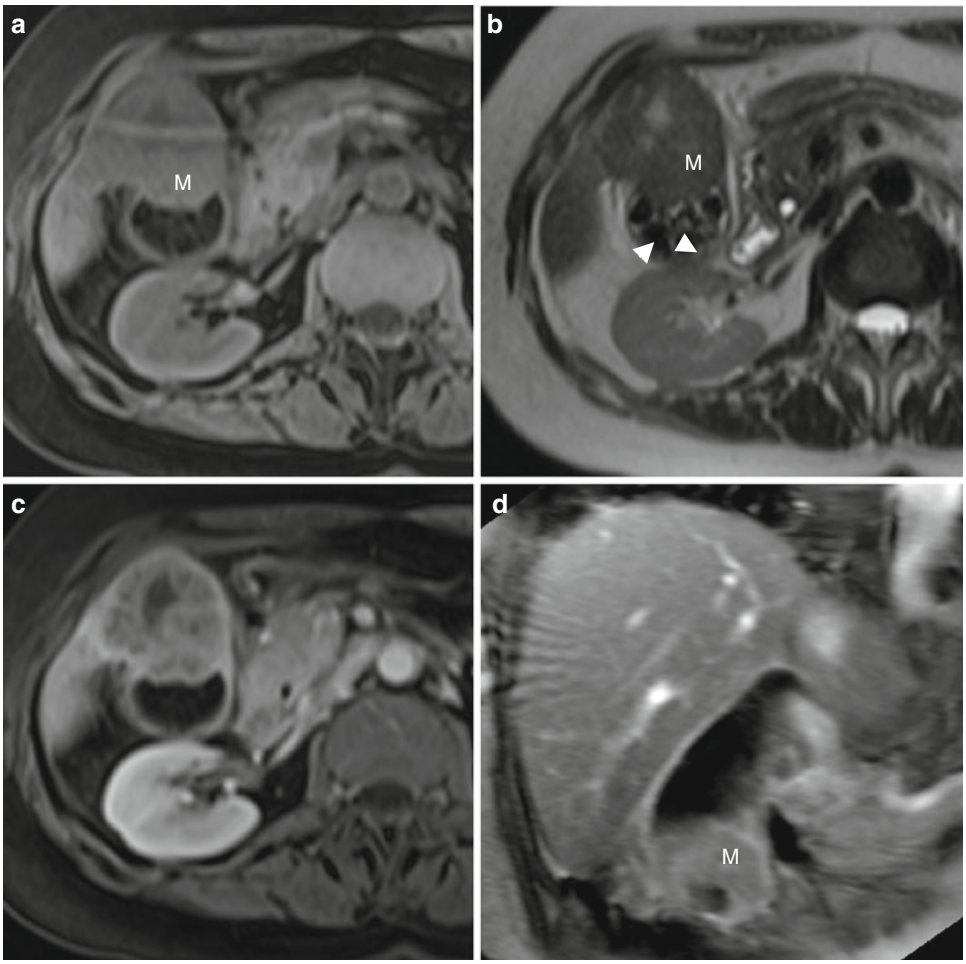


**Fig. 11** A 73-year-old female with primary gallbladder carcinoma. **a** T1 weighted demonstrates an isointense mass-like (M) thickening of the gallbladder fundus with a central hypointense area probably related to central necrosis. **b** The mass is hypointense in T2-weighted images with a central hyperintense area. There are innumerable gallstones (*arrowheads*). **c** There is enhancement of the mass after contrast administration enhancement 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 pathophysioplogical information using MRI.

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# Functional Imaging

Muhammad A. Chaudhry, Lujaien Al-Rubaiey Kadhim, and Richard L. Wahl

## Contents



M. A. Chaudhry
School of Medicine and Medical Director,
Tawam Molecular Imaging Centre,
Johns Hopkins University,
Al-Ain, United Arab Emirates
e-mail: mchaudh9@jhmi.edu

L. A.-R. Kadhim
Research Consultant, Tawam Molecular Imaging Centre, Al-Ain, United Arab Emirates

M. A. Chaudhry · R. L. Wahl (✉)
Division of Radiology, School of Medicine, Johns Hopkins University, Baltimore, USA
e-mail: rwahl@jhmi.edu

**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, $^{18}$F-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[$^{18}$F]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 $^{18}$F-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 $^{18}$F-FDG for tumor metabolism in line with Warburg's observation of increased glucose uptake by malignant cells [6]. The value of $^{18}$F-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

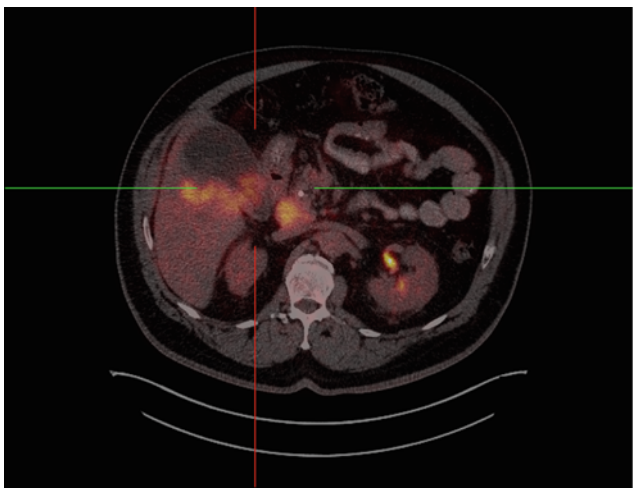

**Fig. 1** 54 year-old male with a newly found gallbladder mass. Axial fused staging 18F-FDG PET/CT image demonstrates intensely FDG-avid gallbladder wall thickening involving the posterior wall (SUV-max 10.1) and the hepatic parenchyma

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).

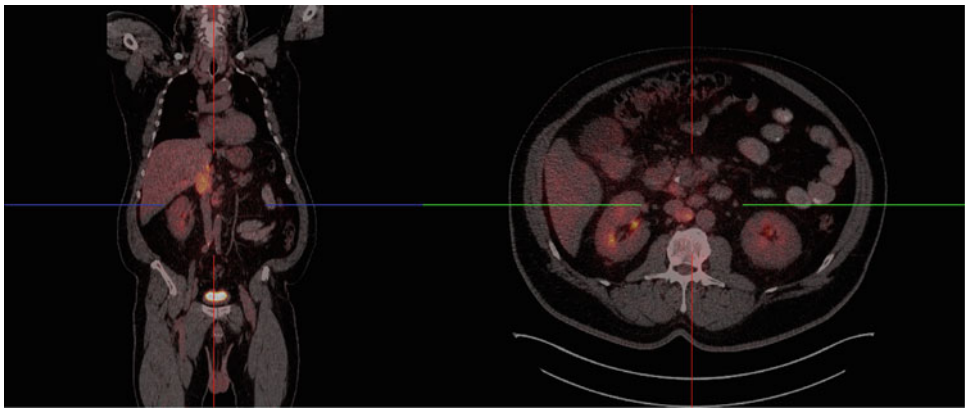

**Fig. 2** Coronal (*left*) and axial (*right*) fused PET/CT images demonstrate FDG-avid lymphadenopathy involving the aortocaval nodes with the highest SUVmax of 6.6

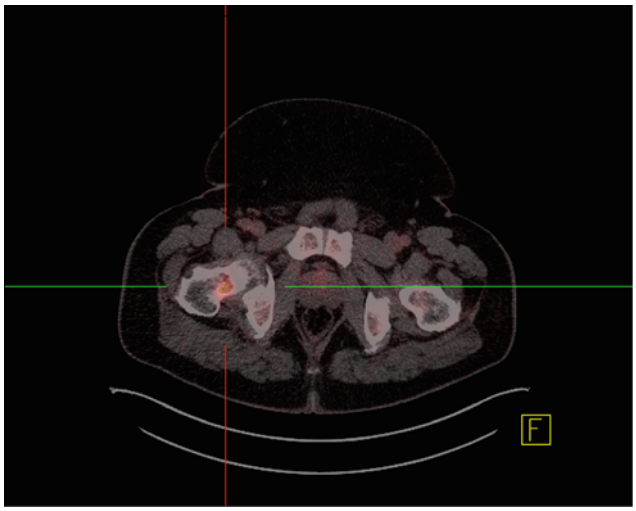


**Fig. 3** Intense focal FDG uptake is noted in a lucent lesion involving the right femoral neck and is highly suspicious for malignant involvement with a SUVmax of 7.2

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 (%) |
|---|---|---|---|---|---|---|---|
| Alkhawaldeh 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

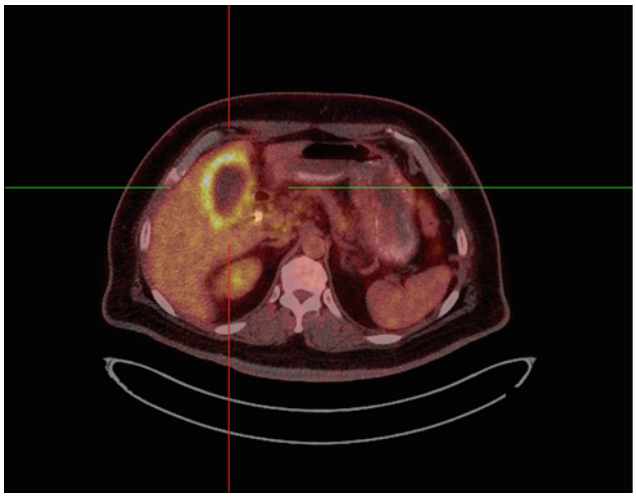

**Fig. 4** 54 year-old male with a history of cholangiocarcinoma with liver metastases. PET/CT scan was indicated for the evaluation of residual disease following completion of chemotherapy. Fused axial 18F-FDG PET/CT image demonstrates intensely FDG-avid thickening noted along the gallbladder with the 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.

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# Computed Tomography

Gory Ballester-Ortiz, Leonardo Marcal, Chitra Viswanathan, and Janio Szklaruk

## Contents



G. Ballester-Ortiz
Section of Diagnostic Radiology,
University of Puerto Rico School of Medicine, PO Box 365067
San Juan, PR 00936-5067, USA

L. Marcal · C. Viswanathan · J. Szklaruk (✉)
Department of Diagnostic Radiology, Unit 1473,
The University of Texas MD Anderson Cancer Center, 1515
Holcombe Blvd, Houston, TX 77030, USA
e-mail: jszklaru@mdanderson.org

**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 y-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,

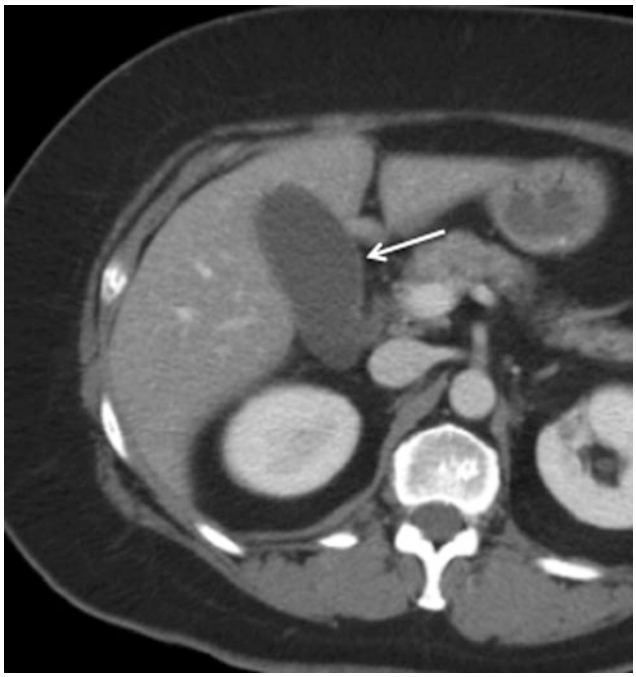

**Fig. 1** Normal gallbladder. Axial MSCT contrast-enhanced image obtained during the portal phase shows a normal gallbladder. Its walls are sharply demarcated from the adjacent liver parenchyma and peritoneal fat. Thin homogeneous wall enhancement (*arrow*) during the arterial or portal phase is a normal finding

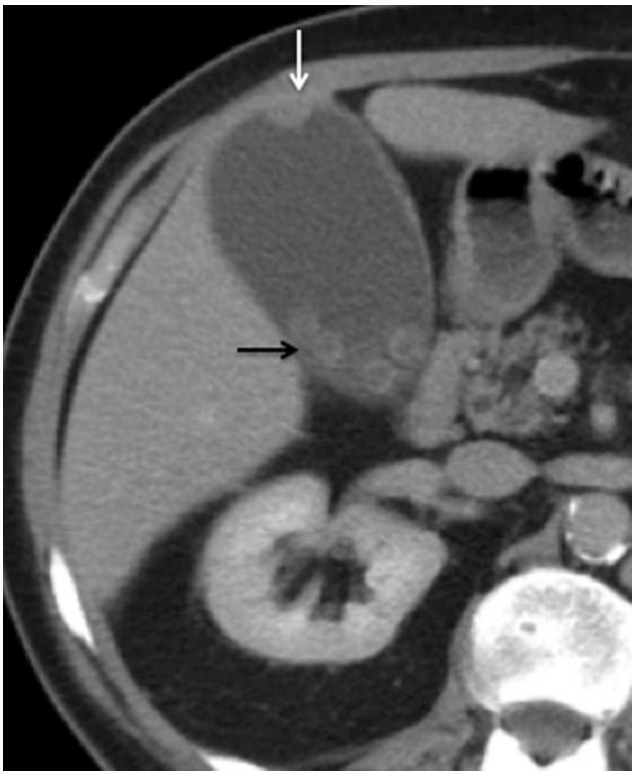

**Fig. 2** Papillary gallbladder cancer: 68-year-old male patient with gallbladder cancer incidentally found during evaluation for abdominal pain. Contrast-enhanced MSCT axial image shows a soft tissue mass protruding from the wall (*white arrow*) into the homogeneous low-attenuation lumen of the gallbladder. Cholelithiasis (*black arrow*) is also present in this patient

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 nonsurgical 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 nonenhanced 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.

## 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.

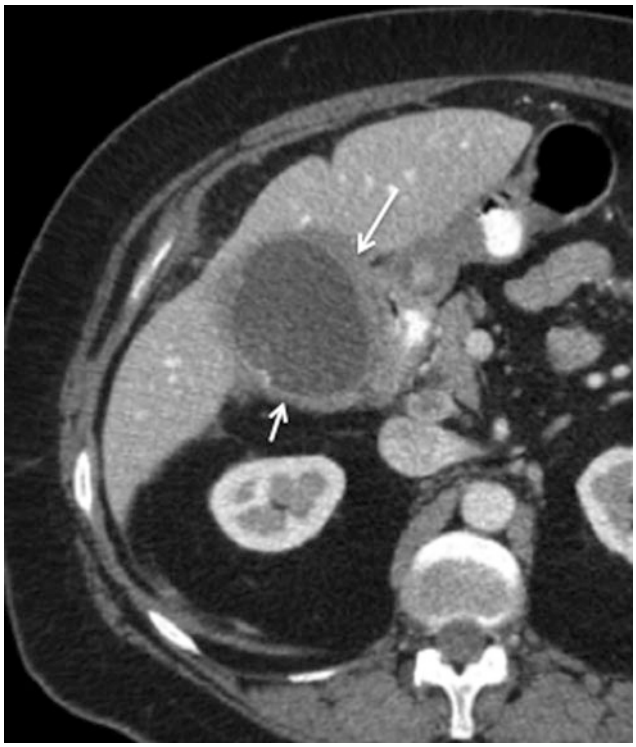

**Fig. 3** Flat gallbladder cancer. Axial MSCT contrast-enhanced image of a 72-year-old male patient showing irregular thickening of the gallbladder wall (*arrows*), representing gallbladder cancer

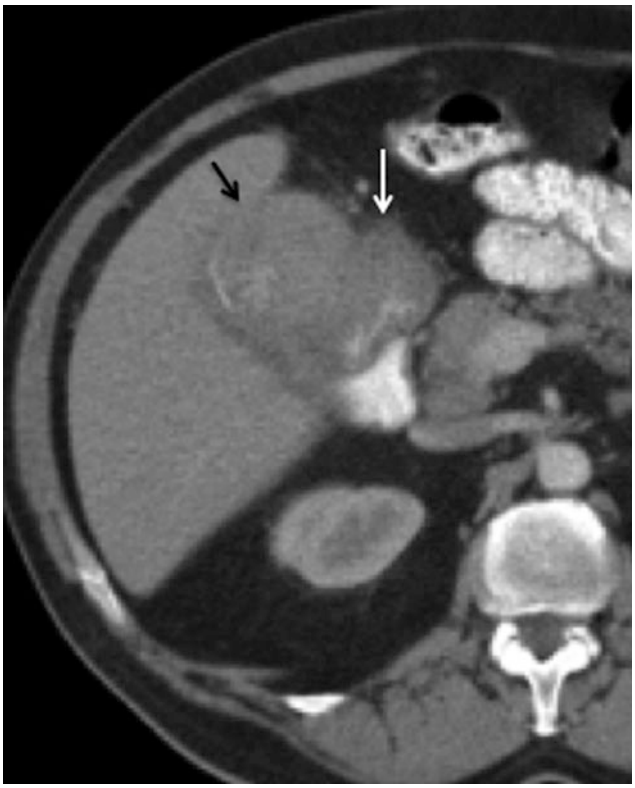

**Fig. 4** Massive gallbladder cancer. 59-year-old female patient presenting with anorexia and weight loss. Contrast-enhanced MSCT axial image shows a large soft tissue mass (*black arrow*) occupying most of the lumen of the gallbladder. Note is also made for irregular thickening of the proximal duodenum (*white arrow*), representing neoplastic invasion

## 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

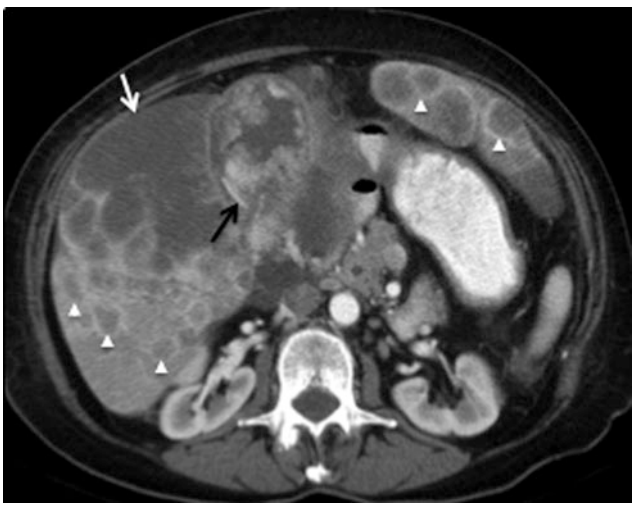

**Fig. 5** Advanced gallbladder cancer. 60-year-old female patient presenting with jaundice and hepatomegaly. Axial MSCT contrast-enhanced image shows a large heterogeneous mass centered in the gallbladder (*black arrow*). Associated invasion of the liver with irregular peripheral enhancement and central hypodensity (*white arrow*), compatible with necrosis. Multiple hypodense masses with peripheral rim enhancement (*arrowheads*) are appreciated throughout both hepatic lobes, compatible with hematogenous metastases

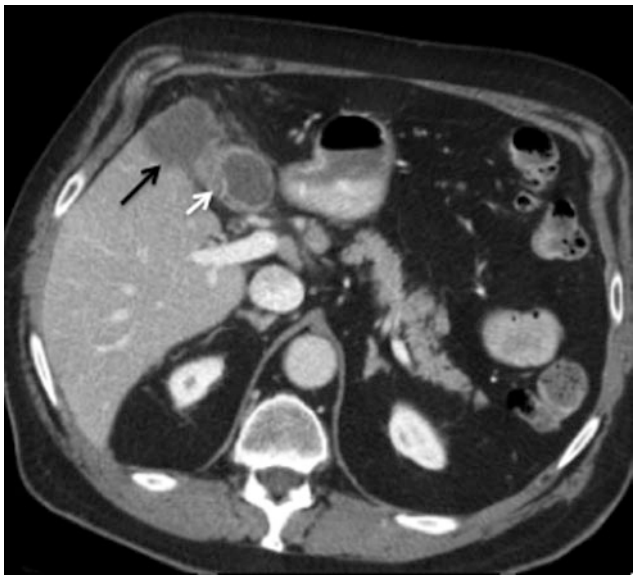

**Fig. 6** Invasive gallbladder cancer. Contrast-enhanced MSCT axial image showing irregular gallbladder wall thickening (*white arrow*), with more than 2 cm of invasion into segment V (*black arrow*) of the right liver lobe (T4 tumor). Tumor in the gallbladder fundus has propensity for early invasion of segments IVb and V

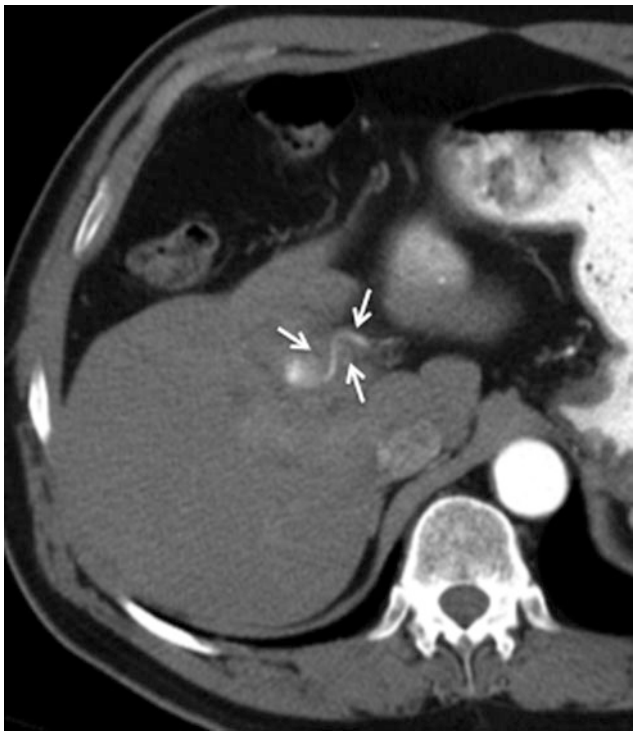

**Fig. 7** Vascular involvement of tumor. 68-year-old male with gallbladder cancer. Axial MSCT image in arterial phase shows soft tissue infiltration and encasement (*arrows*) of the hepatic artery at the hepatic hilum

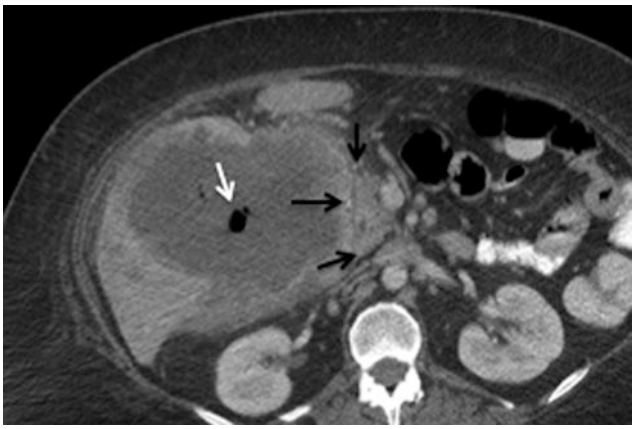

**Fig. 8** Adjacent organ invasion. 44-year-old female patient with gallbladder cancer. Contrast-enhanced MSCT axial image demonstrates a large mass invading the second portion of the duodenum (*black arrow*). It shows few air bubbles (*white arrow*) that correlate with fistulous communication

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

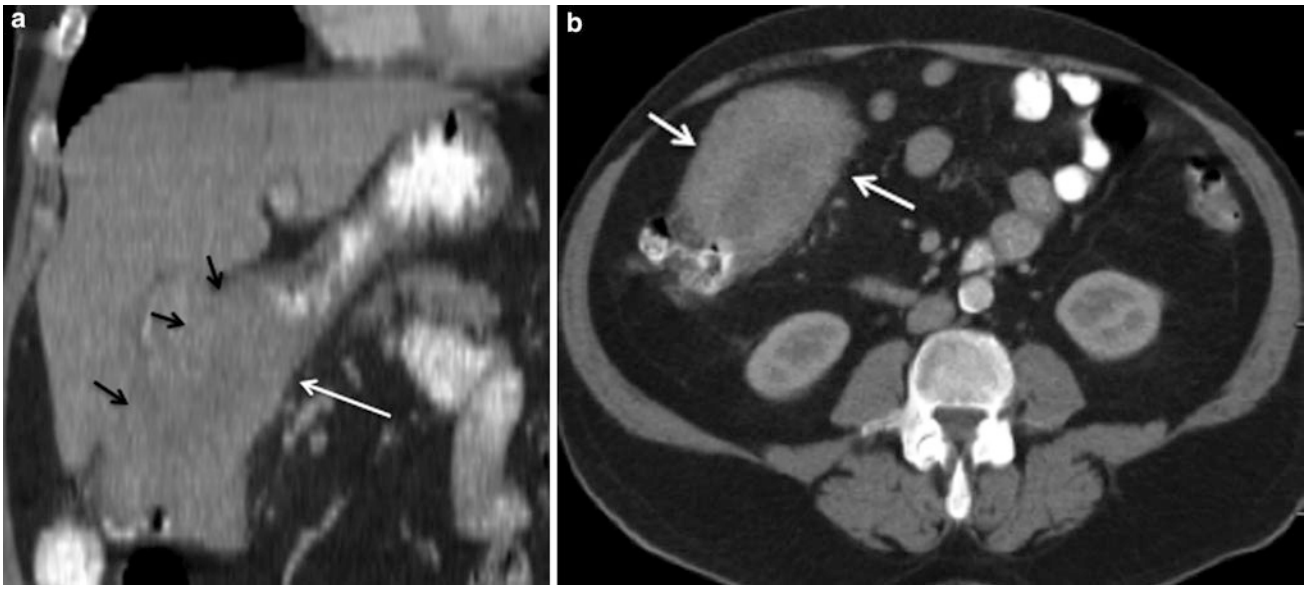


**Fig. 9** Adjacent organ invasion. 59-year-old female patient with advanced gallbladder cancer. **a** Contrast-enhanced MSCT coronal image demonstrates a large mass (*black arrows*) invading the hepatic flexure (*white arrow*). **b** Contrast-enhanced MSCT axial image shows irregular thickening and 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

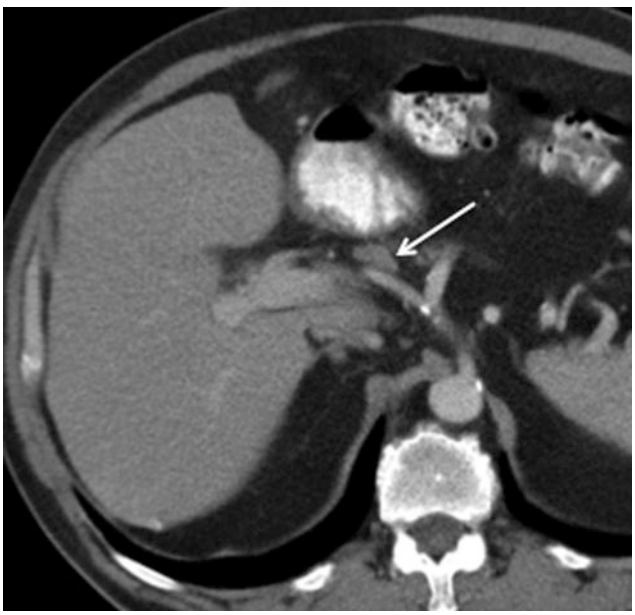

**Fig. 10** Lymphatic spread of gallbladder cancer. Contrast-enhanced MSCT axial image shows a lymph node in the hepatic artery nodal station (*arrow*), consistent with N1 node. Although this node does not exceed 1 cm in short axis diameter, because it is larger than the adjacent nodes and is located 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

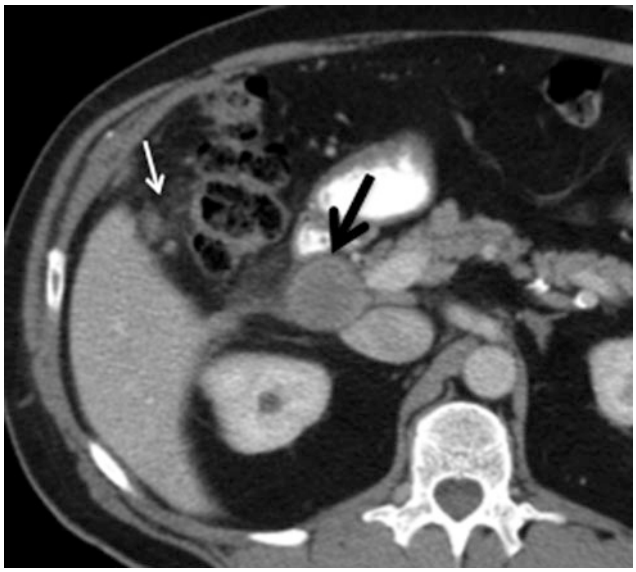

**Fig. 11** Lymphatic spread of gallbladder cancer. Contrast-enhanced MSCT axial image shows an enlarged lymph node in the portocaval nodal station (*black arrow*), consistent with N1 node. Nodes larger than 1 cm in short axis or with central low density (necrosis) are more likely metastatic. There is also nodularity and increased attenuation of the mesocolon adjacent to the hepatic flexure (*white arrow*), representing peritoneal infiltration

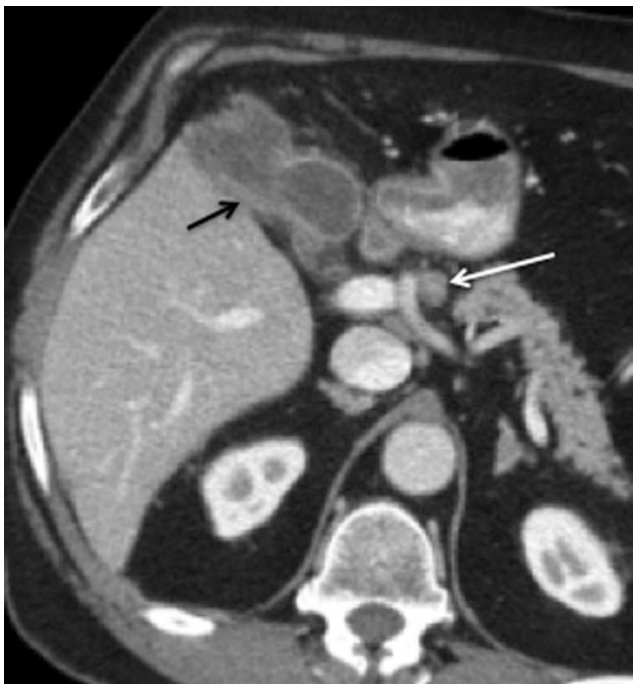

**Fig. 12** Lymphatic spread of gallbladder cancer. Axial MSCT contrast-enhanced image shows irregular thickening (*black arrow*) of the gallbladder wall, compatible with tumor. There is also an enlarged lymph node in the celiac nodal station (*white arrow*), consistent with a metastatic N2 node

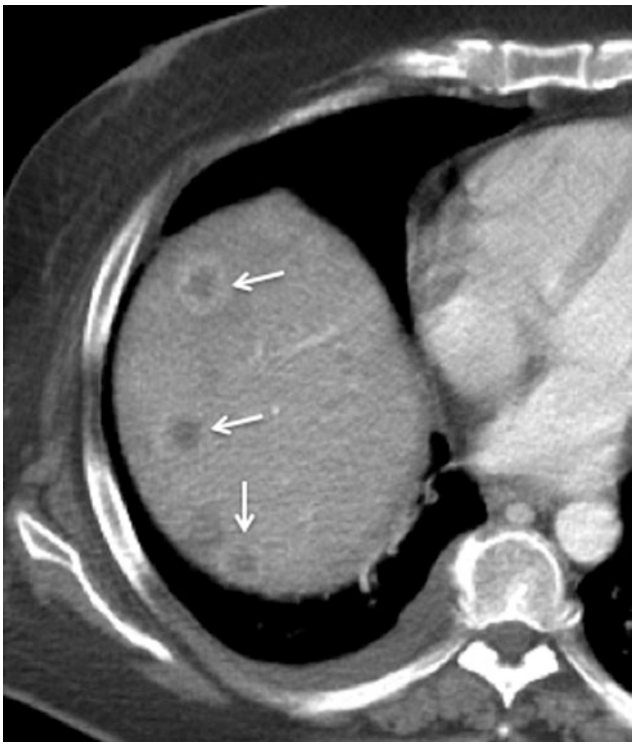

**Fig. 13** Hematogenous metastasis in gallbladder cancer; 44-year-old female with advanced gallbladder carcinoma. Contrast-enhanced MSCT axial image shows several masses with central low-attenuation and peripheral rim enhancement (*arrows*) in the right hepatic lobe, representing hematogenous metastases

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].

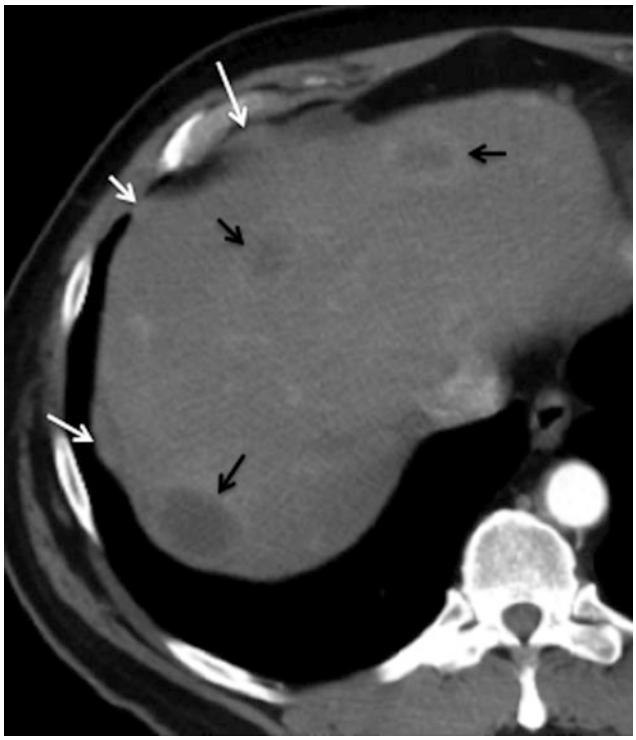

**Fig. 14** Peritoneal metastasis; 70-year-old male with advanced gallbladder carcinoma. Axial contrast-enhanced MSCT image shows few areas of nodular soft tissue thickening in the perihepatic sub diaphragmatic wall (*white arrows*), consistent with metastatic deposits. Multiple rim-enhancing liver lesions (*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

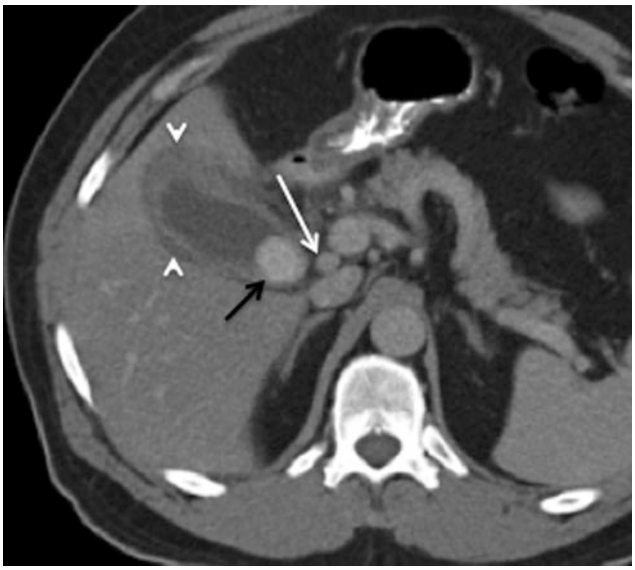

**Fig. 15** Cholelithiasis and gallbladder cancer; 65-year-old female with gallbladder carcinoma presenting with chronic right upper quadrant pain. Contrast-enhanced MSCT axial image shows a large intraluminal calcified stone (*black arrow*), compatible with cholelithiasis. Irregular thickening (>1 cm) 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

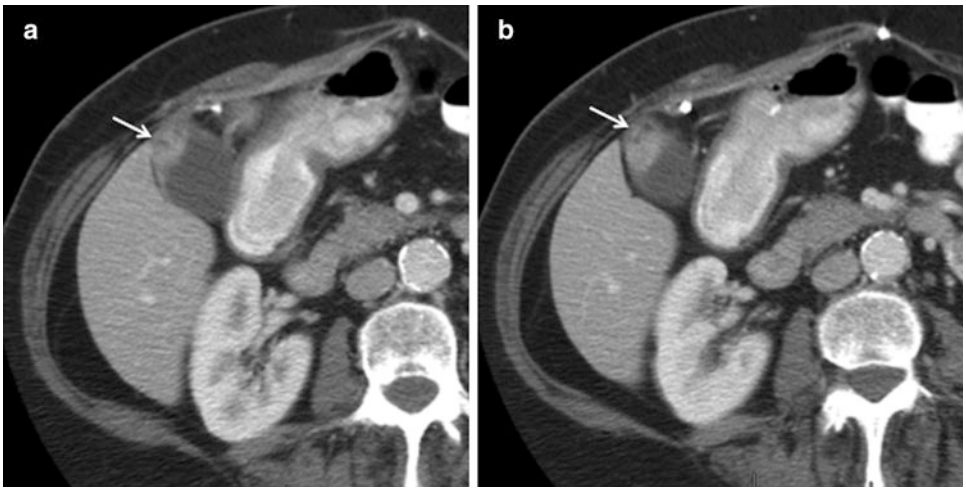


**Fig. 16** Gallbladder adenomyomatosis. Sequential contrast-enhanced axial MSCT images show focal thickening with cystic spaces in the gallbladder fundus (*arrows*). Note the thin homogeneous enhancement of the remaining gallbladder wall

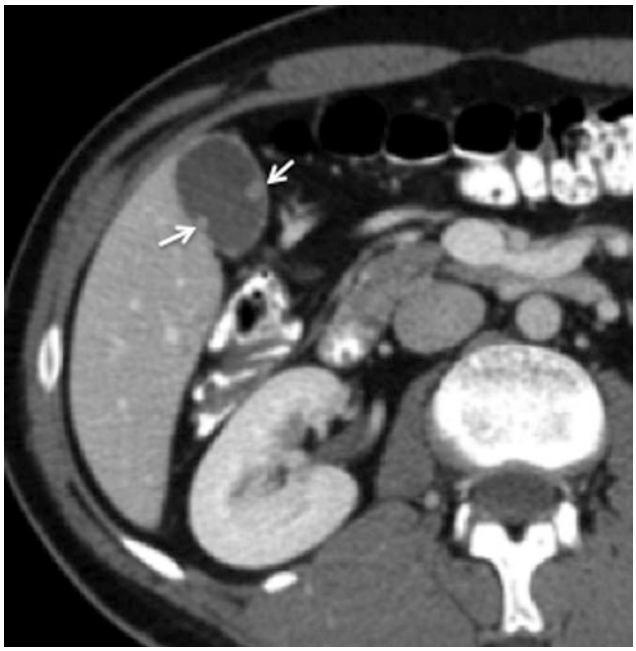

**Fig. 17** Gallbladder polyps. Axial contrast-enhanced MSCT image shows two subcentimeter nodular-enhancing lesions in the gallbladder wall (*arrows*), protruding into the lumen. Note the thin homogeneous enhancement of the remaining gallbladder wall

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

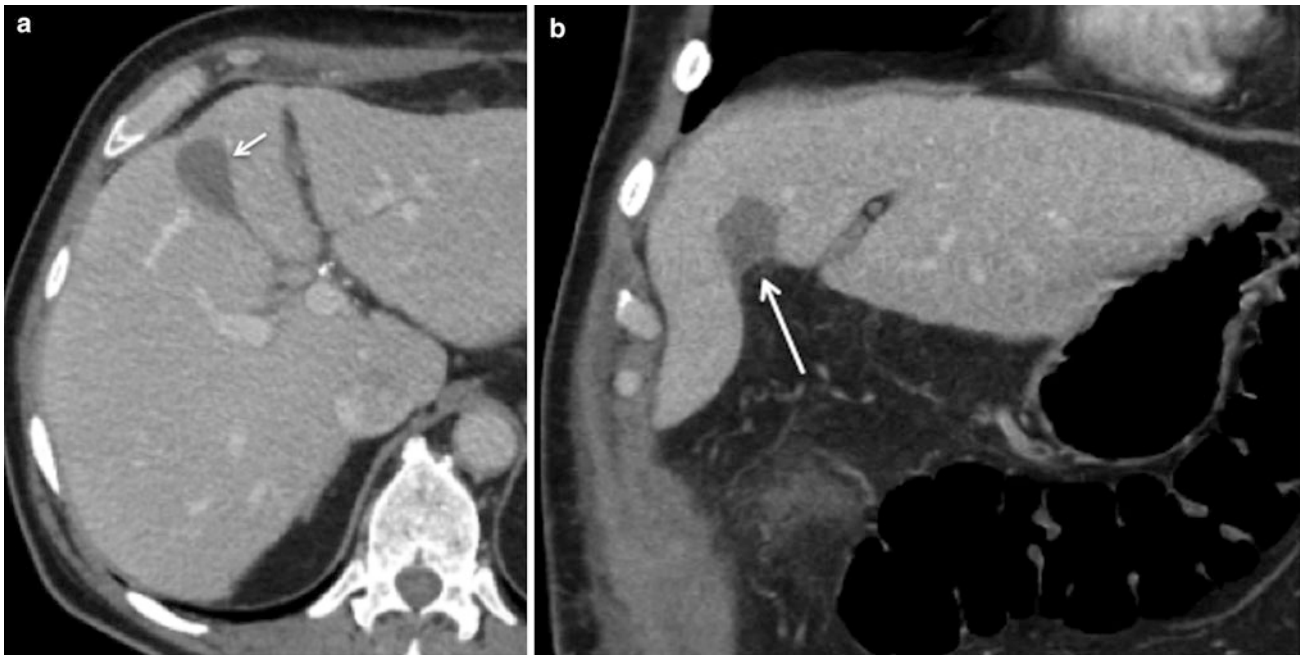


**Fig. 18** Postoperative seroma following surgery for gallbladder cancer. 68-year-old male's status postsurgery for gallbladder cancer on routine follow-up. **a** Axial and **b** coronal images from contrast-enhanced MSCT reveals a small fluid collection without peripheral enhancement in the surgical 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

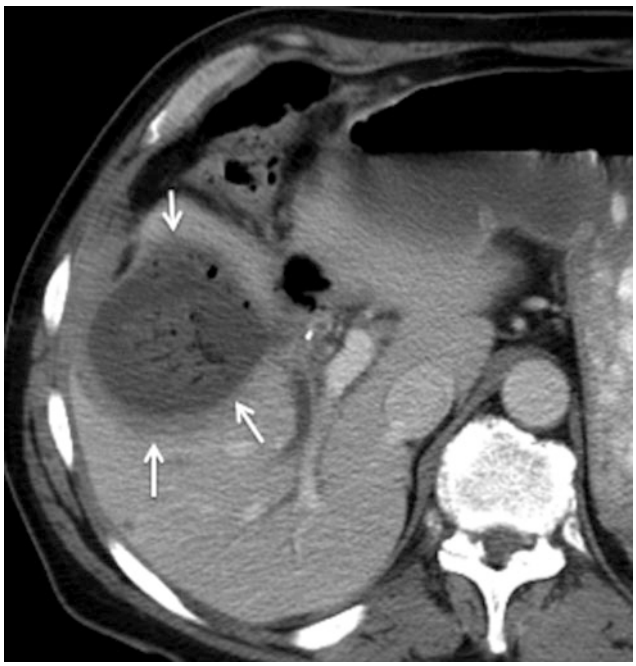

**Fig. 19** Postoperative abscess following surgery for gallbladder cancer. 67-year-old male's status postsurgery for gallbladder cancer with fever and leukocytosis. Axial contrast-enhanced MSCT image reveals a fluid collection with a thick rim of peripheral enhancement and internal gas bubbles in the 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].

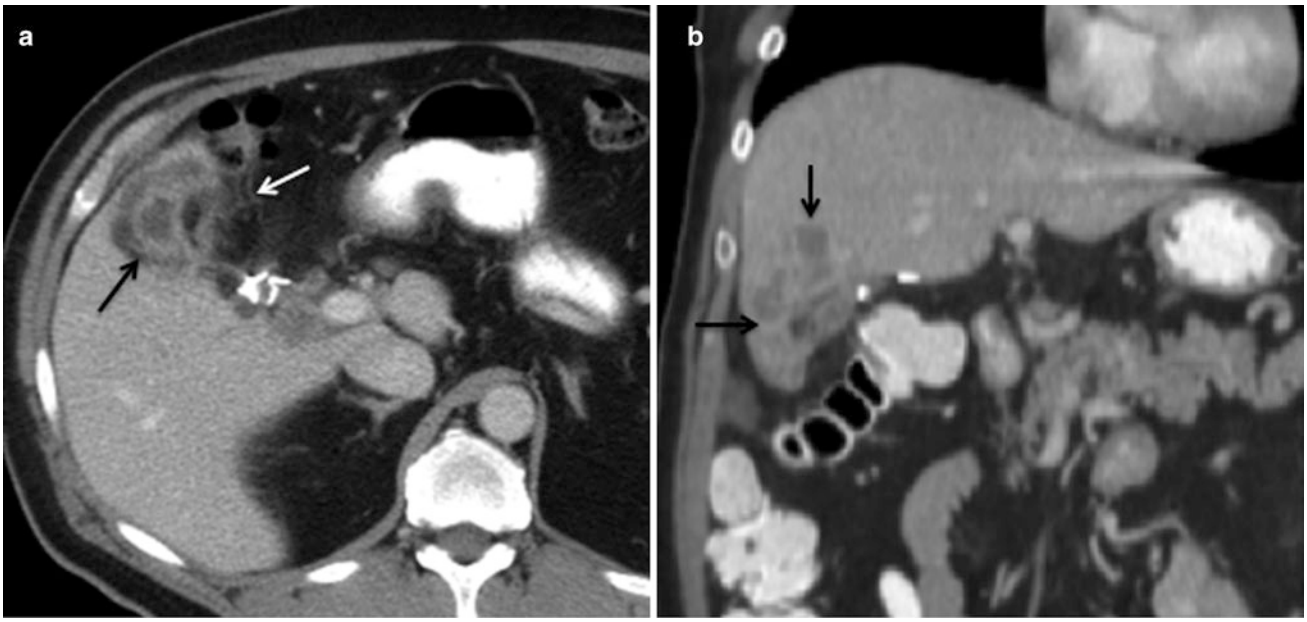


**Fig. 20** Recurrent disease after surgery for gallbladder cancer. **a** Axial contrast-enhanced MSCT image shows heterogeneous enhancement in the surgical bed (*black arrow*), with associated fat stranding and nodular thickening of the adjacent peritoneal fat (*white arrow*), consistent with tumor recurrence. **b** Coronal contrast-enhanced MSCT image reveals invasion of segment V at the resection margin of the liver (*black arrows*)

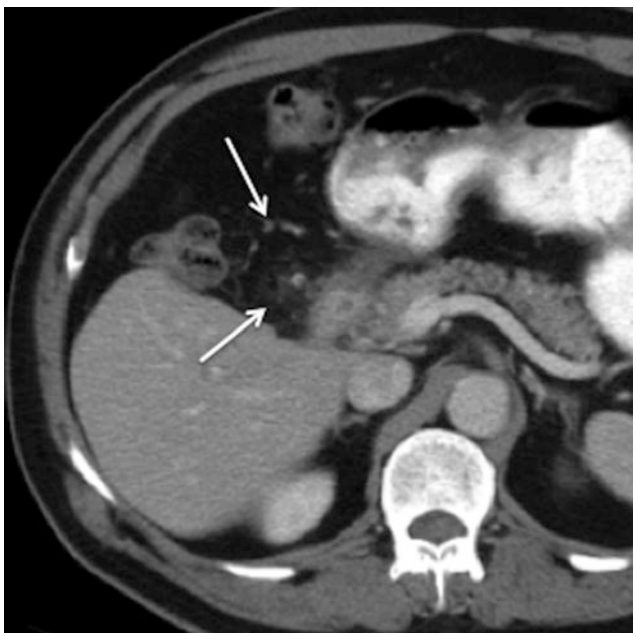

**Fig. 21** Recurrent gallbladder cancer; 68-year-old male with surgery for gallbladder cancer 1 year prior to the study. Axial contrast-enhanced MSCT image demonstrates subtle nodularity and increased fat attenuation near the gallbladder fossa and in the gastroepiploic region (*white arrow*), worrisome 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.

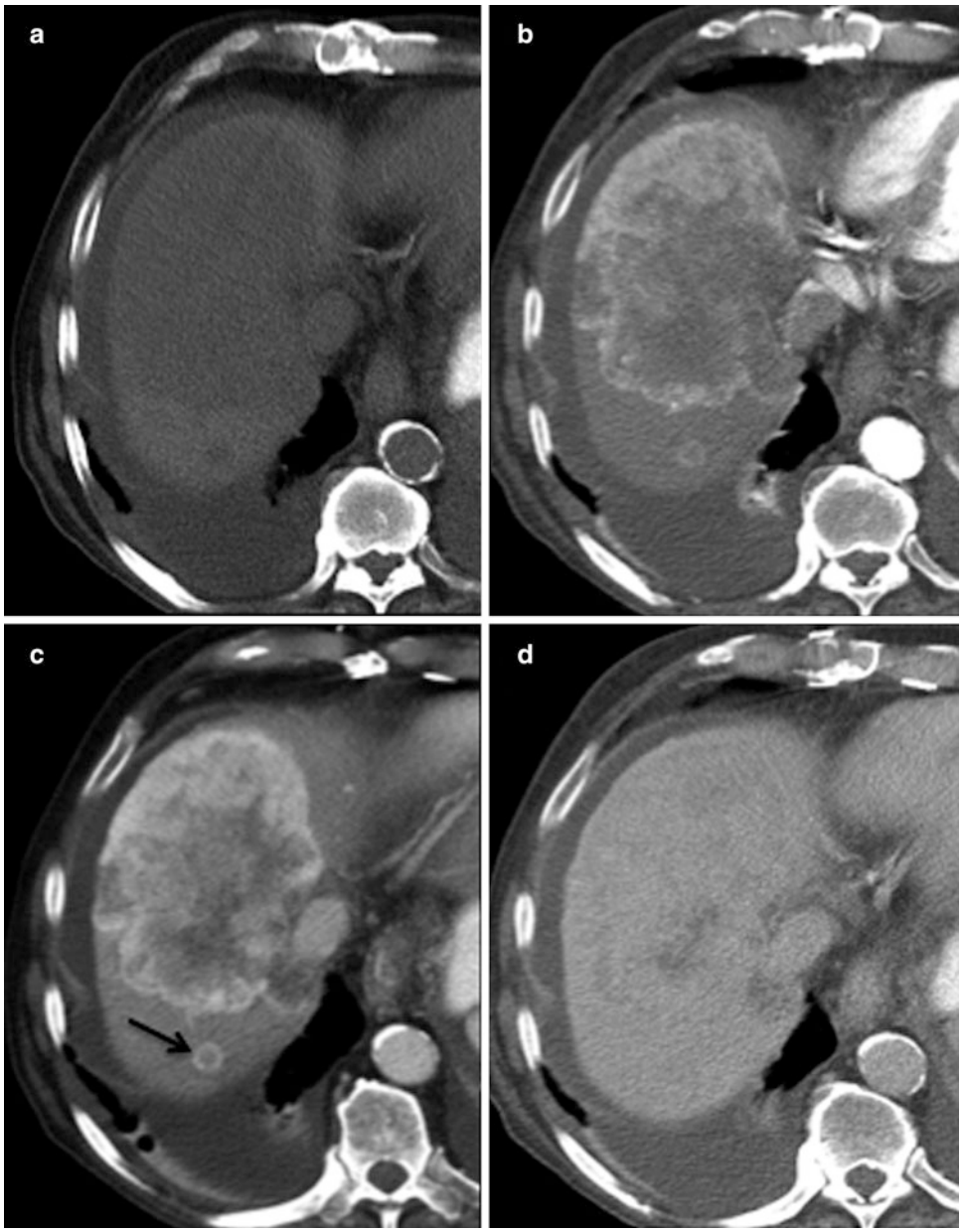


**Fig. 22** ICC enhancement pattern; 81-year-old male presenting with weight loss. **a** Pre-contrast MSCT image shows hypodense mass involving segments VII and VIII of the right liver lobe. **b** Postcontrast MSCT image in late arterial phase shows hypodense center with only mild peripheral enhancement. **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, hypoattenuating 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

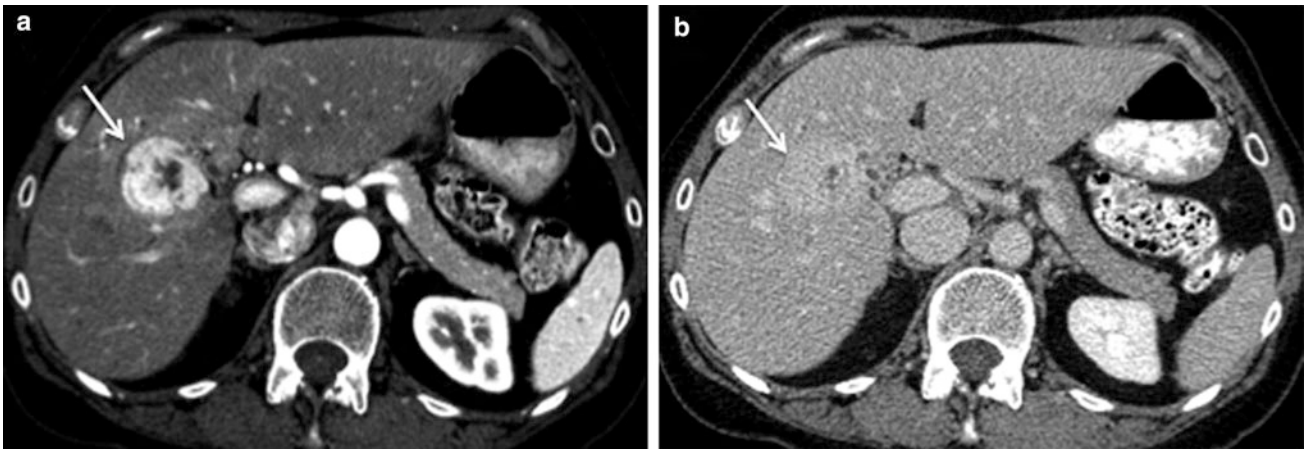


**Fig. 23** Enhancement pattern; 62-year-old female with ICC. **a** Postcontrast MSCT image shows mass in segment V of the right liver lobe (*arrow*) with avid contrast enhancement in late arterial phase. **b** Postcontrast MSCT image in delayed phase shows progressive contrast enhancement with almost complete filling (*arrow*) of the tumor. ICC may present with arterial enhancement similar to other hypervascular liver tumors

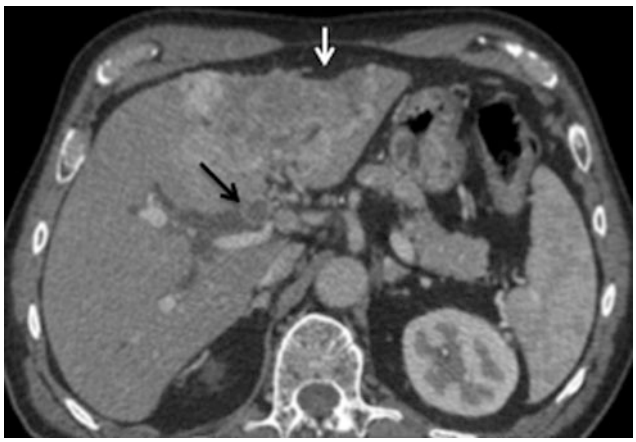

**Fig. 24** Capsular retraction; 72-year-old male presenting with weakness and anorexia. Axial portal venous phase MSCT image shows large, heterogeneous enhancing space-occupying lesion in segment II and III of the left liver lobe with associated capsular retraction (*white arrow*), due to desmoplastic 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

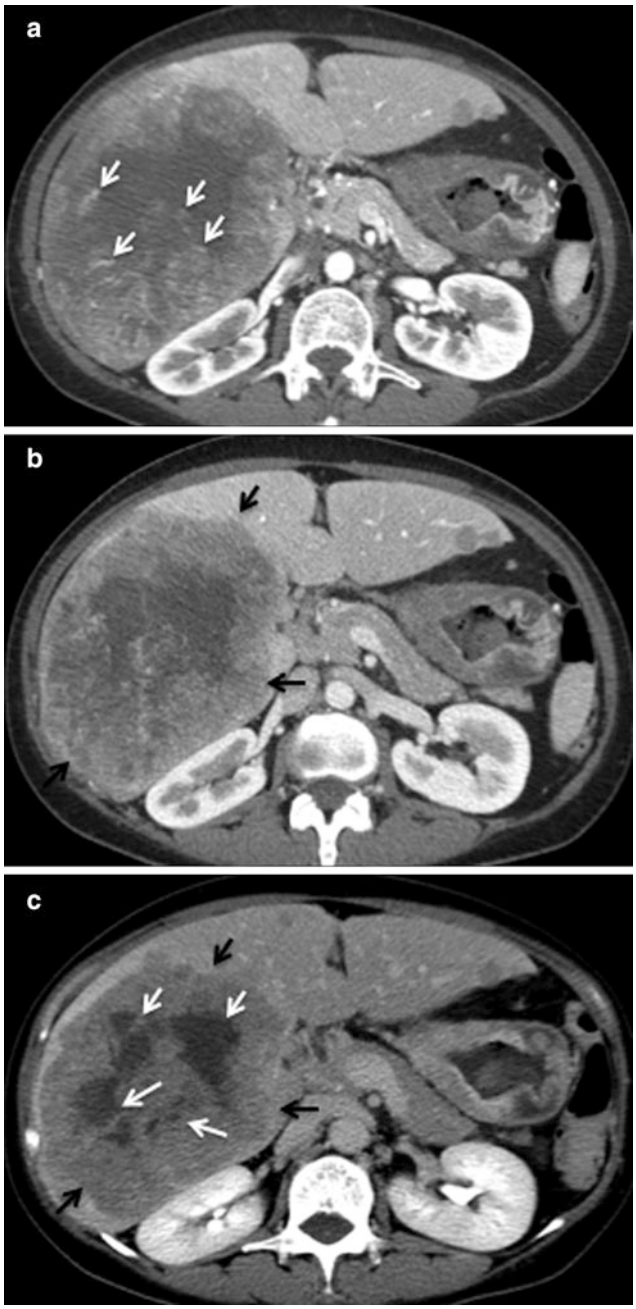


**Fig. 25** Enhancement pattern; 65-year-old male with cHCC-ICC. **a** Postcontrast MSCT image in late arterial phase shows few central focal areas of increased vascularity (*white arrows*) and minimal peripheral enhancement. **b** Postcontrast MSCT image in portal venous phase shows increased peripheral 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 cHCC-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

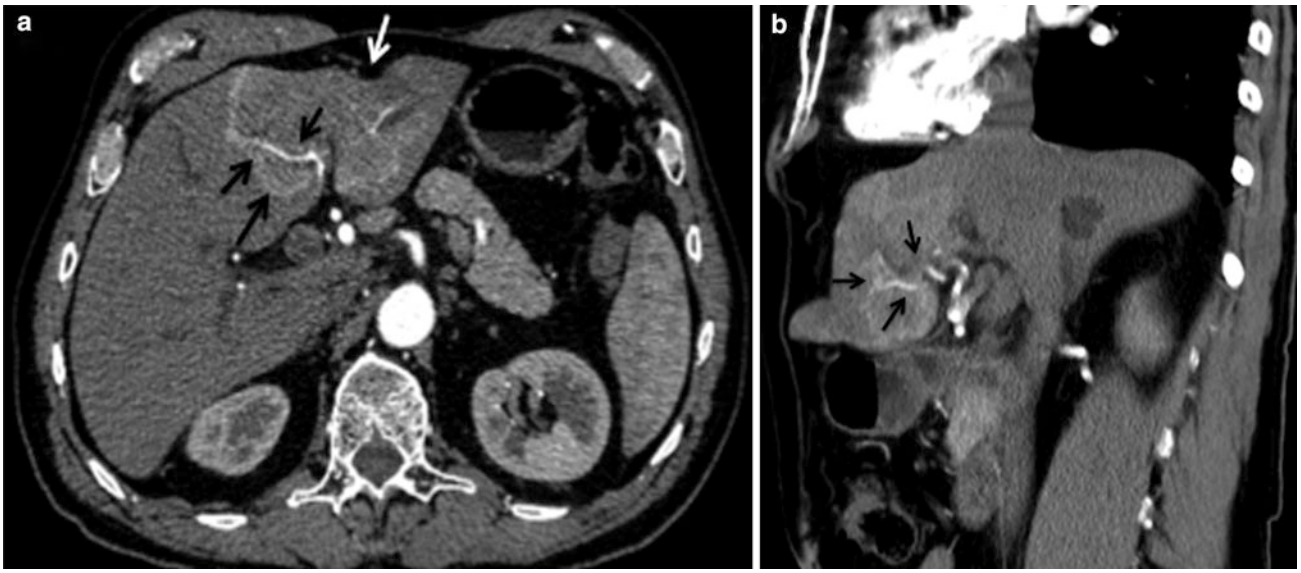


**Fig. 26** Hepatic artery involvement; 72-year-old male with weakness and anorexia. **a** Axial and **b** coronal MSCT images in late arterial phase show heterogeneous enhancing mass in the left liver lobe with encasement (*black arrow*) of the left hepatic artery. Capsular retraction (*white arrow*) is appreciated as well

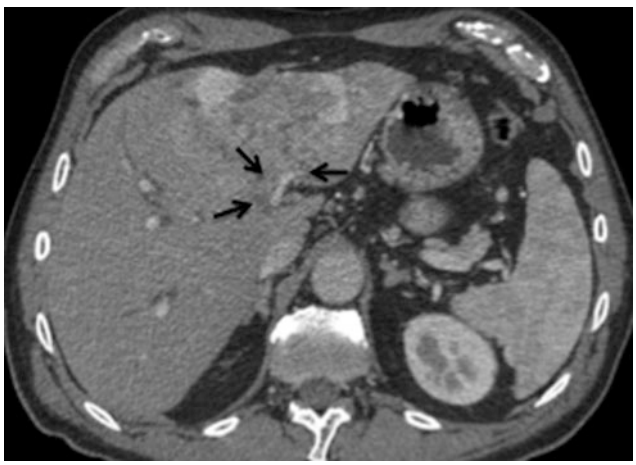

**Fig. 27** Vascular involvement; 72-year-old male with ICC. Axial MSCT image in portal venous phase shows heterogeneous mass with encasement of the left portal vein (*arrows*)

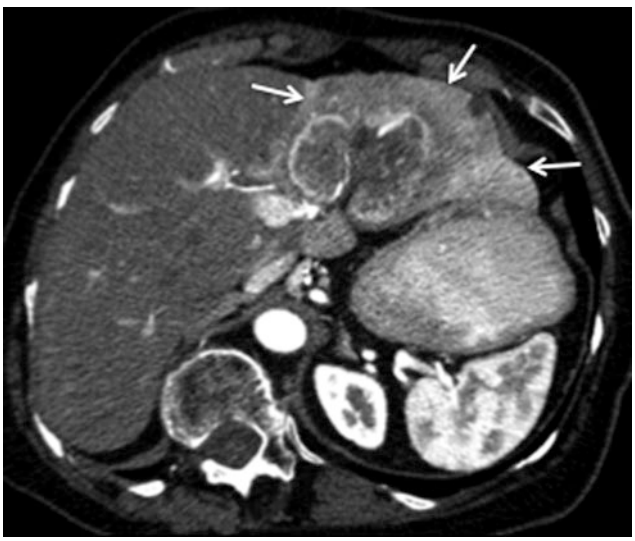

**Fig. 28** Transient asymmetric hepatic enhancement secondary to portal vein invasion; 79-year-old female presenting with chronic right upper quadrant pain. Late arterial phase axial MSCT image demonstrates large mass with enhancement of the surrounding liver parenchyma of the left liver lobe (*arrows*), representing preferential arterial flow secondary to portal vein invasion

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).

## 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

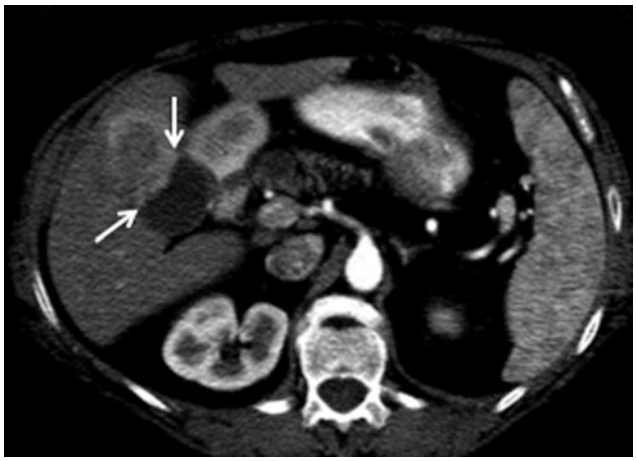

**Fig. 29** Adjacent organ involvement; 62-year-old female with ICC. Axial MSCT image in late arterial phase shows heterogeneous mass in segment V of the left liver lobe (*arrows*) invading the gallbladder by direct extension

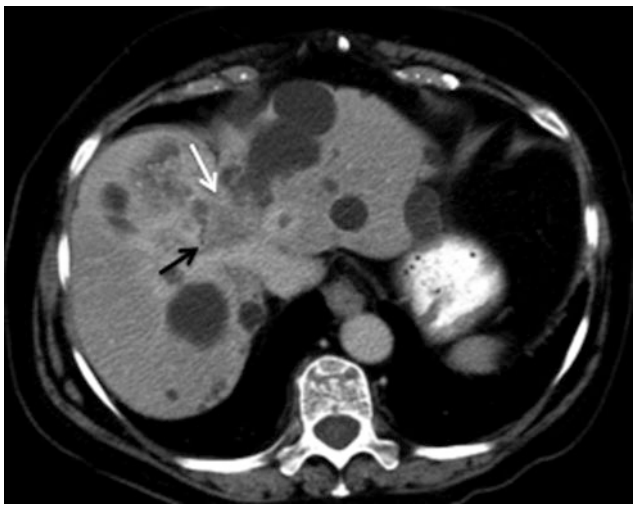

**Fig. 30** Invasive ICC; 83-year-old female with polycystic kidney disease and ICC. Axial MSCT image in delayed phase shows heterogeneous mass in segments VIII and IVA of the liver with encasement of the mid-hepatic vein (*white arrow*). Bile duct invasion is also appreciated with mild biliary ductal 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,

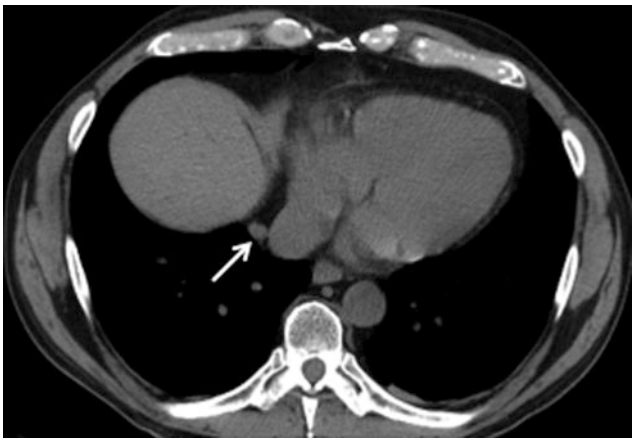

**Fig. 31** Lymphatic spread of ICC. 61-year-old male with ICC in the left hepatic lobe. Noncontrast MSCT axial image shows a mildly enlarged lymph node in the middle diaphragmatic nodal station (*arrow*), consistent with N1 node. The node is enlarged when compared to prior exam, favoring neoplastic involvement

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

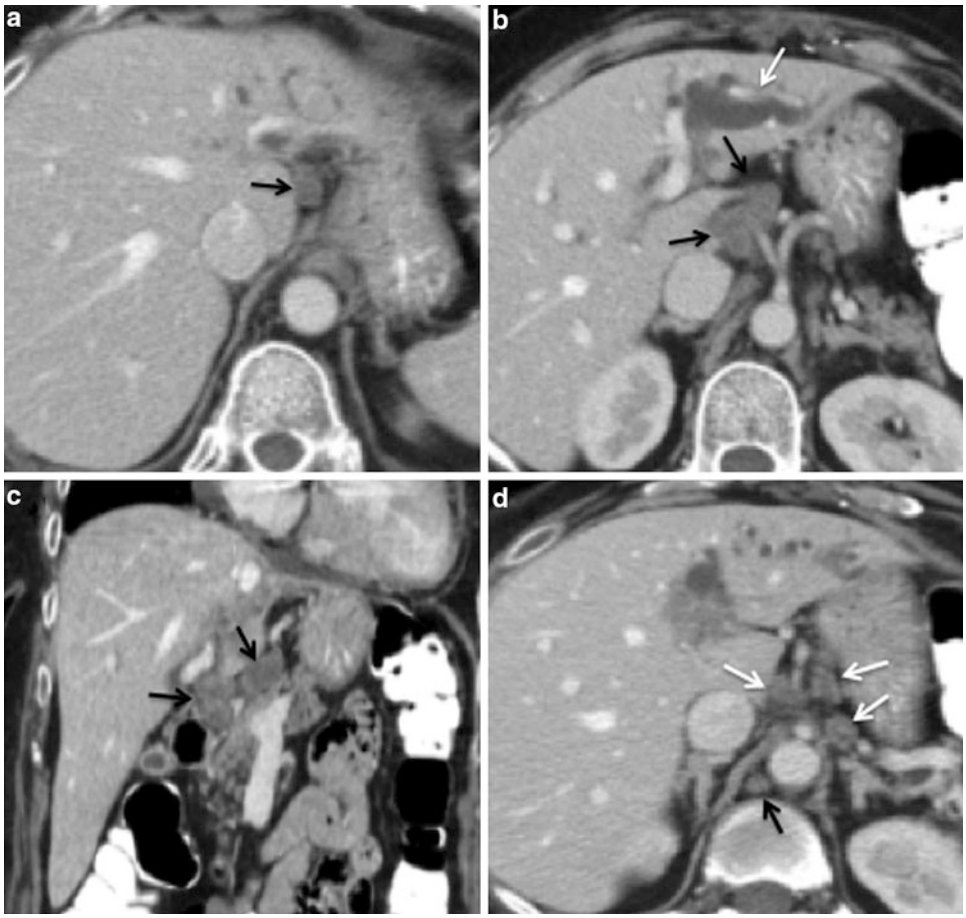


**Fig. 32** Lymphatic spread of ICC; 60-year-old female with left ICC. **a** Contrast-enhanced MSCT axial image shows an enlarged hypodense lymph node in the gastrohepatic nodal station (*arrow*), consistent with N1 node. **b** Contrast-enhanced MSCT axial image shows few enlarged hypodense lymph nodes 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

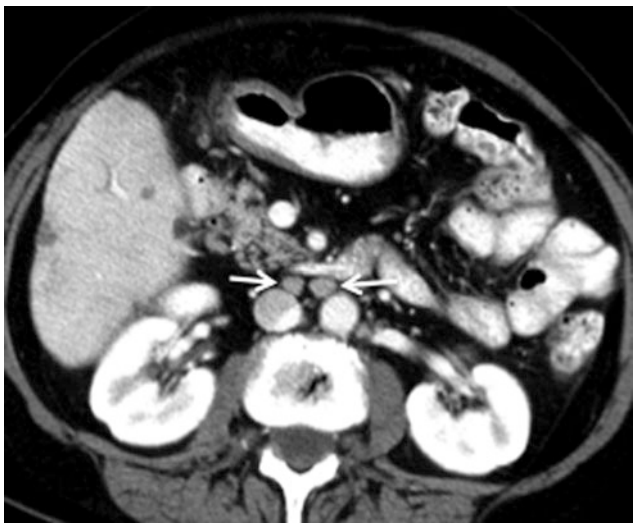

**Fig. 33** Lymphatic spread of ICC. Contrast-enhanced MSCT axial image shows two enlarged lymph nodes in the periaortic and pre-caval retroperitoneal nodal stations (*arrows*), consistent with N2 nodes. Few metastatic lesions identified in the right liver lobe as well

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].

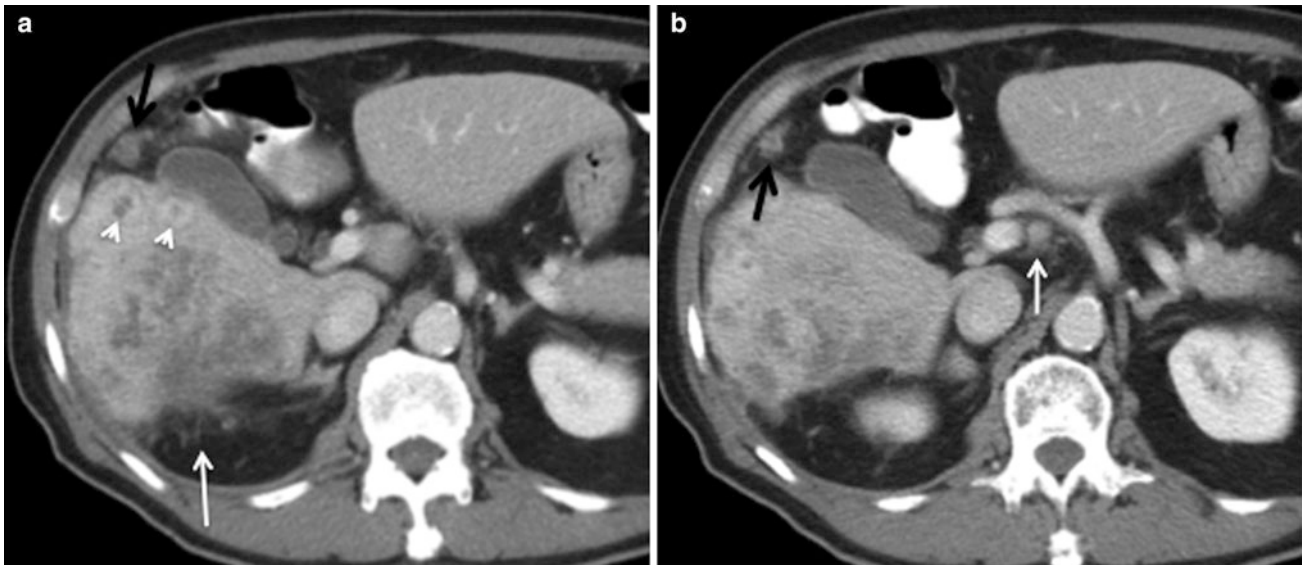


**Fig. 34** Advanced ICC. 73-year-old male patient with ICC. **a** Axial MSCT contrast-enhanced image shows a large heterogeneous mass in the posterior right liver lobe with invasion and extension through the liver capsule (*white arrow*). Few satellite hypoattenuating peripheral enhancing nodules (*arrowheads*) 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*)

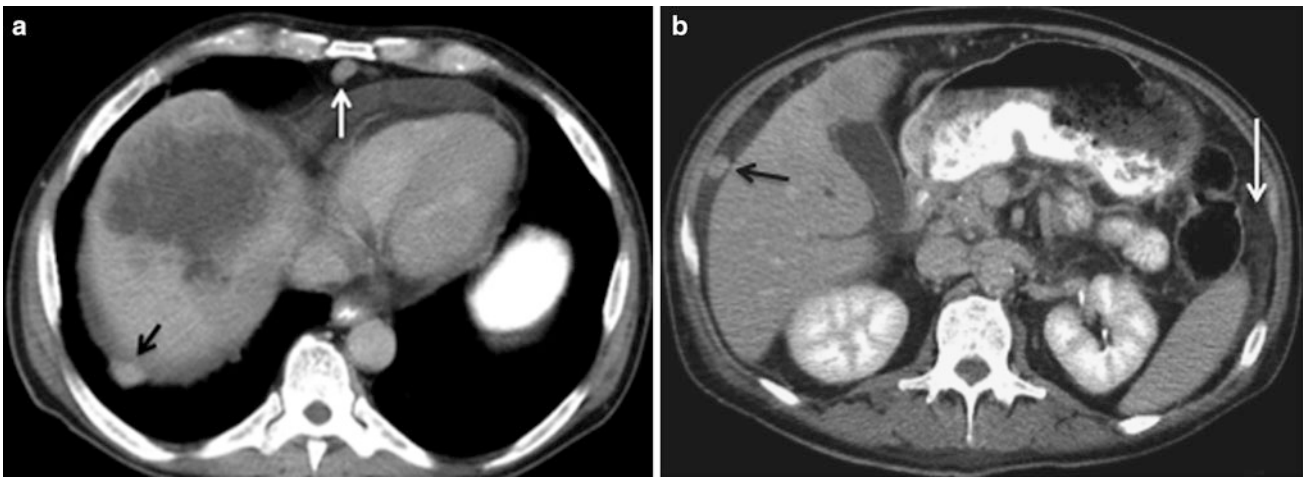


**Fig. 35** Peritoneal carcinomatosis from ICC. **a** Axial MSCT contrast-enhanced image shows a large heterogeneous mass with central necrosis in the liver. Perihepatic metastatic implant seen (*black arrow*). Anterior diaphragmatic node also appreciated (*white arrow*). **b** Nodular perihepatic soft 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

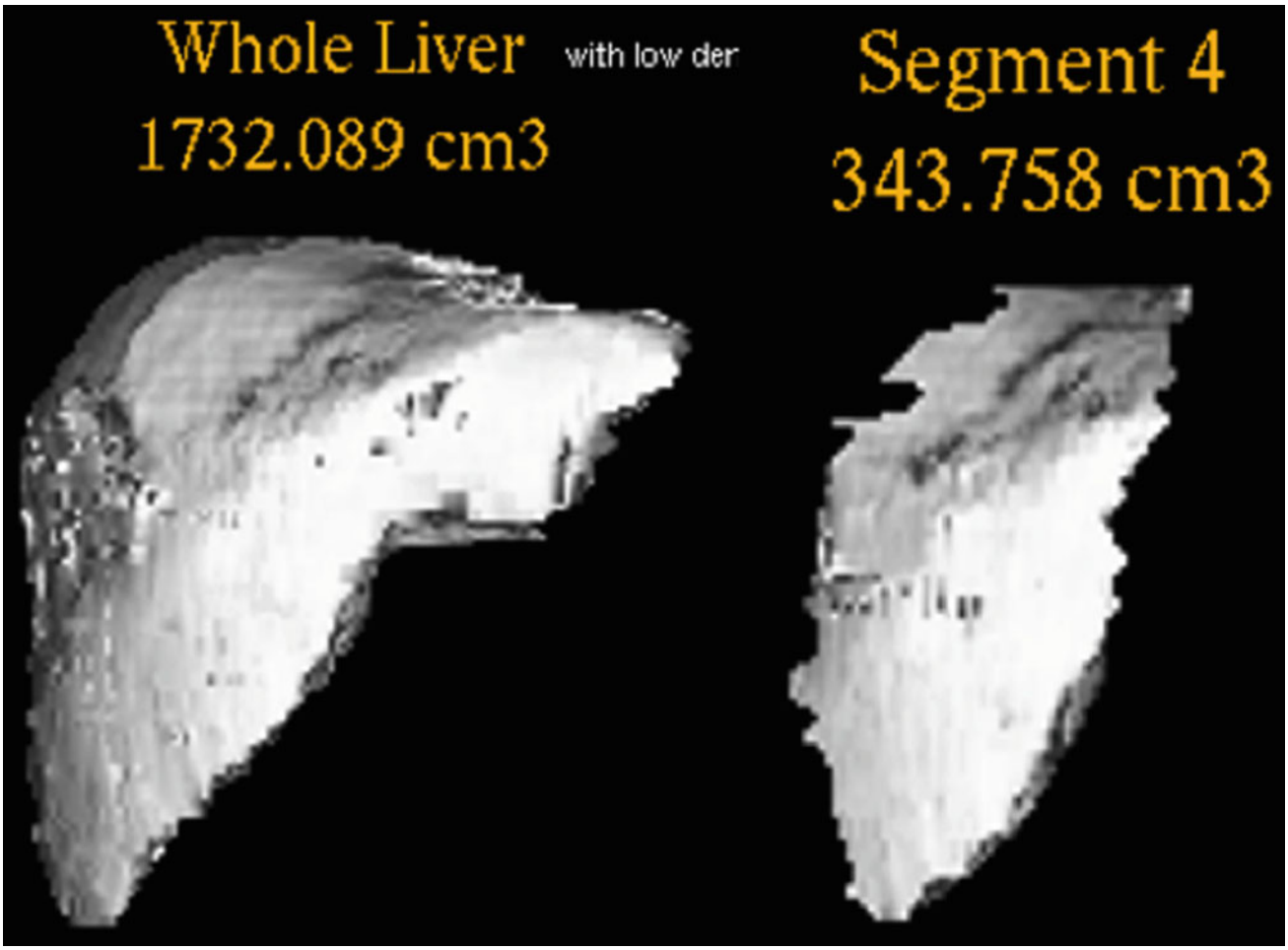


**Fig. 36** Liver volume calculation; 45-year-old male patient with cholangiocarcinoma. Multiple volume-rendered images of the whole liver and residual liver segment; whole liver volume of approximately 1,732 cc and segment IV volume of approximately 344 cc

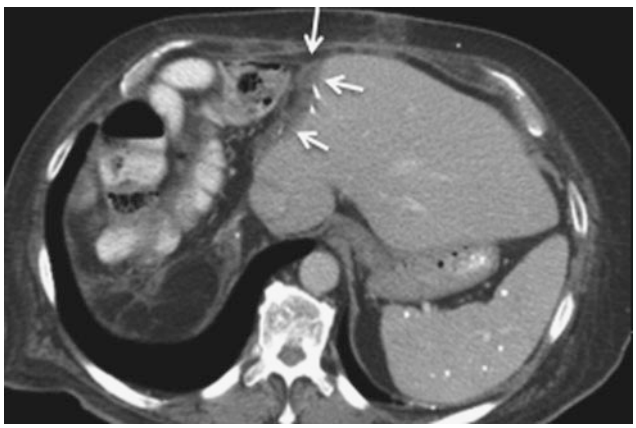

**Fig. 37** Postoperative seroma following extended right hepatectomy for ICC; Axial contrast-enhanced MSCT image reveals a small fluid collection without peripheral enhancement in the surgical bed (*arrows*), compatible with seroma

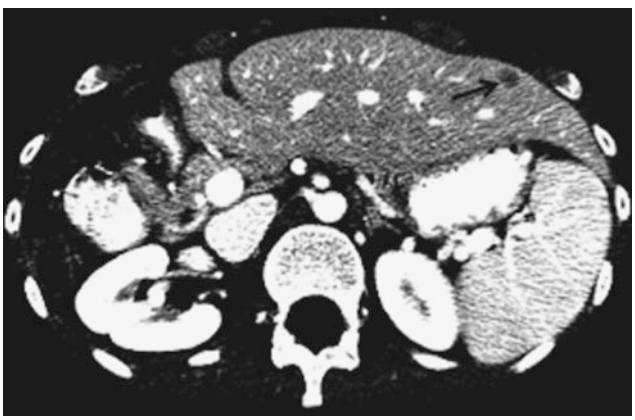

**Fig. 38** Recurrent tumor; 29-year-old female with ICC status post-right hepatectomy. MSCT contrast-enhanced image shows a small hypoattenuating rim-enhancing lesion in segment III of left liver lobe (*arrow*), in keeping with recurrent disease

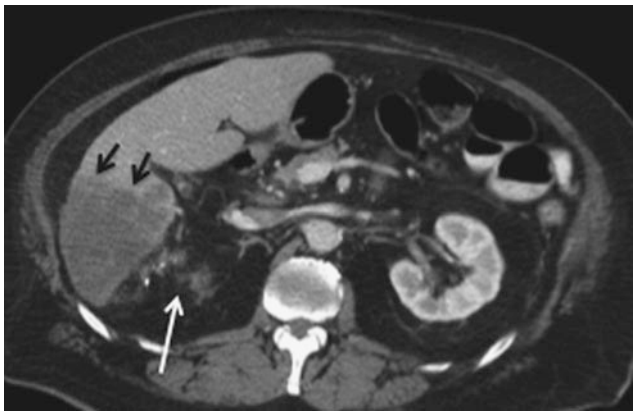

**Fig. 39** Postchemotherapy changes; 66-year-old male with ICC after 1 year of chemoradiation. Axial contrast-enhanced MSCT image shows a sharply demarcated low-attenuation region in segment VI of right liver lobe (*black arrows*) without significant enhancement, correlating with chemoradiation changes. 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

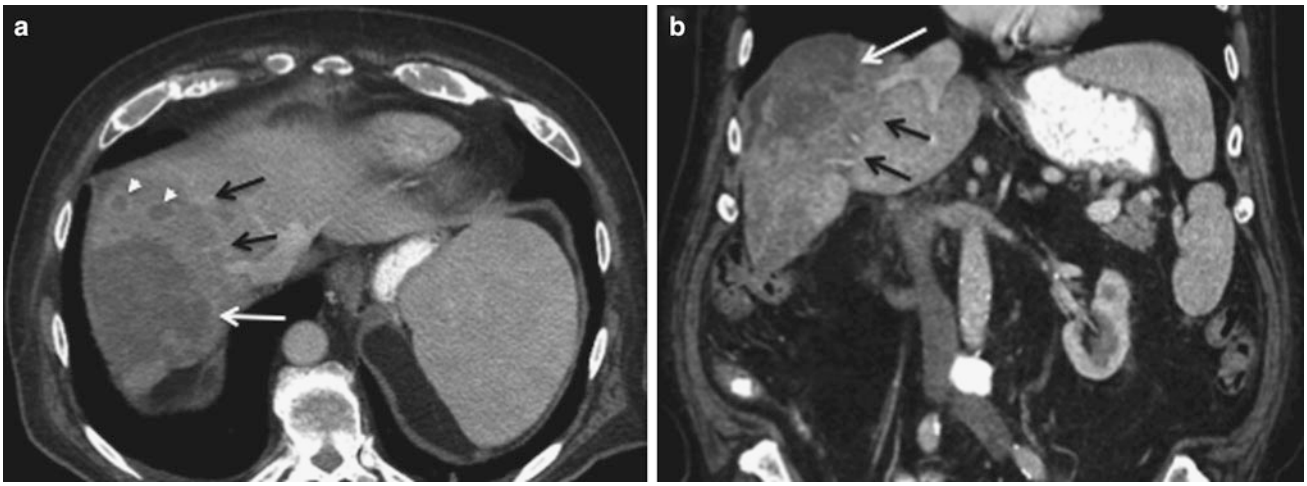


**Fig. 40** Radiotherapy changes; 77-year-old male with right liver lobe ICC with satellite nodules after 8 months of radiation. **a** Axial and **b** coronal contrast-enhanced MSCT images show a sharply demarcated low-attenuation area in the right liver lobe (*black arrows*), correlating with edema 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

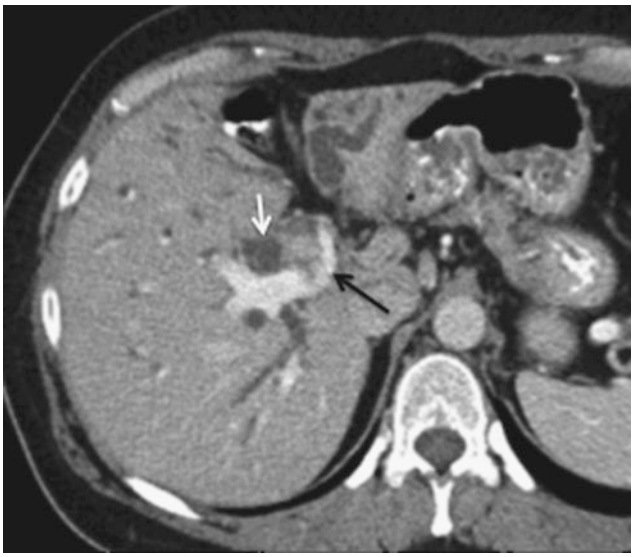

**Fig. 41** T4 hilar cholangiocarcinoma; vascular and biliary involvement. Axial MSCT image in portal venous phase shows heterogeneous mass in the hepatic hilum with encasement and invasion of the portal vein (*black arrow*). Bile duct invasion is also appreciated with associated biliary ductal dilation (*white arrow*)

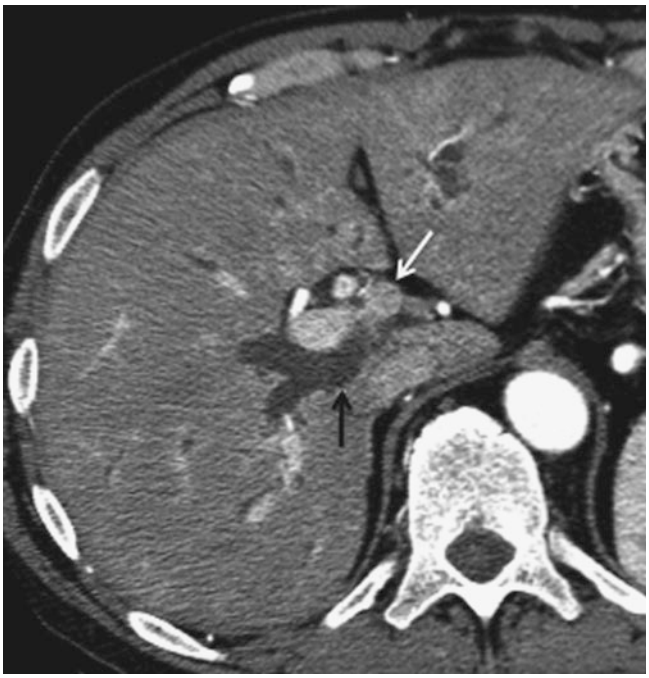

**Fig. 42** Hilar ECC Type I. Axial MSCT image in late arterial phase shows heterogeneous mass in the common hepatic duct within 1 cm of the bifurcation (*white arrow*). There is associated biliary ductal dilation; bile duct to segments VII and VIII, coursing posterior to the portal vein (*black arrow*), anatomical variant

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.

## 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

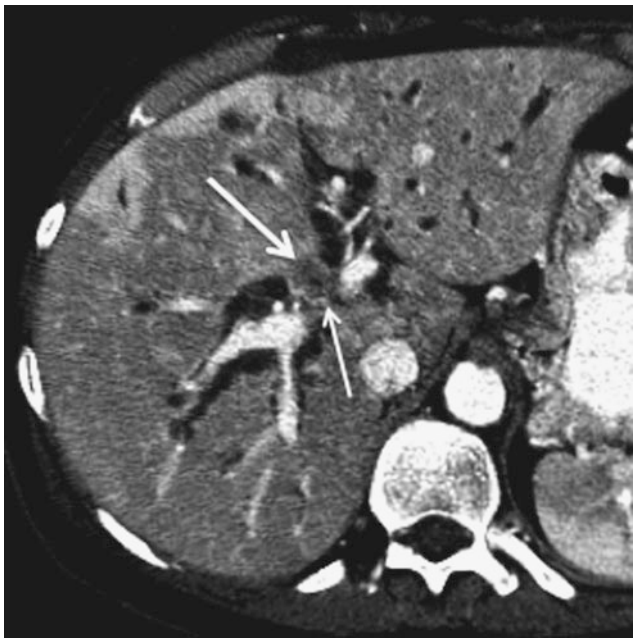

**Fig. 43** Hilar ECC Type IV. Axial MSCT image in late arterial phase shows heterogeneous mass involving both the right and left hepatic ducts (*arrows*). There is associated bilateral intrahepatic biliary ductal dilation

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 cm) 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.

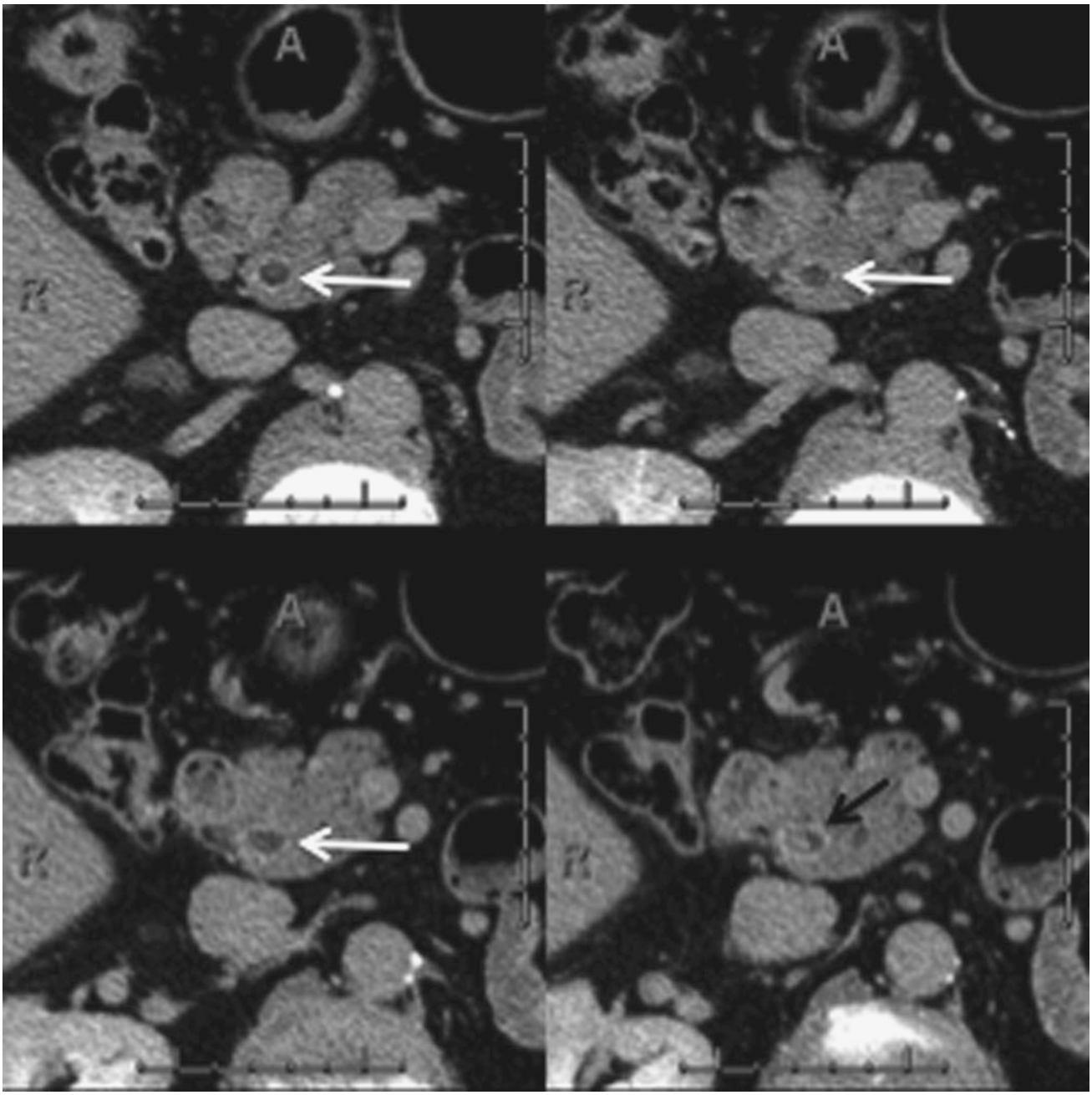


**Fig. 44** Periductal-infiltrating ECC. Sequential contrast-enhanced axial MSCT images in delayed phase of a 76-year-old male show circumferential thickening and enhancement of the distal common bile duct (*white arrow*). This is always an abnormal finding and should raise concerns for tumor involvement 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-infiltratingc (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

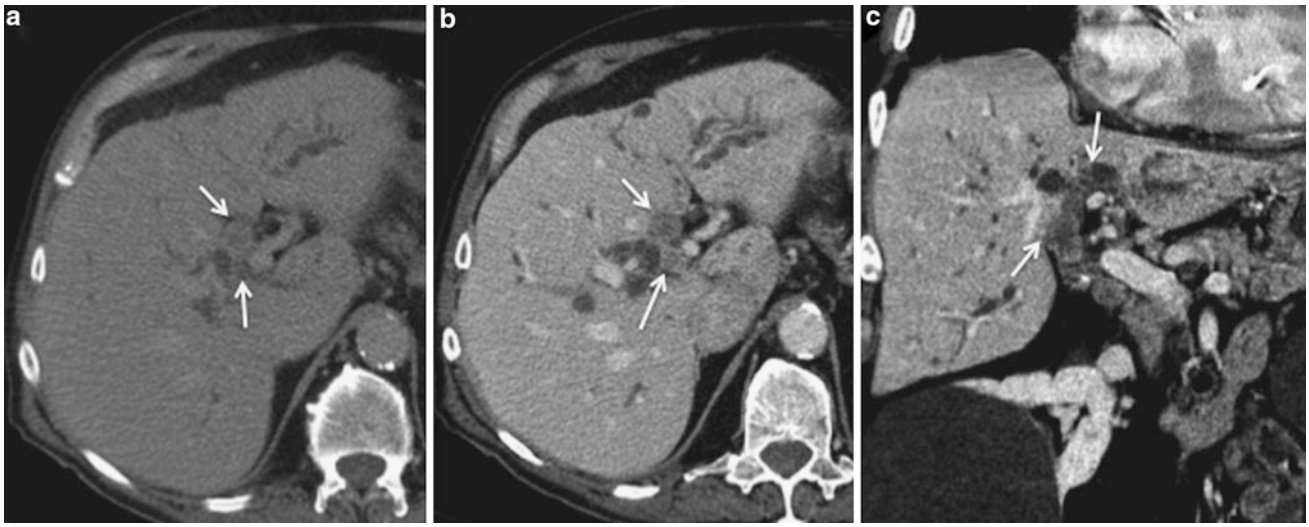


**Fig. 45** Mass-forming hilar cholangiocarcinoma. 80-year-old male with jaundice and anorexia. **a** Axial noncontrast, and contrast-enhanced **b** axial and **c** coronal MSCT images show a soft tissue mass involving both the right and left hepatic ducts (*arrows*), compatible with type IV hilar cholangiocarcinoma. There is associated bilateral intrahepatic biliary ductal dilation

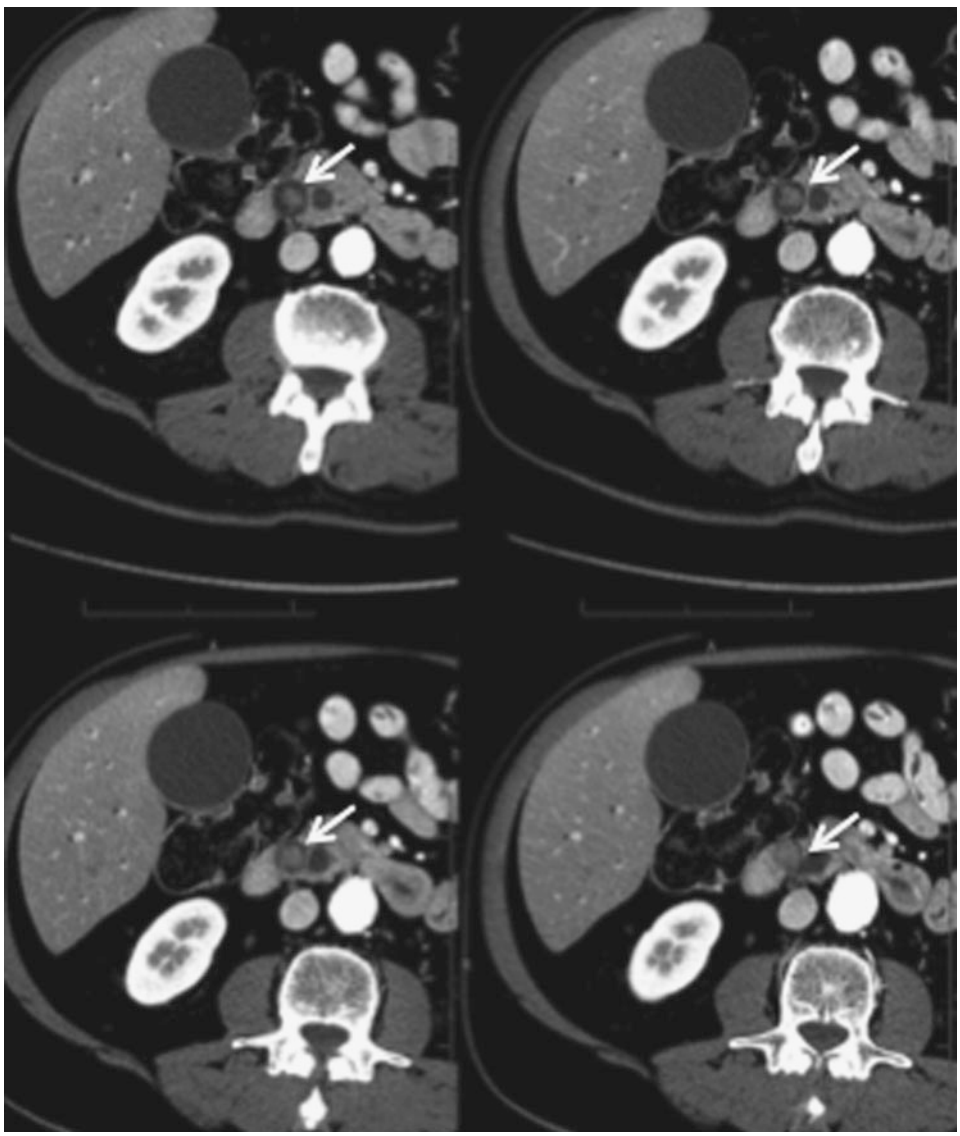

**Fig. 46** Intraductal ECC. Sequential contrast-enhanced axial MSCT images show intraluminal enhancing soft tissue density partially filling the lumen of the CBD (*arrows*)

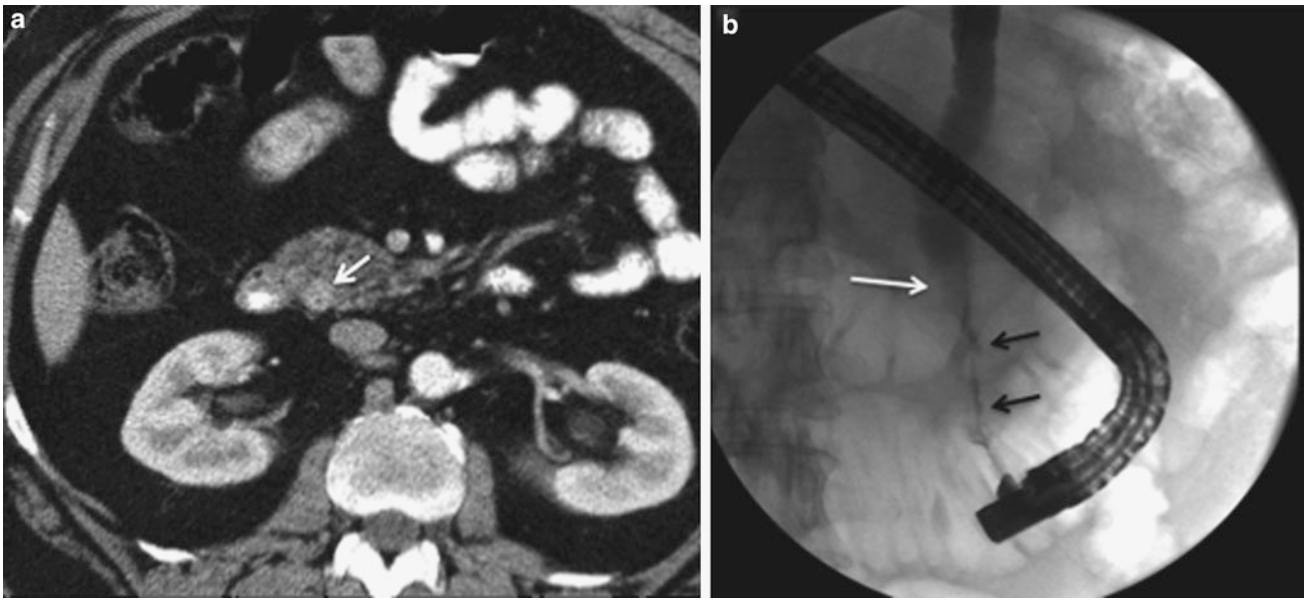


**Fig. 47** Periductal-infiltrating ECC. 55-year-old male with jaundice. **a** Contrast-enhanced axial MSCT image shows annular thickening and enhancement (*arrow*) of the distal CBD, consistent with cholangiocarcinoma. **b** Spot fluoroscopic image of an ERCP shows bile duct dilation with abrupt change 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.

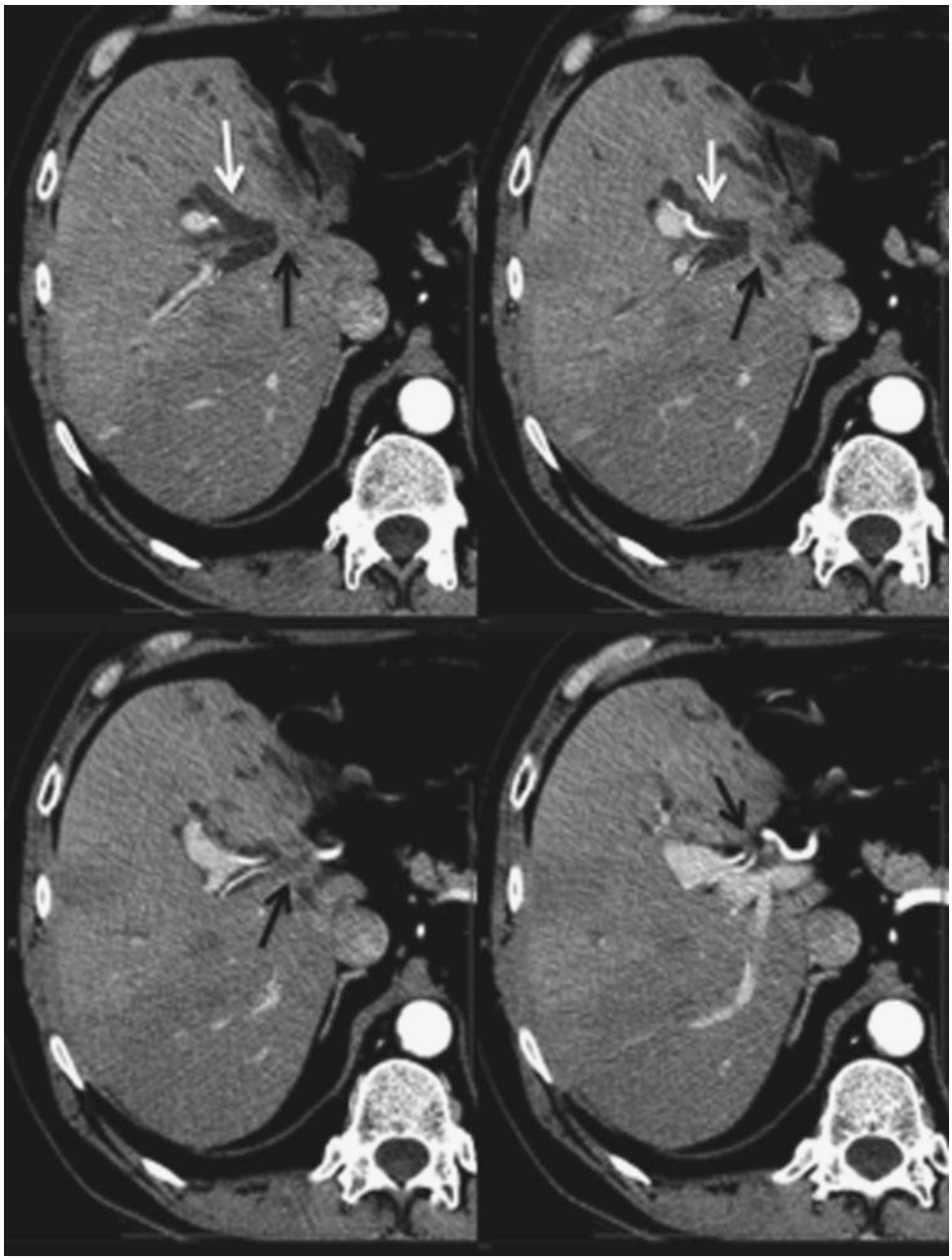

**Fig. 48** Water and soft tissue attenuation in bile duct with cholangiocarcinoma. Sequential contrast-enhanced axial MSCT of a 63-year-old male shows dilation of bile ducts (*white arrows*). Note abrupt transition from fluid to soft tissue attenuation in the right hilar fissure extending to the main 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 or 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 hemangiomata, can generally be confidently

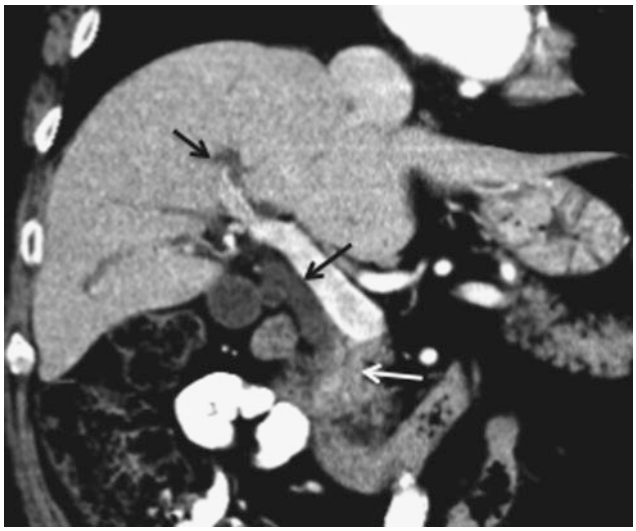

**Fig. 49** Periductal-infiltrating cholangiocarcinoma. Coronal contrast-enhanced MSCT image in portal venous phase shows luminal narrowing, circumferential thickening and enhancement of the distal common bile duct (*white arrow*). There is associated proximal biliary ductal dilation (*black arrow*). This type of tumor infiltrates the periductal soft tissues and causes a desmoplastic response that results in significant enhancement

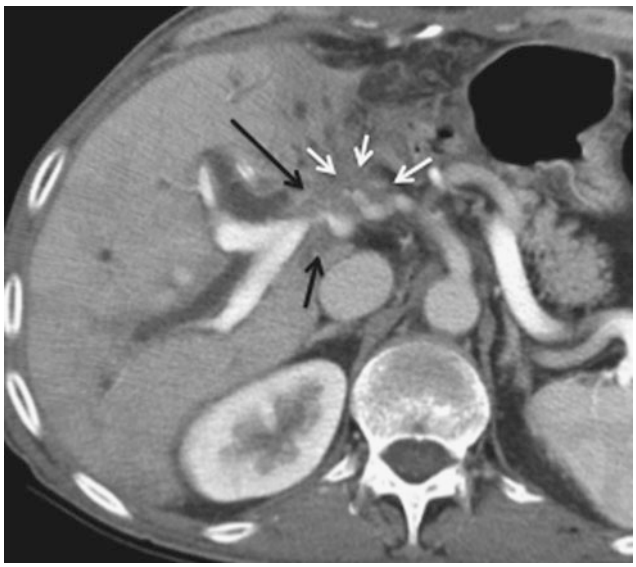

**Fig. 50** Periarterial perineural spread in cholangiocarcinoma. Axial MSCT image in portal venous phase shows infiltrating soft tissue encasing the hepatic artery (*white arrows*) in the hilar fissure. Note atrophy of the left liver lobe. There is also encasement and invasion of the main portal vein (*black arrow*). Periarterial perineural spread of tumor may cause angulation, narrowing or occlusion depending on the degree of invasion into the adventitia

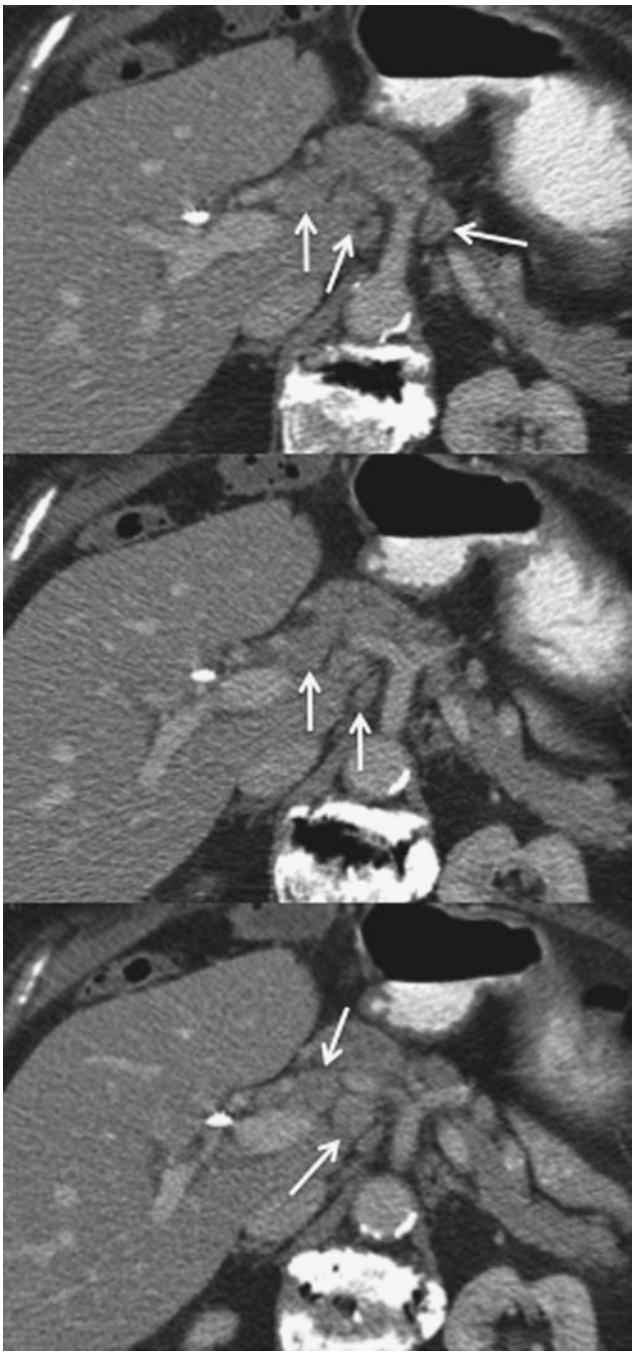

**Fig. 51** Lymphangitic spread in ECC. Sequential axial contrast-enhanced images of a patient with cholangiocarcinoma show multiple enlarged lymph nodes along the common hepatic, periportal (*black arrows*), and celiac nodal stations (*white arrows*), consistent with N1 and N2 nodes, respectively

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 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

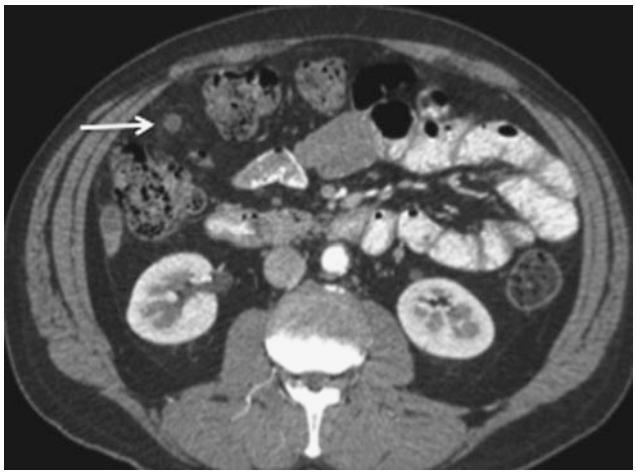

**Fig. 52** Peritoneal carcinomatosis; 50-year-old male with metastatic cholangiocarcinoma. Axial contrast-enhanced image shows large enhancing soft tissue nodule in the omentum in the right side (*arrow*). There is mild stranding and infiltration of the surrounding fat

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

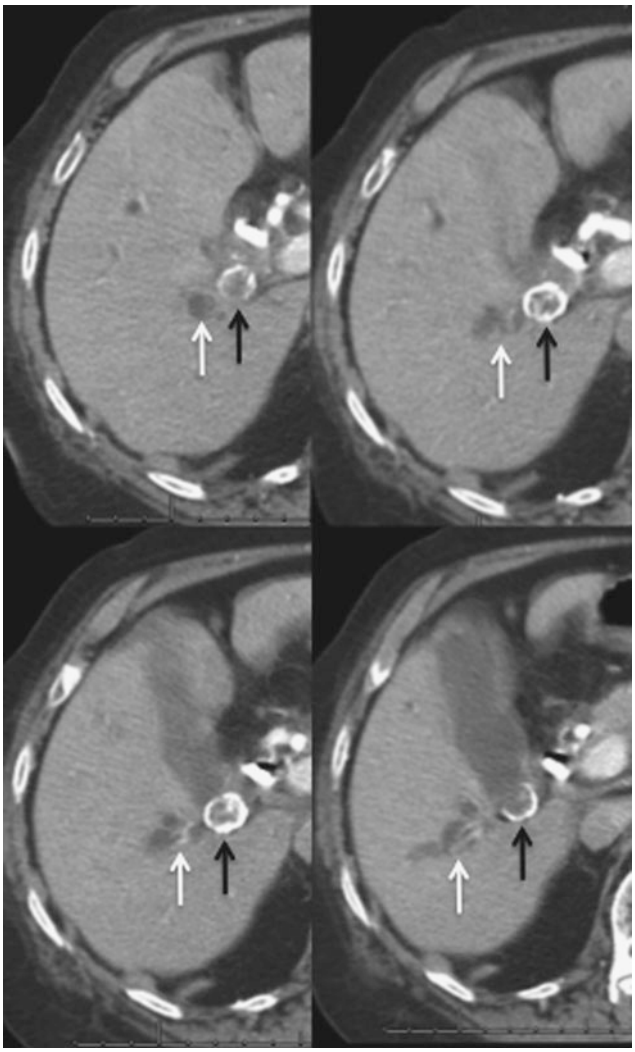

**Fig. 53** Mirizzi's syndrome; Sequential contrast-enhanced axial MSCT images show a large rim-calcified stone in the gallbladder neck (*black arrows*) with associated intrahepatic biliary ductal dilation (*white arrows*), representing Mirizzi's syndrome

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

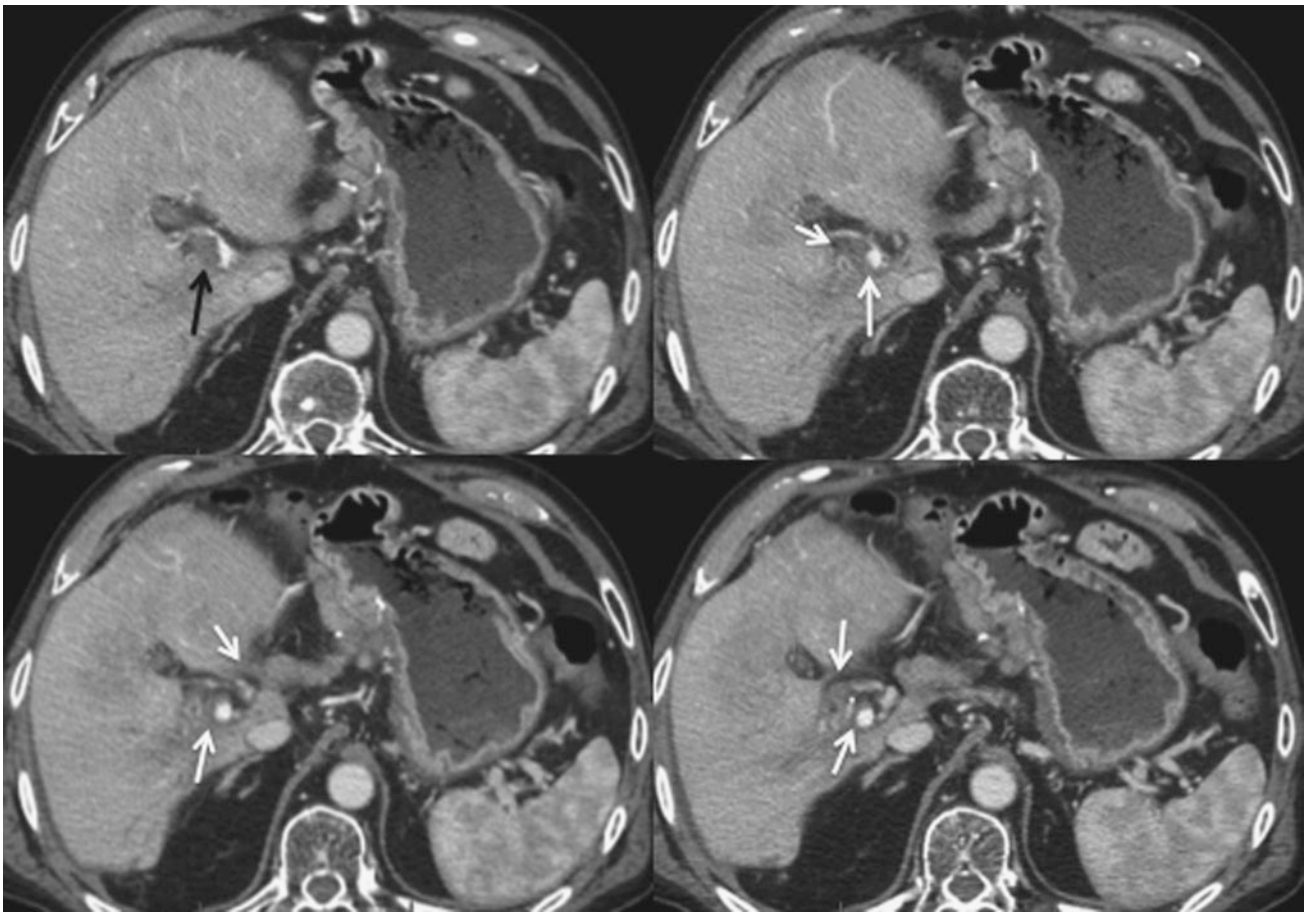

**Fig. 54** Recurrent hilar cholangiocarcinoma. Sequential contrast-enhanced axial images of a patient with history of cholangiocarcinoma show soft tissue infiltration surrounding the portal vein and hepatic artery (*white arrows*) along the hilar fissure, compatible with recurrent disease. Note also 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

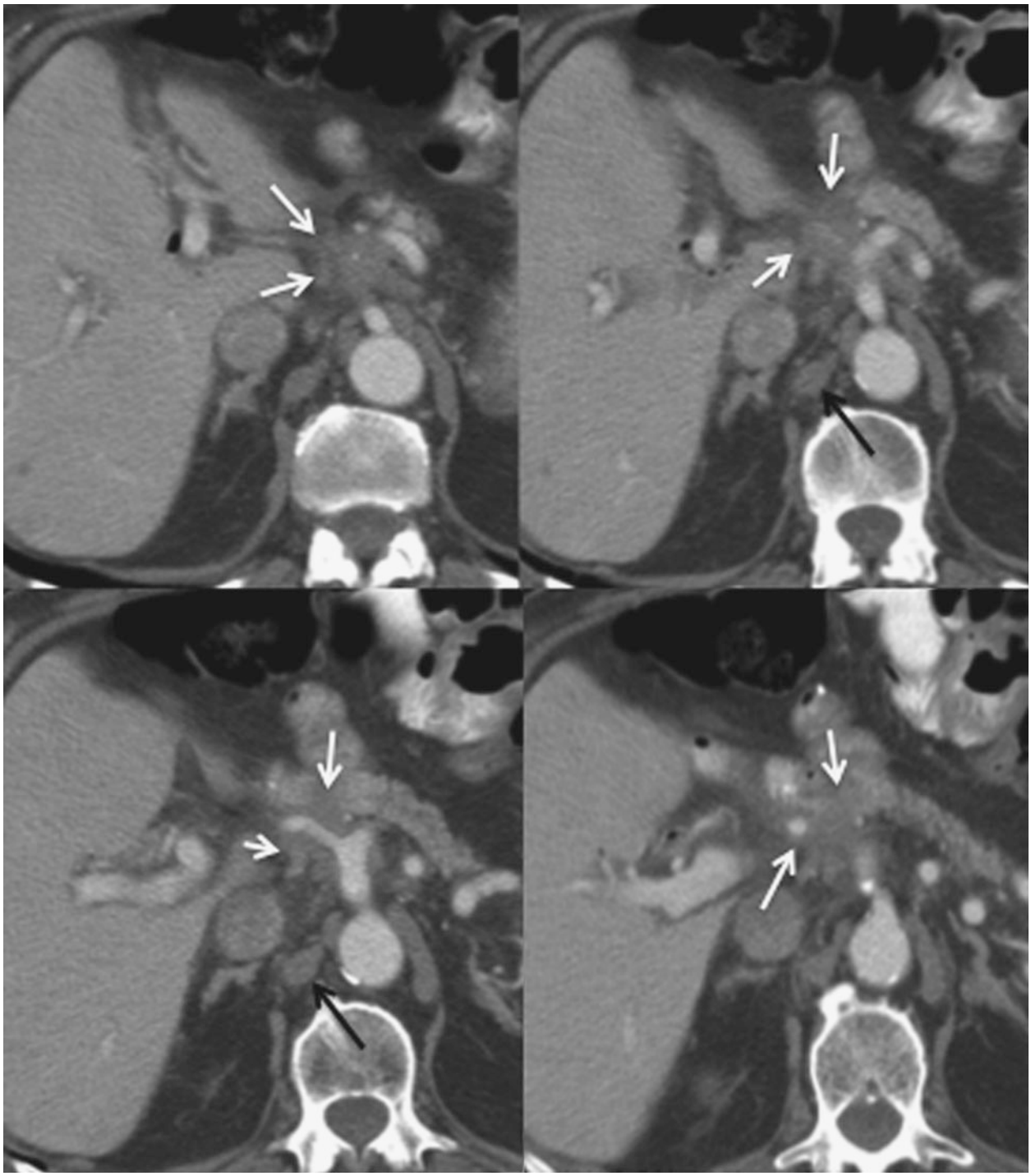

**Fig. 55** Recurrent distal cholangiocarcinoma; 78-year-old male patient with history of distal cholangiocarcinoma. Sequential contrast-enhanced axial images show soft tissue infiltration surrounding the celiac artery and common hepatic artery (*white arrows*), representing recurrent disease. Note lymph 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 preoperative 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

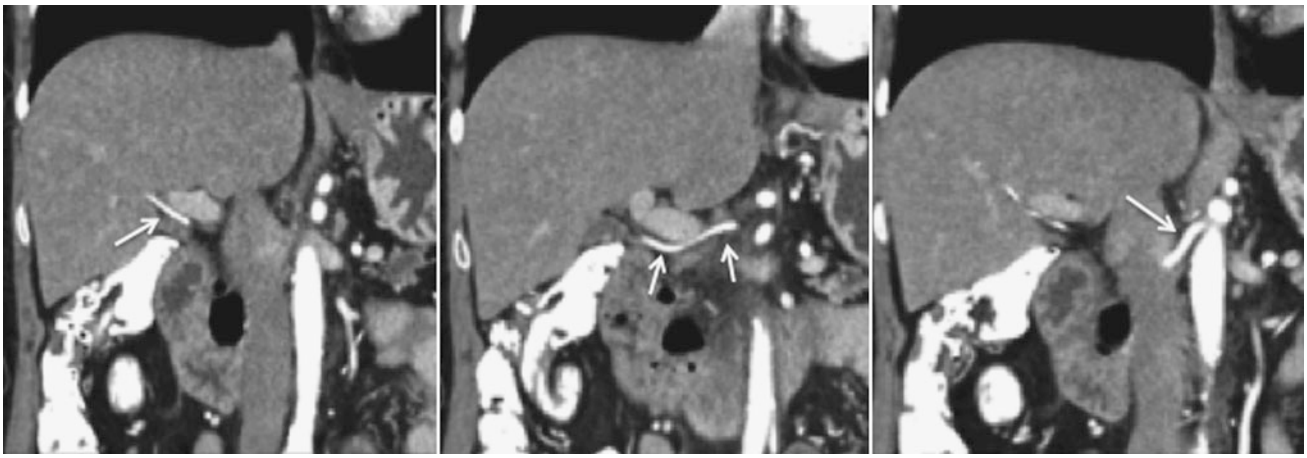

**Fig. 56** Variant vascular anatomy; replaced right hepatic artery (*RHA*). Coronal MSCT contrast-enhanced images of a 70-year-old female show a RHA (*arrows*) arising from the SMA

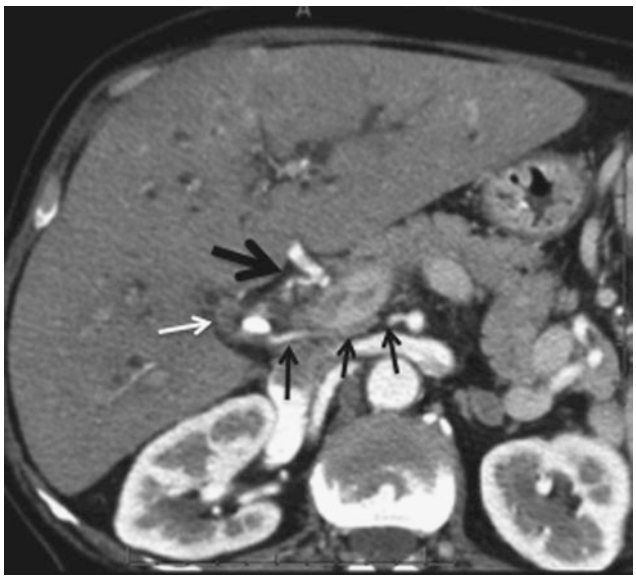

**Fig. 57** Variant vascular anatomy; accessory right hepatic artery (*RHA*) in a patient with hilar cholangiocarcinoma (*white arrow*). Axial contrast-enhanced MSCT image show a RHA (*thin black arrows*) arising from the SMA. The main right and left hepatic arteries are also observed (*thick black arrow*)

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

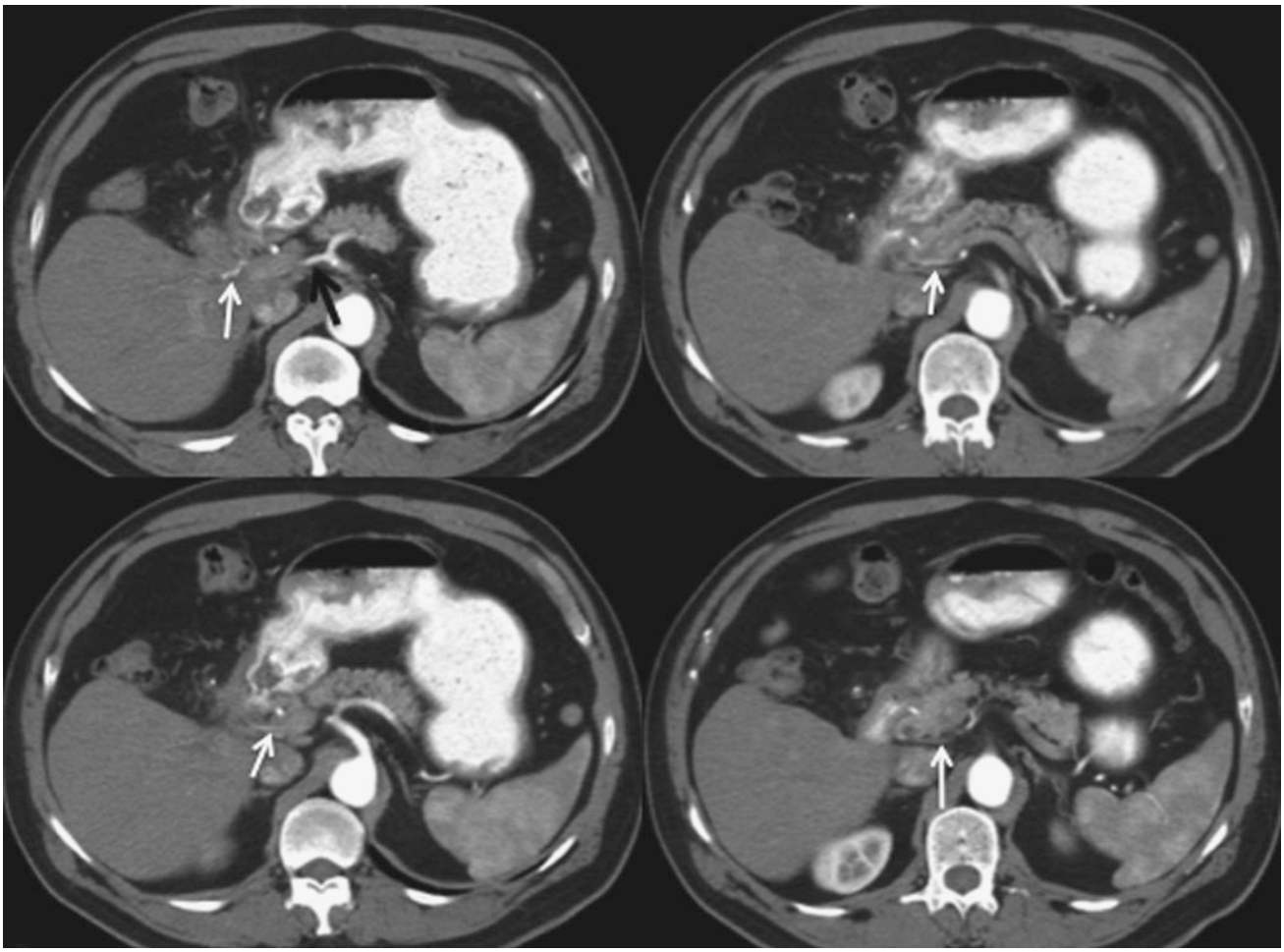

**Fig. 58** Variant vascular anatomy. Sequential axial contrast-enhanced MSCT images show an accessory right hepatic artery (*white arrows*) from the common hepatic artery (*black arrow*)

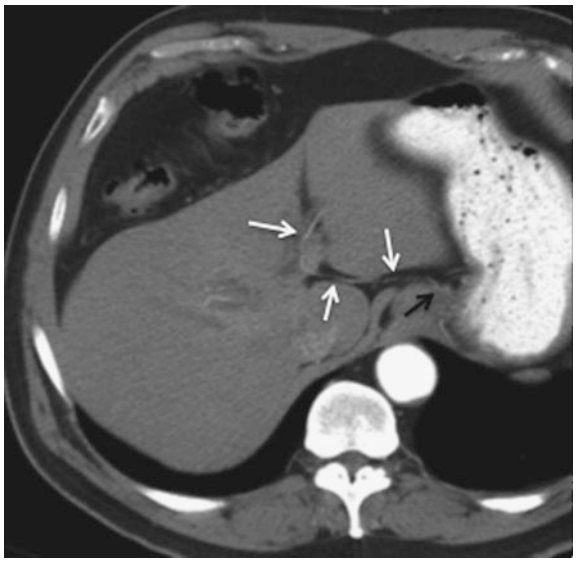

**Fig. 59** Variant vascular anatomy; replaced left hepatic artery (*HA*). Axial contrast-enhanced MSCT image shows the left HA (*arrows*) arising from left gastric artery (*black arrow*) within the gastrohepatic ligament

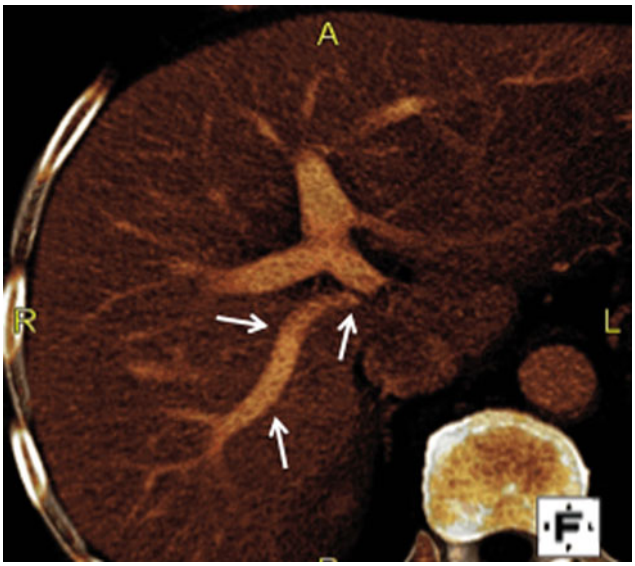


**Fig. 60** Variant vascular anatomy. Axial MSCT volume-rendered image shows the portal vein to segments VI and VII of the right liver lobe (*arrows*) arising from the main portal vein

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.

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# Interventional Radiology Management of Unresectable Intrahepatic Cholangiocarcinoma

Adam D. Talenfeld, Daniel J. Holzwanger, and David C. Madoff

## Contents




**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.


A. D. Talenfeld · D. J. Holzwanger · D. C. Madoff (✉)
Department of Radiology, Division of Interventional Radiology, New York-Presbyterian Hospital, Weill Cornell Medical Center, 525 E. 68th St.,P-518, New York, NY 10065, USA
e-mail: dcm9006@med.cornell.edu

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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 99 mTc-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 hepaticoenteric 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 hepaticoenteric 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 hepaticoenteric 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, 99 mTc-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

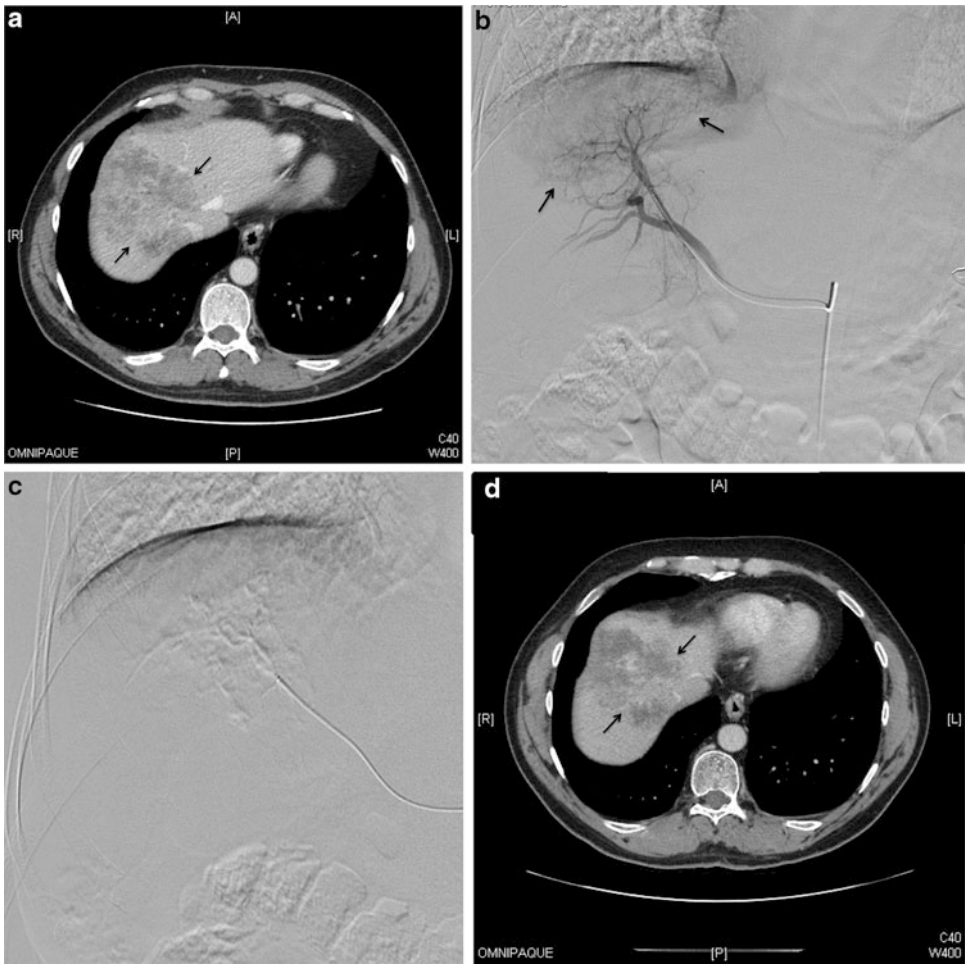



**Fig. 1** 49-year-old man with unresectable, liver-dominant intrahepatic ICC. **a** Axial portal-phase post-contrast CT image demonstrating a heterogeneously hypoenhancing mass centered in segments 7 and 8, measuring up to 9.8 cm. **b** Segmental right hepatic artery angiography demonstrating tumor blush and discrete neovascularity despite relative hypovascularity by CT. **c** Follow-up angiography immediately after embolization to segmental vessel stasis with 100 micron Embozene microsphere 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 subsegmental 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.

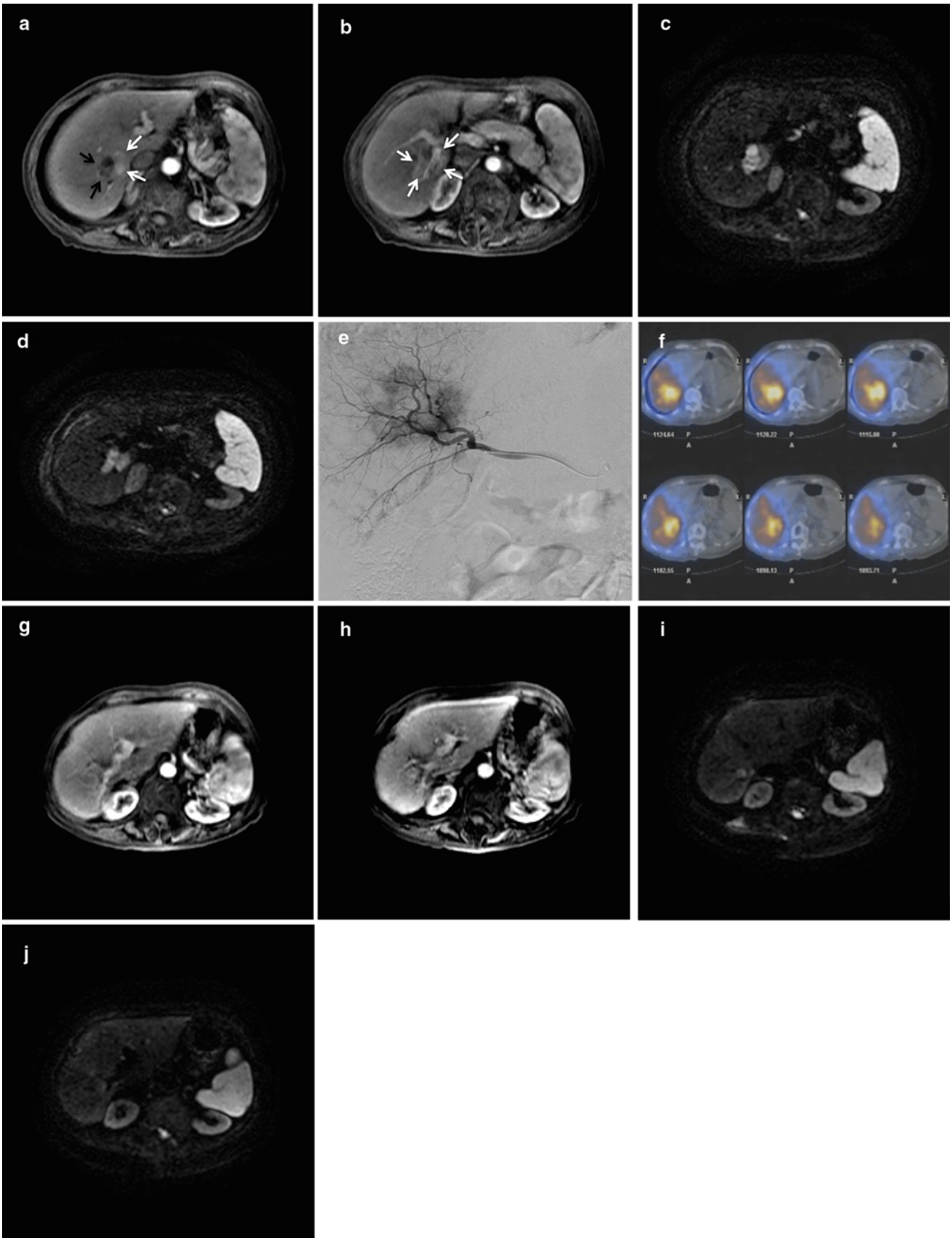
TARE, also known as selective internal radiotherapy (SIRT) or radiomicrobrachytherapy (RMB), makes use of neoplastic arterial supply to deposit small glass or starch–resin microspheres within the target tumor(s) that emit beta radiation from directly within the malignancy. Two devices are marked in North 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 $1.2 \times 10^6$ 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 40–80 $\times 10^6$ 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)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◀ **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 ± doxirubicin, 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: gemcitabine | 12 | 6 | | 25 | 75 | 0 | 100 | 8 | |
| | | | cTACE: gemcitabine + 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: gemcitabine, 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 | | | 54 | 41 | 5 | 0 | 0 | 0 |
| Kiefer | 2011 | CS | cTACE: cisplatin, doxorubicin and mitomycin C with PVA spheres | 62 | 2.7 | | 89 | 10 | 1 | 29 | 3 | 5 |
| Shen | 2011 | CC | fluorouracil or carboplatin, epirubicin and hydroxycamptothecin TACI ± Lipiodol cTACE | 53 | | 60 | | | | | | |
| | | | 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)/31/14 | 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/[+++]/[+++] | 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 chemoinfusion, *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 cholangiocarcioma 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/m$^2$ 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/m$^2$ [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).

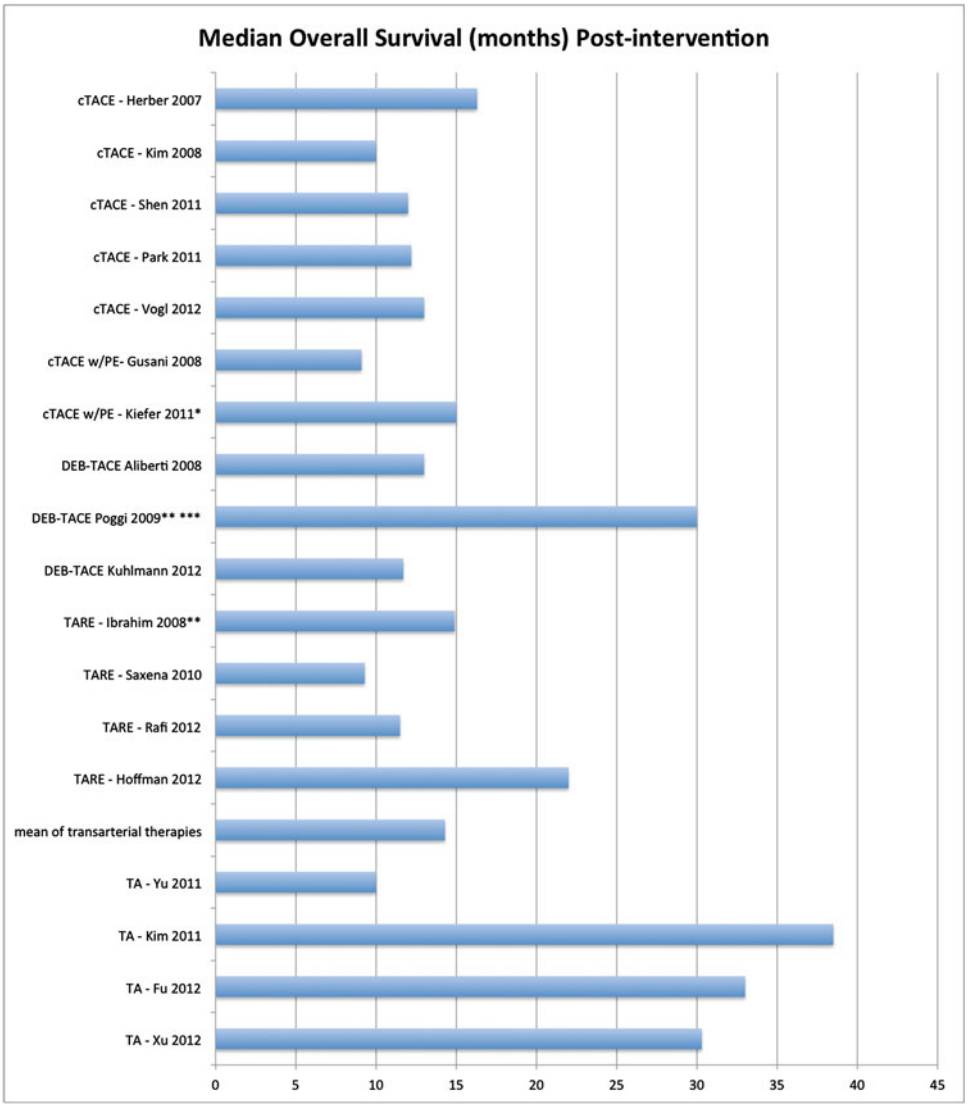


**Fig. 3** Median overall survival post-intervention by studies cited in this chapter. *cTACE* conventional transarterial chemoembolization with temporary embolic (Lipiodol and/or gelfoam or starch microspheres), *cTACE w/ PE* cTACE with permanent embolic material (tris-acryl gelatin or polyvinyl alcohol); *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 | Embolic therapy | Gem-cis dual-agent cTACE | (+) RECIST response |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Vogl | 2006 | TACI/ TACE | 12 | | | | | | | | | | | | <0.01 | | |
| 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 | | | <0.001 OR or SD vs. PD |
| Hoffman | 2012 | TARE | 33 | | <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 chemoinfusion, *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

All *p* values by multivariate regression analysis unless indicated by *, in which case univariate analysis was used

** 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.

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# Overview of Emerging Strategies in the Surgical Management of Biliary Tract Tumors

Felipe José Fernández Coimbra, Héber Salvador de Castro Ribeiro, Igor Correia de Farias, André Luis de Godoy, and Wilson Luiz da Costa Junior

## Contents



**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

F. J. F. Coimbra (✉) · H. S. de Castro Ribeiro · I. C. de Farias · A. L. de Godoy · W. L. da Costa Junior
Surgical Oncology, Department of Abdominal Surgery, AC Camargo Cancer Center, São Paulo, Brazil
e-mail: drfelipecoimbra@gmail.com

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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

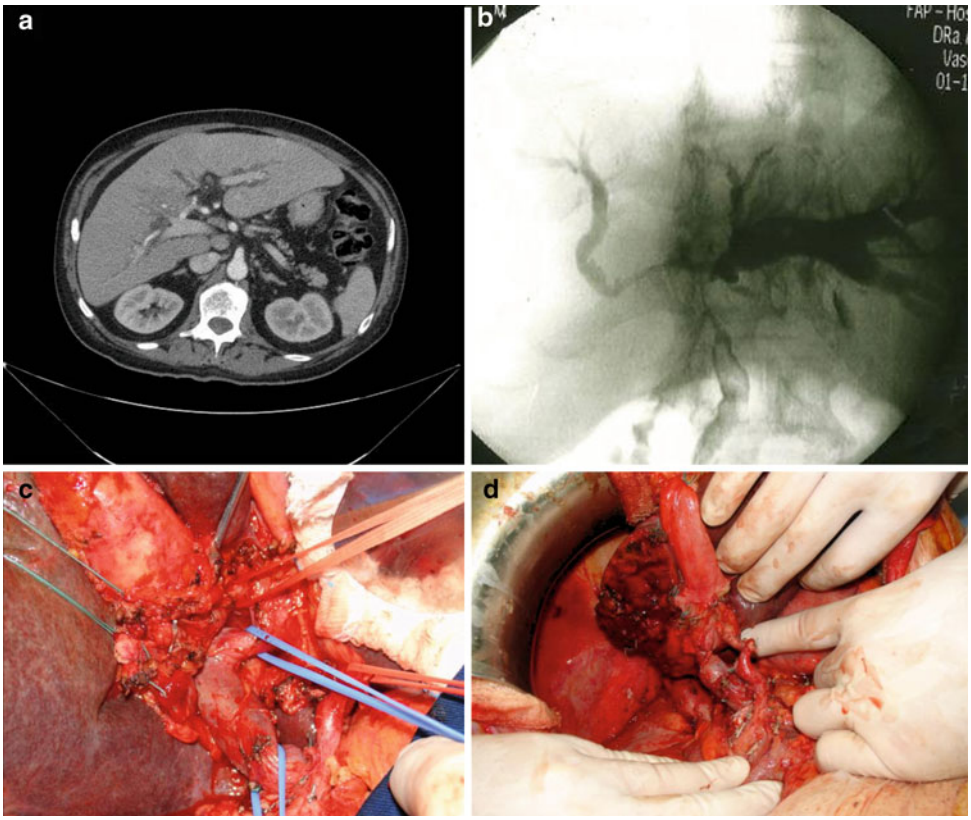


**Fig. 1** A Hilar cholangiocarcinoma with portal vein confluence encasement. **a** Abdominal CT showing the conduct of the mass with the portal vein bifurcation and the narrowing of the right anterior branch. **b** Percutaneous transhepatic cholangiogram detecting a IIIa tumor. **c** and **d** Intraoperative aspects before and after portal vein resection with end to end anastomosis

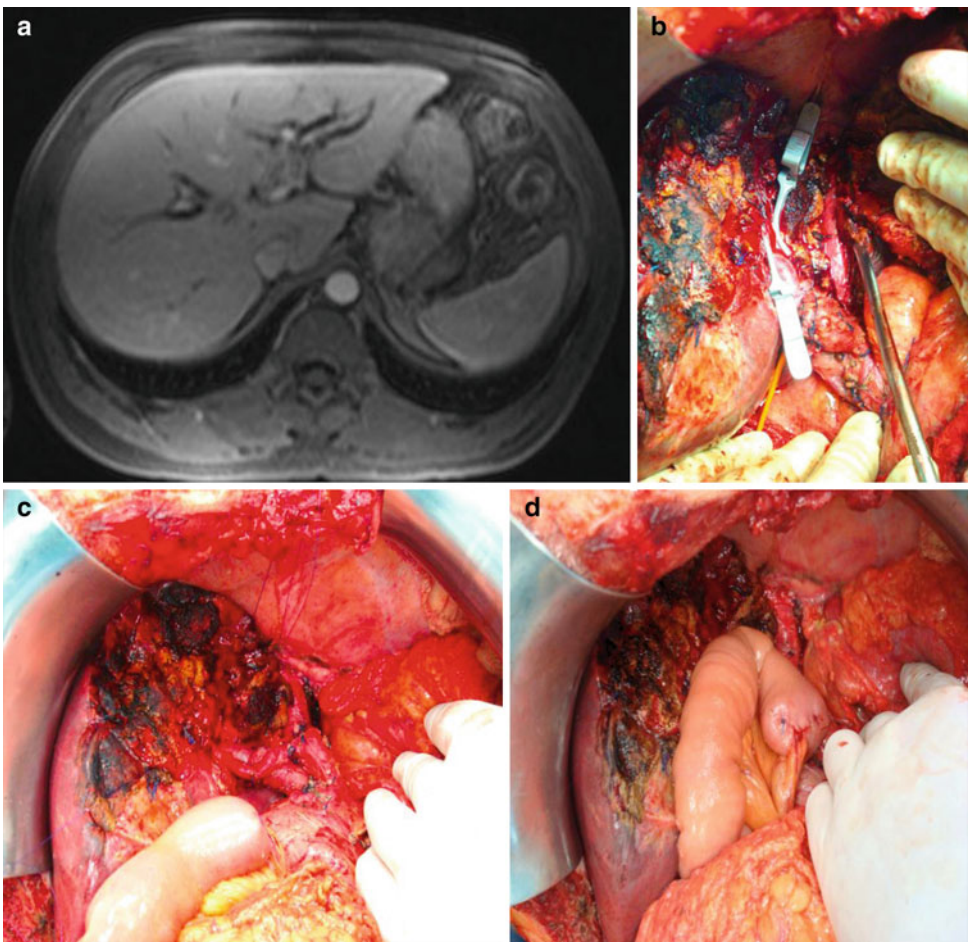


**Fig. 2** A Hilar cholangiocarcinoma Bismuth–Corlette type IIIB treated by left lobectomy and caudate resection. **a** Abdominal CT showing the extension of the tumor to the left main duct. **b–d** Intraoperative view after liver transection with hanging liver approach, resection of the caudate lobe, and intrahepatic bilioenteric anastomosis

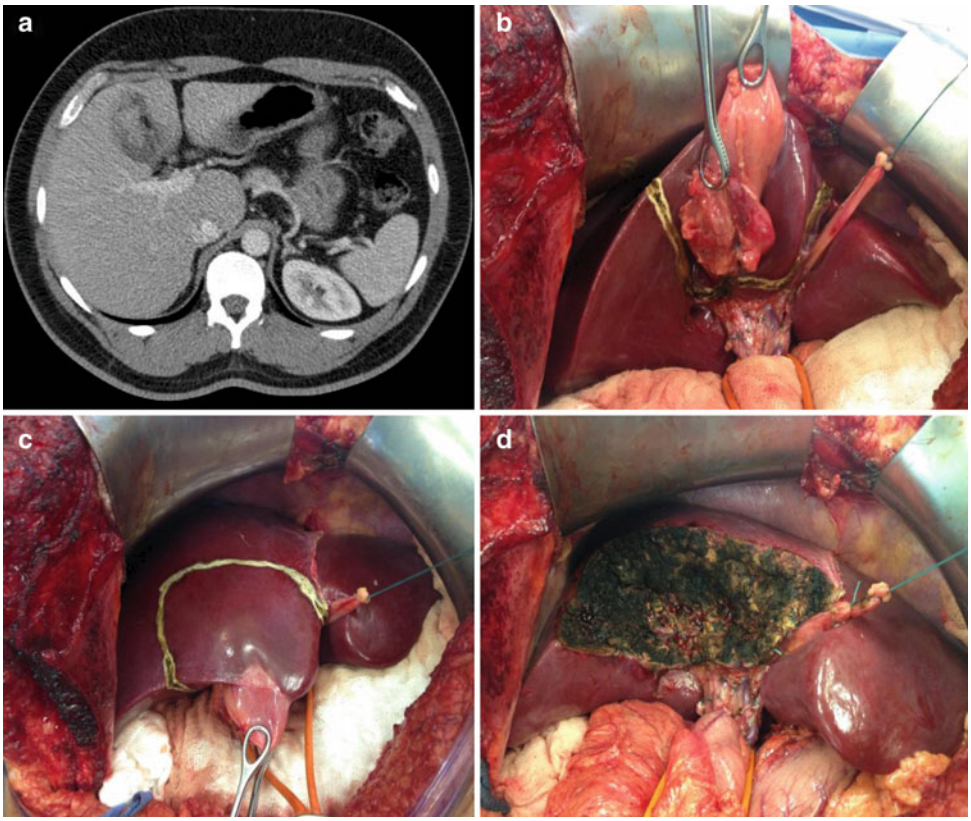


**Fig. 3** Radiological and intraoperative aspects of gallbladder cancer. **a** Abdominal CT with a heterogeneous mass in the gallbladder fundus. **b–d** Radical cholecystectomy with *en bloc* resection of segments IVB and V and hilar lymphadenectomy

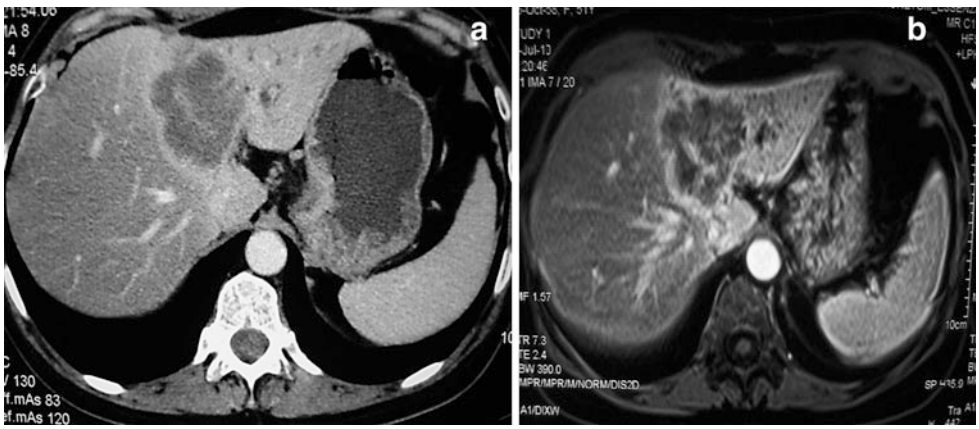


**Fig. 4** Radiological aspects on CT and MRI of intrahepatic cholangiocarcinoma. Note the heterogeneous enhancement, left lobe atrophy and biliary dilatation

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 rational 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 neo-adjuvant 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 experimented 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 hepatobiliar 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 periampular 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 hepatopancreatoduodenectomy (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 $\leq$ 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 then 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.

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# Surgical Management of Intra-Hepatic Cholangiocarcinoma

Kimberly M. Brown and David A. Geller

## Contents




**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


K. M. Brown
Department of Surgery, University of Texas Medical Branch, Galveston, TX, USA

D. A. Geller (✉)
Liver Cancer Center, University of Pittsburgh Medical Center, 459 Fifth Ave. UPMC Montefiore, 7 South Pittsburgh, Pittsburgh, PA 15213-2582, USA
e-mail: gellerda@upmc.edu


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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 extrahepatic 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, intraoperative 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

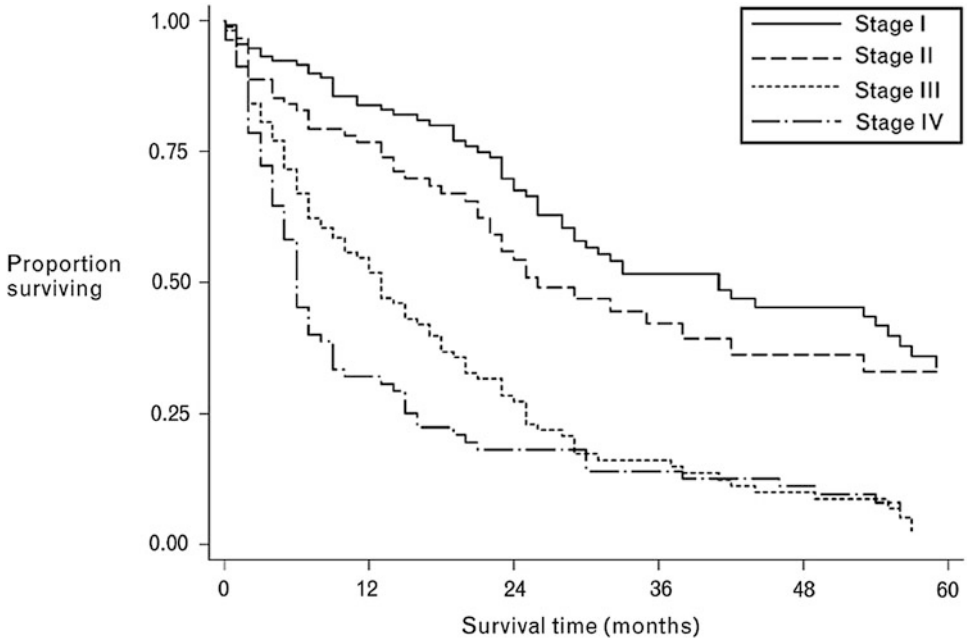


**Fig. 1** Kaplan–Meier survival curve for ICC, stratified by AJCC 7th edition stage (with permission, from Ref [13])

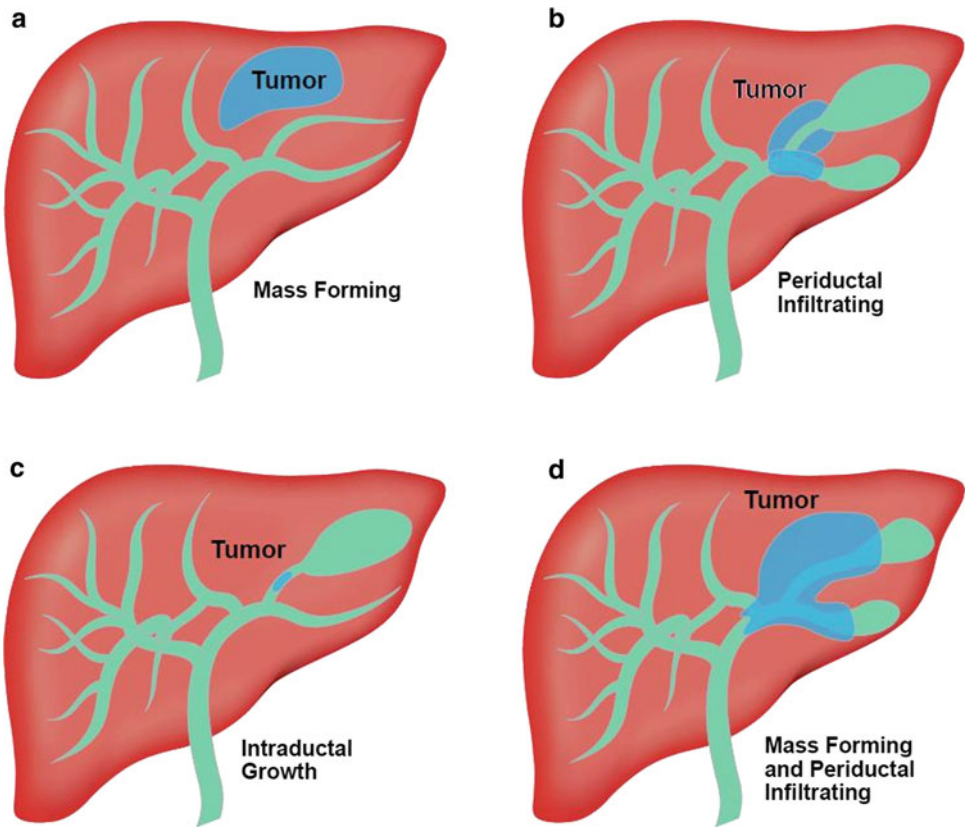


**Fig. 2** Macroscopic subtypes of ICC

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

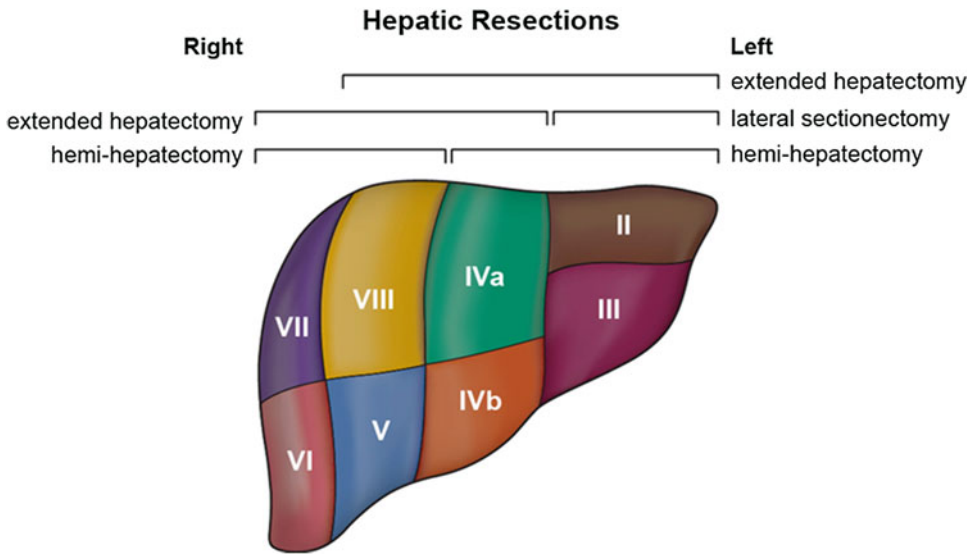


**Fig. 3** Anatomic basis for liver resection procedures, according to Brisbane terminology

patients (54 %) were initially unresectable, based on the presence of multiple tumors (70), locally advanced intrahepatic 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 $33 \pm 8$ % to $43 \pm 8$ %. Among patients who ultimately underwent

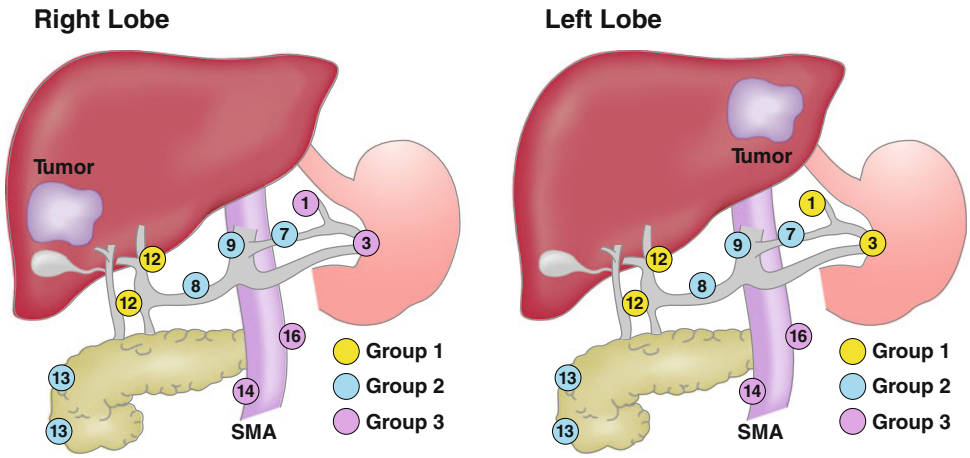


**Fig. 4** Patterns of lymphatic spread for *left-sided* and *right-sided* hepatic tumors

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, lymphovascular 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 multifocal 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/m$^2$) on days 1, 8 and 15 of a 4-week cycle, or cisplatin (25 mg/m$^2$) and gemcitabine (1,000 mg/m$^2$) 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.

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# Surgical Techniques for Extrahepatic Biliary Tract Cancers

Junichi Shindoh, Giuseppe Zimmitti, and Jean-Nicolas Vauthey

## Contents



J. Shindoh · G. Zimmitti · J.-N. Vauthey (✉)
The University of Texas, MD Anderson Cancer Center, Texas, USA
e-mail: jvauthey@mdanderson.org

**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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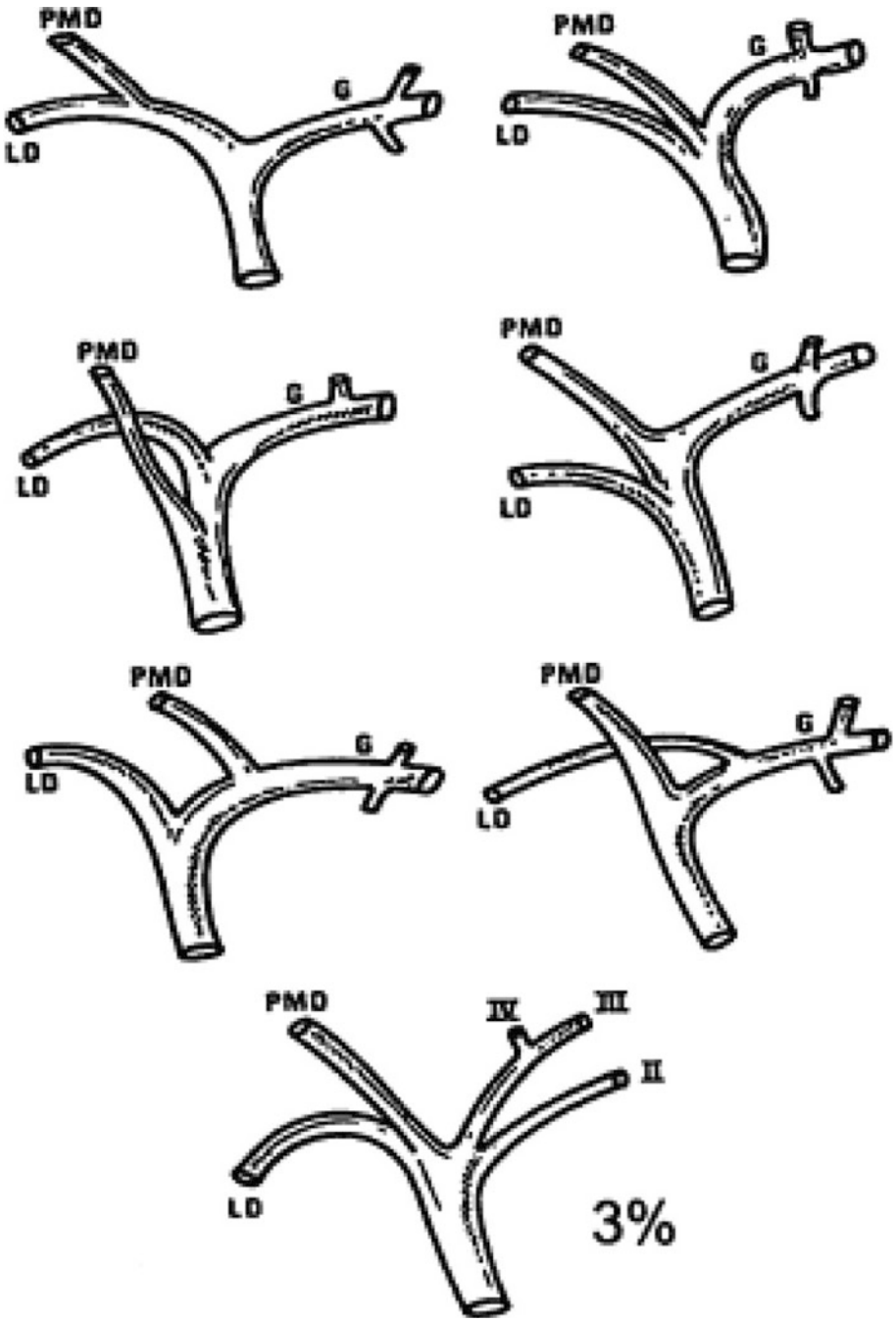


**Fig. 1** **Variations in biliary anatomy**. The left hepatic duct is long and present in 97 % of patients. In types I, II, and IIIa hilar cholangiocarcinomas, an extended right hepatectomy permits placement of a safe and single anastomosis away from the confluence, minimizing the probability of positive 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

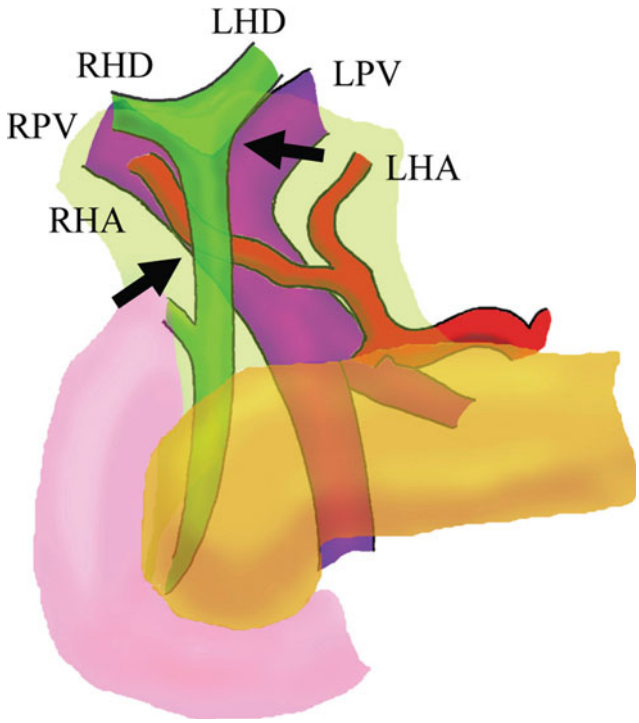


**Fig. 2** **Vascular anatomy at the hepatic hilum**. Right hepatic artery and portal bifurcation are located just behind the extrahepatic biliary tract, and they are subjected to invasion by extrahepatic biliary cancers at these points (*arrows*). *LHA* left hepatic artery; *LHD* left hepatic duct; *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

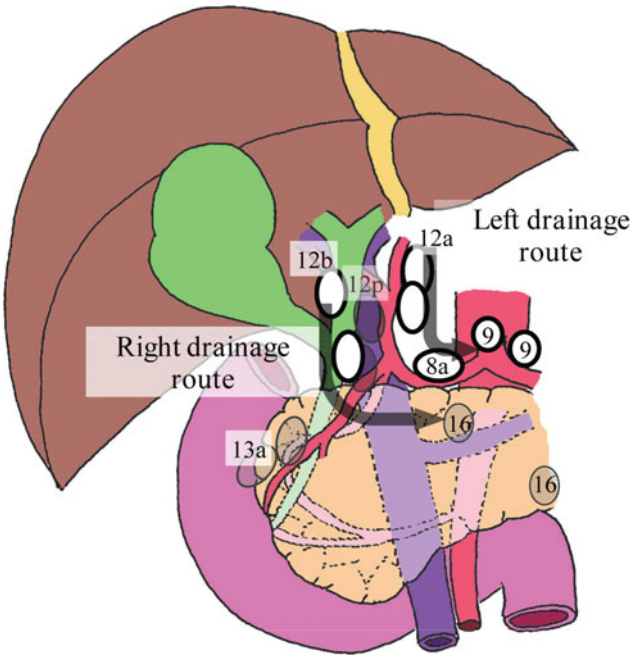


**Fig. 3** Schematic drawing of the biliary lymphatic system and supposed major drainage routes (Adapted from [29])

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

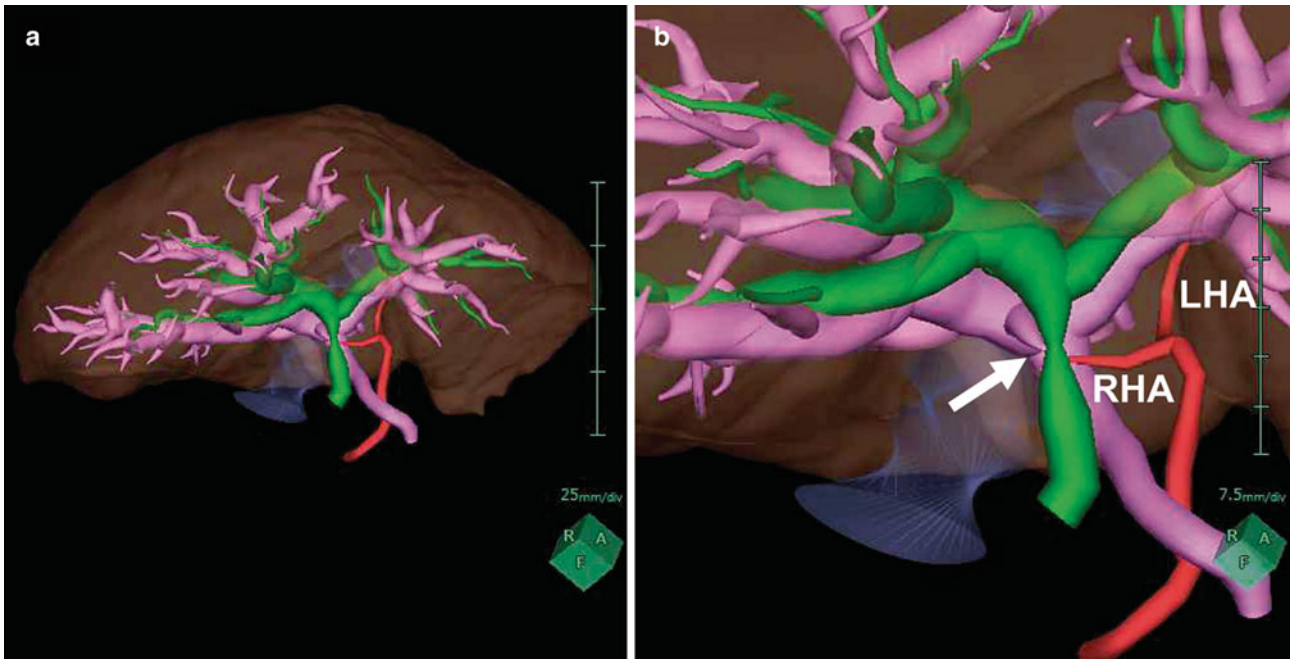


**Fig. 4** 3-D visualization of anatomic structures for surgical planning of hilar cholangiocarcinoma (courtesy to Dr. Yoshihiro Mise). **a** 3-D intrahepatic vascular structures (*pink* portal vein; *green* biliary tract; *red* hepatic artery). **b** Magnified view of hepatic hilum. Cancerous stricture 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

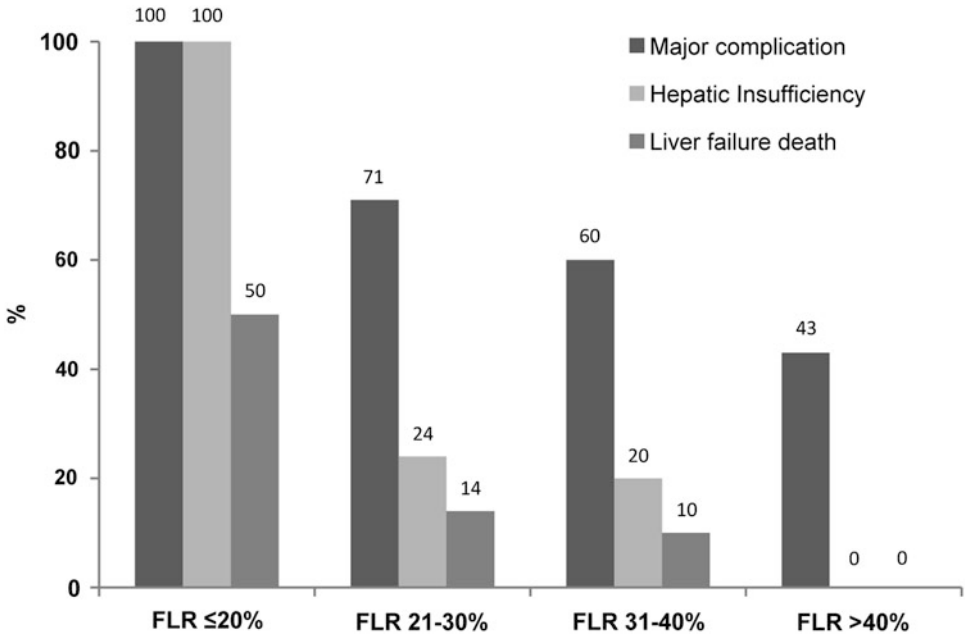


**Fig. 5** Incidences of major complication, hepatic insufficiency, and liver failure death after resection of hilar cholangiocarcinoma (MD Anderson Cancer Center 1997–2011, $n = 47$)

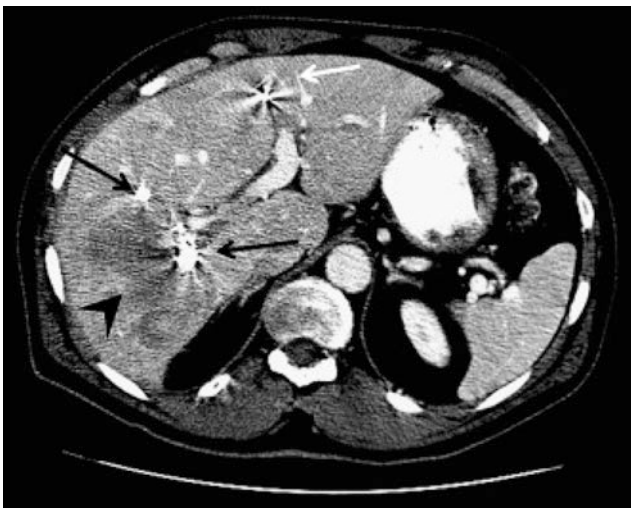

**Fig. 6** CT image after right PVE + Segment IV embolization. Note that major S4 branches, in addition to right portal vein branches, were distally embolized (*white arrow*). *Black arrows*, coils used to embolized right portal vein branches; *black arrowhead*, tumor (From [37], with permission)

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

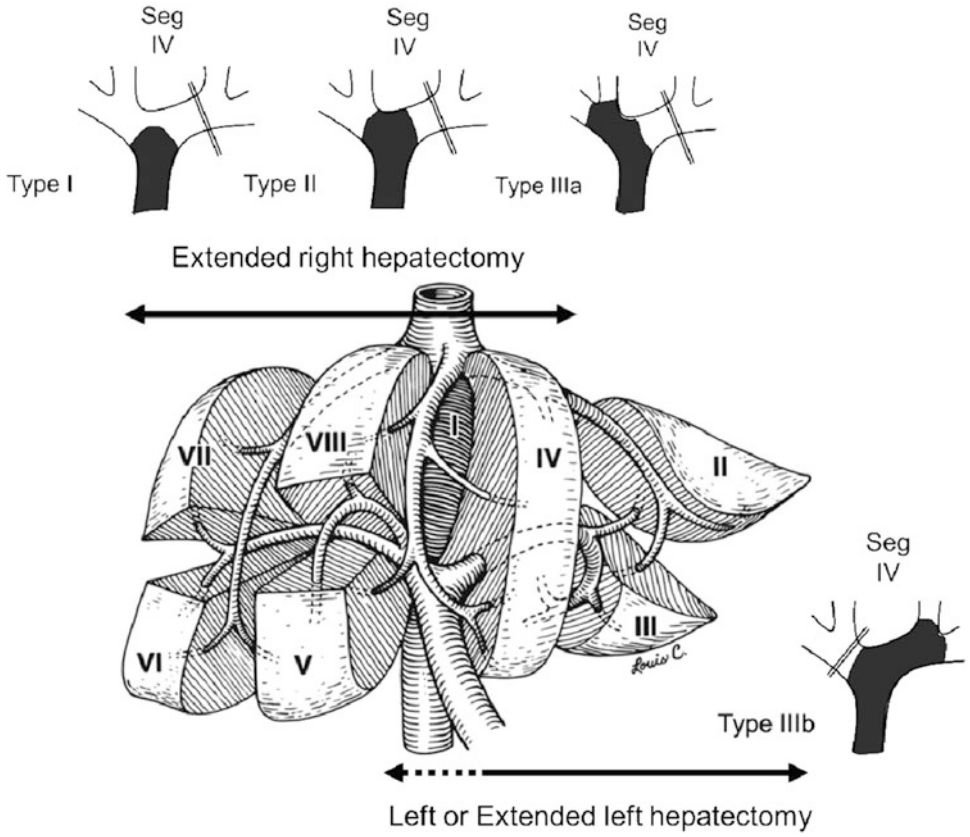


**Fig. 7** Extent of hepatic resection according to Bismuth–Corlette classification of hilar cholangiocarcinoma (adapted from [62] with permission)

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

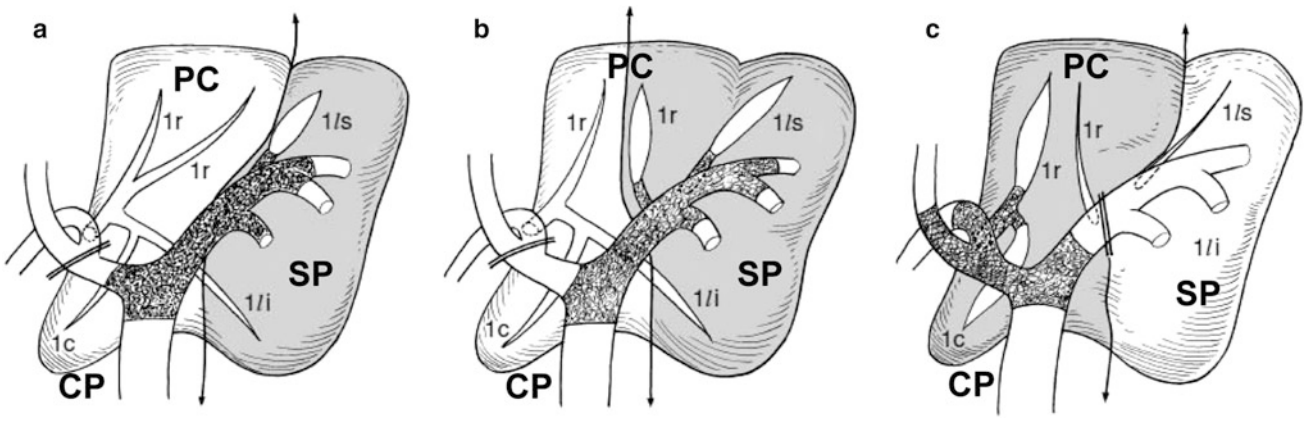


**Fig. 8** Partial preservation of the caudate lobe based on the biliary anatomy and tumor invasion (Adapted from [59] Tan-to-sui 26:309–314 with permission). **a** Preservation of right caudate lobe in extended left hepatectomy for type IIIb tumor without invasion to 1r and 1c. **b** Partial preservation 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,

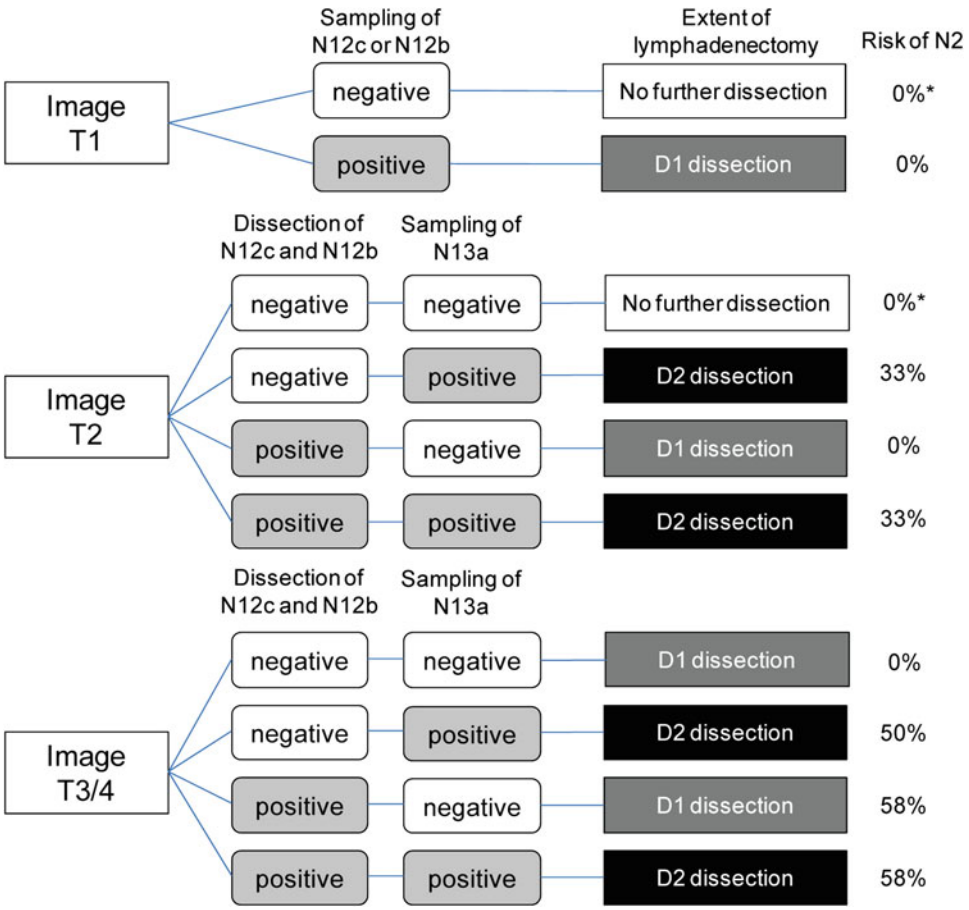


**Fig. 9** Algorithm for the extent of lymph node dissection based on preoperative diagnosed T factor (Image T) and intraoperative frozen section of key lymph nodes. (Adapted from [39] with permission). D1 dissection, lymphadenectomy of first-echelon lymph nodes (N12c and N12b); D2 dissection, lymphadenectomy 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].

## 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 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.

#### 7.1.1 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 patent 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.

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# Surgical Management of Gallbladder Cancer

Amudhan Pugalenthi and Yuman Fong

## Contents




**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


A. Pugalenthi
Department of Surgery, Memorial Sloan–Kettering Cancer Center, New York, NY, USA

Y. Fong (✉)
Department of Surgery, Murray F. Brennan Chair in Surgery, Memorial Sloan–Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA
e-mail: fongy@mskcc.org


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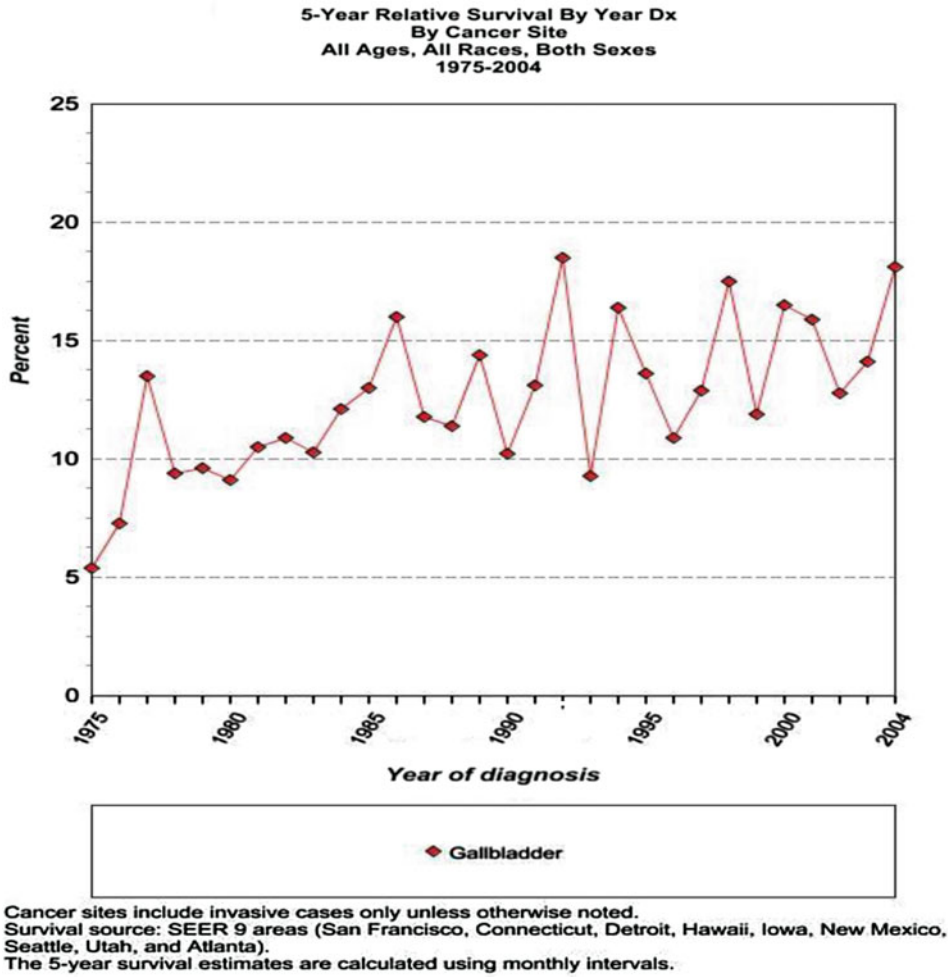


**Fig. 1** Five-year survival for gallbladder cancer generated from surveillance epidemiology and end results (SEER) database [4]

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 hepatique"). 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

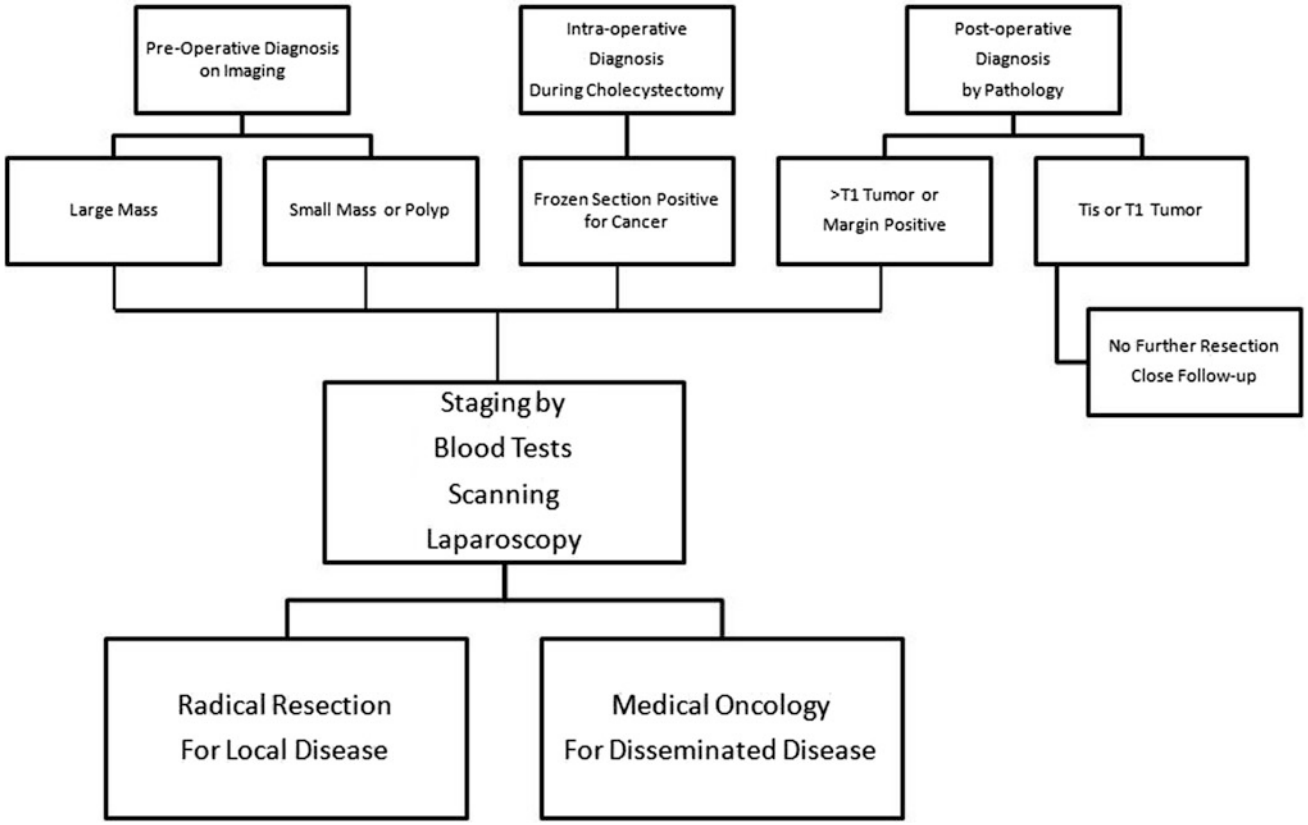


**Fig. 2** Algorithm of management of gallbladder cancer

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 | | | |
|---|---|---|---|
| *Primary Tumor (T)* | | | |
| TX—primary tumor cannot be assessed<br>T0—no evidence of primary tumor<br>Tis—carcinoma in situ<br>T1—tumor invades lamina propria or muscular layer<br>T1a—tumor invades lamina propria<br>T1b—tumor invades muscular layer<br>T2—tumor invades perimuscular connective tissue; no extension beyond serosa or into liver<br>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<br>T4—tumor invades main portal vein or hepatic artery or invades two or more extrahepatic organs or structures<br>*Regional Lymph Nodes (N)*<br>N0—no regional lymph node metastases<br>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<br>*Distant Metastasis (M)*<br>M0—no distant metastasis<br>M1—distant metastasis | | | |
| *Anatomic stage/prognostic groups* | | | |
| 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 | MO |
| Stage IVA | T4 | N0-1 | MO |
| Stage IVB | Any T<br>Any T | N2<br>Any N | M0<br>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 Aretxabala 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<br>11 | III<br>IV | 11<br>9 | NR<br>NR | |
| Onoyama, 1995 [42], Japan | 12<br>14 | III<br>IV | 44*<br>8* | 44*<br>8* | |
| Nakamura, 1999 [60], Japan | 23 | IV | 17 | 11 | Morbidity—60 %<br>Mortality—nil |
| Fong, 2000 [22], USA | 58 | III/IV | 28 | 28 | |
| Kondo, 2002 [40], Japan | 9<br>29 | III<br>IVa(M0)<br>IVb(M1) | 44<br>24<br>7 | 33<br>17<br>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, ≥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.

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# Systemic Therapy: Current Strategies and New Directions

Melanie B. Thomas

## Contents



**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.

M. B. Thomas (✉)
Charleston, SC, USA
e-mail: thomasmb@musc.edu

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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] / TTP[c] (months) | Overall survival (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 / TTP (months) | Overall survival (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 carcinogenetic 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.

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# External Beam Radiation Therapy: 3D-Conformal, Intensity-Modulated, and Proton Beam

Anusha Kalbasi and Edgar Ben-Josef

## Contents




**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

A. Kalbasi (✉) · E. Ben-Josef
Department of Radiation Oncology,
Perelman Center for Advanced Medicine,
Hospital of the University of Pennsylvania,
Philadelphia PA, USA
e-mail: anusha.kalbasi@uphs.upenn.edu

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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 ± 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 ± adriamycin; 5-FU ± mitomycin | 12 |
| Morganti et al. [15] | 20 | 39.6–50.4 Gy EBRT ± 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 ± 15 Gy ILBT (14 patients) | None | 7 |
| Crane et al. [34] | 52 | 30–85 Gy; EBRT ± ILBT | 5-FU CI in 38 patients | 10 |
| Buskirk et al. [55] | 34 | 45–55 Gy EBRT ± 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 ± 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 veno-occlusive 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

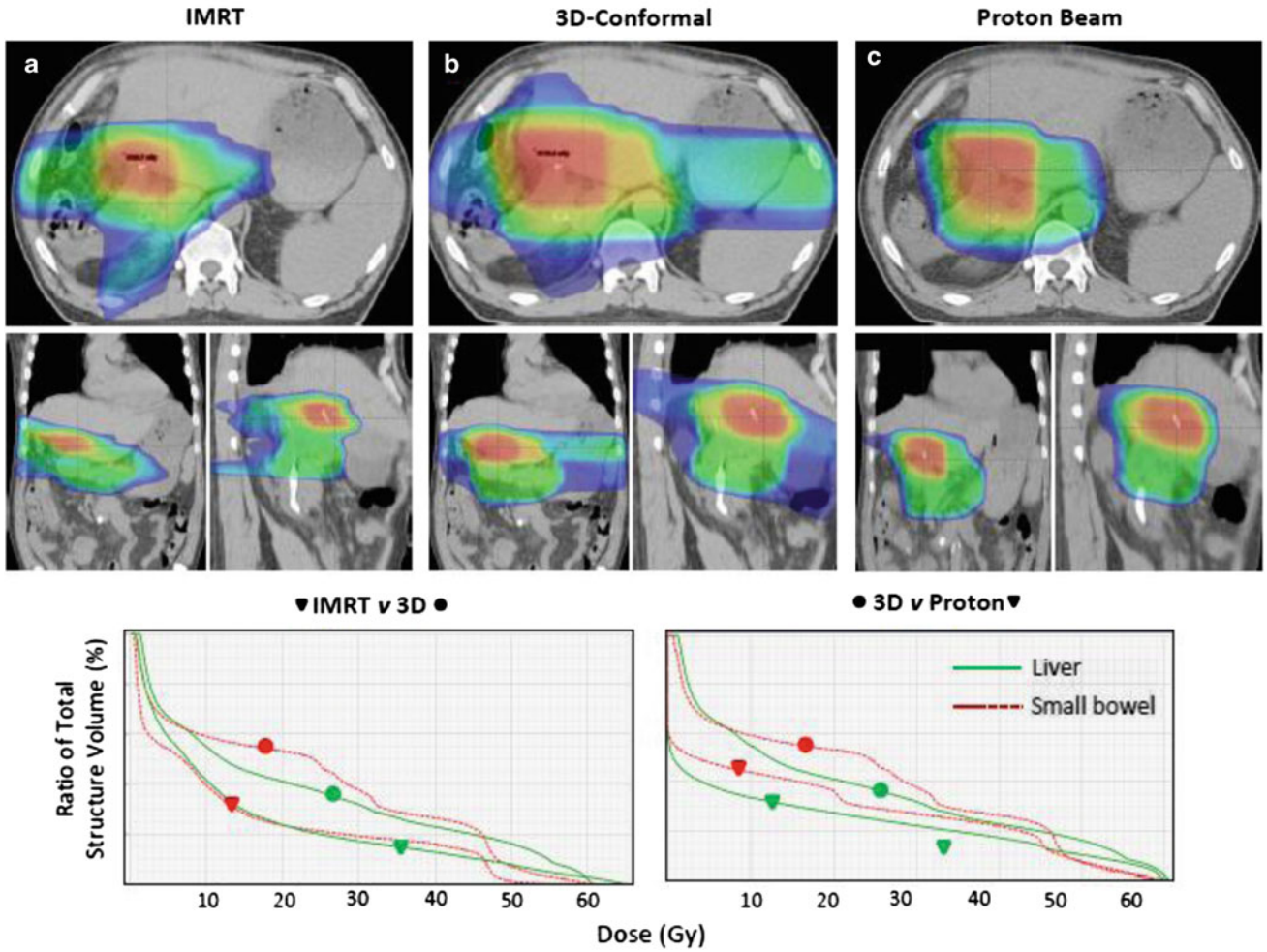


**Fig. 1** Dose distribution for postoperative treatment for extrahepatic cholangiocarcinoma using **a** IMRT, **b** 3D-conformal therapy with four fields, and **c** proton radiotherapy with two fields. Axial (*top panel*), coronal, and sagittal (*middle panel*) views are shown. A dose gradient of 25 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 $\geq$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.

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# Brachytherapy in Hepatobiliary Malignancies

Subir Nag, L. Matthew Scala, and Andrew S. Kennedy

## Contents




**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 $^{125}$I seeds, HDR brachytherapy, or intraluminal $^{192}$Ir to the unresected tumor or to the tumor bed after surgical resection. Diffuse or multiple primary or metastatic liver tumors can be palliated with $^{90}$Y 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,


S. Nag (✉) · L. Matthew Scala
Kaiser Permanente Radiation Oncology, 3800 Homestead Road, Santa Clara, CA 95051, USA
e-mail: Subir.nag@kp.org

A. S. Kennedy
Radiation Oncology, Sarah Cannon Research Institute, Nashville, TN 37203, USA


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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

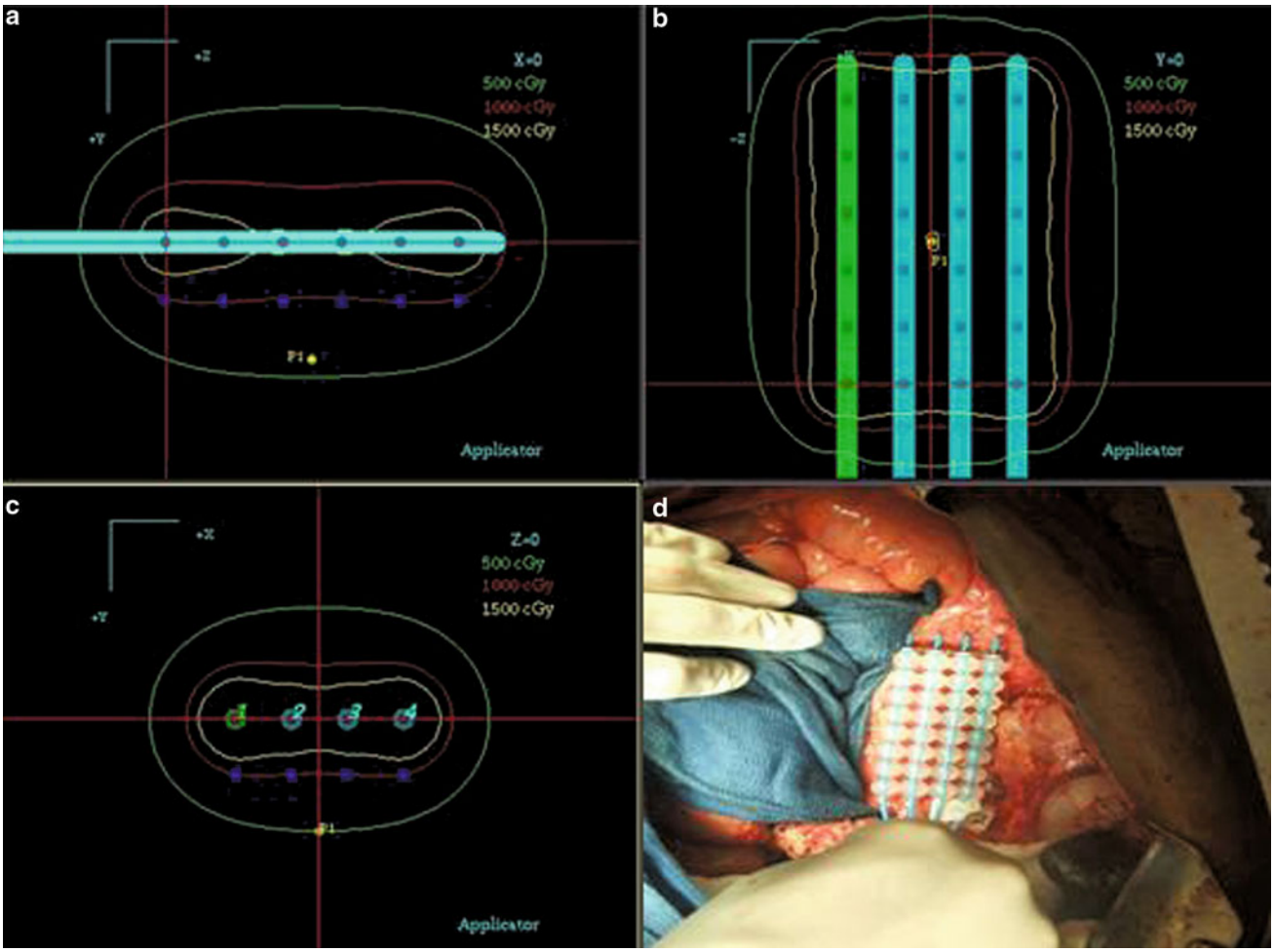


**Fig. 1** A typical plan used for intraoperative radiation therapy using high-dose-rate brachytherapy (*HDR-IORT*). Dose distribution of an IORT plan prescribing 10 Gy to the surface in the (**a**) sagittal (**b**) coronal, and (**c**) axial planes. In (**d**), we see the catheters are placed in a silicone Freiburg flap that can be cut to conform to the tumor bed. 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

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. 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

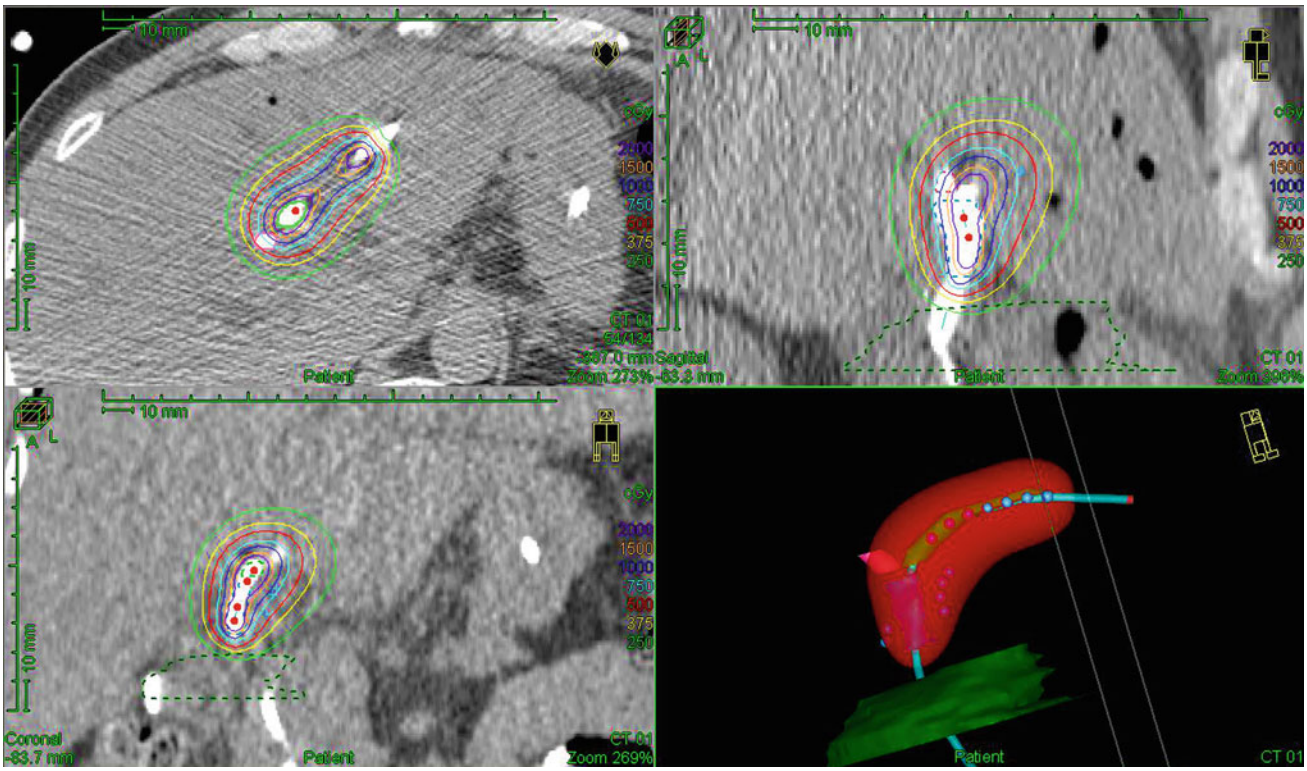


**Fig. 2** Planning of intraluminal brachytherapy for biliary malignancy

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 area 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 $\leq$20, 21–64, and $\geq$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 reposted 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

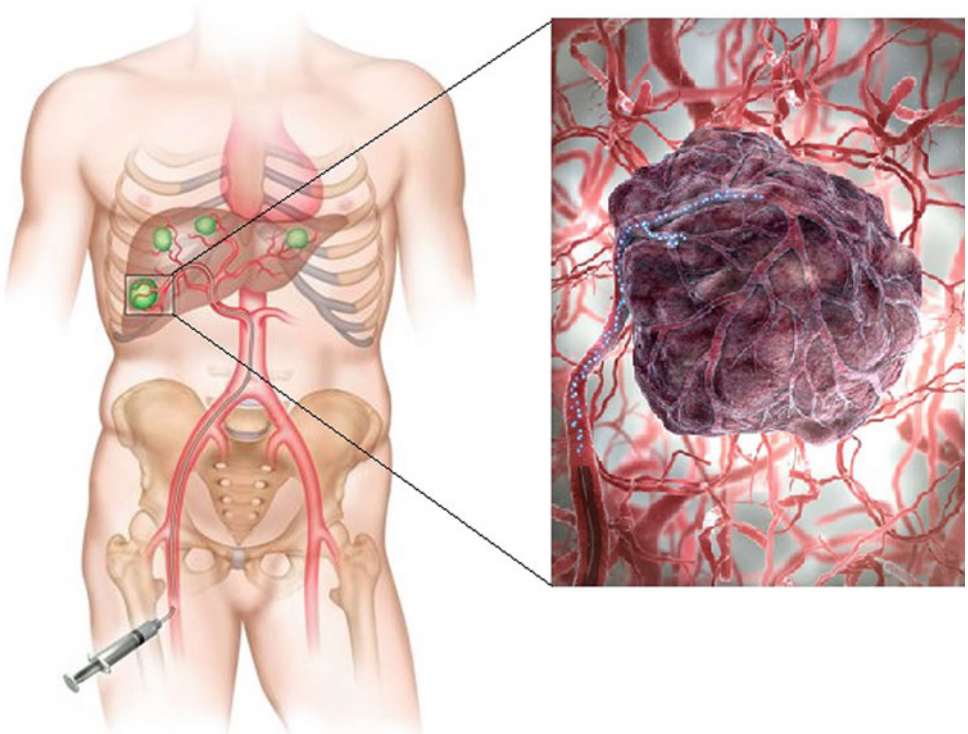

**Fig. 3** An injection of microspheres embedded with $^{90}$Y into the hepatic artery causes cancer cells to die through the emission of beta particles (Yttrium-90 microsphere radioembolization—cancer treatment. Ria Endovascular. http://www.riaendovascular.com/services-procedures/cancer-interventional-oncology/yttrium-90-radioembolization/. 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 $^{99m}$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 anterograde 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 anterograde blood flow is noticed during the procedure.

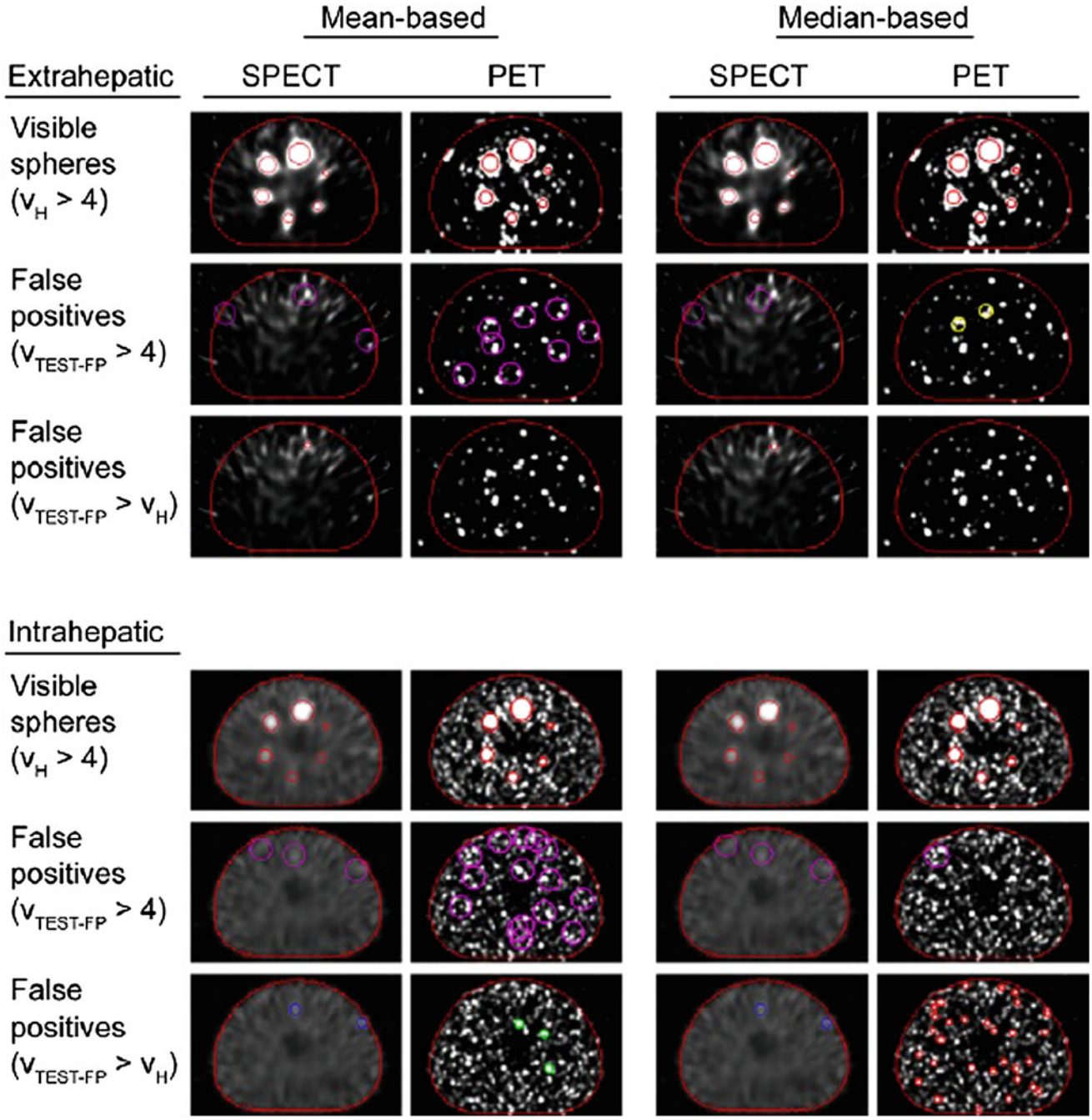


**Fig. 4** A visual comparison of FDG-PET versus SPECT imaging for the assessment of intrahepatic and extrahepatic microsphere distribution after radioembolization. FDG-PET imaging often has superior quality than that of SPECT although both methods can provide useful data regarding dosimetry and disease 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)<br>Child-Pugh B = 10.3 mo (CI 7.4–12.6 mo) | Child-Pugh A = 17.2 mo (CI 14.9–24 mo)<br>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 $^{90}$Y 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 $^{90}$Y 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, $^{90}$Y 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 maker carcinoembryonic antigen (CEA).

Prospective clinical trials have also shown promising results for the use of $^{90}$Y microspheres. One such study, published by Gray et al. [84], was a phase III trial studying the use of resin $^{90}$Y microspheres in chemotherapy-naive 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 $^{90}$Y 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 $^{90}$Y 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.

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# Emerging Techniques in Image-Guided Radiation Therapy and Stereotactic Body Radiation Therapy

John G. Phillips, John A. Wolfgang, and Theodore S. Hong

## Contents




**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].


J. G. Phillips (✉)
Harvard Radiation Oncology Program, Boston, MA, USA
e-mail: tshong1@partners.org

J. A. Wolfgang · T. S. Hong
Department of Radiation Oncology, Massachusetts General Hospital, Boston, MA, USA


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**Table 1** Projected doses for partial liver tolerance in the Enami 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 |

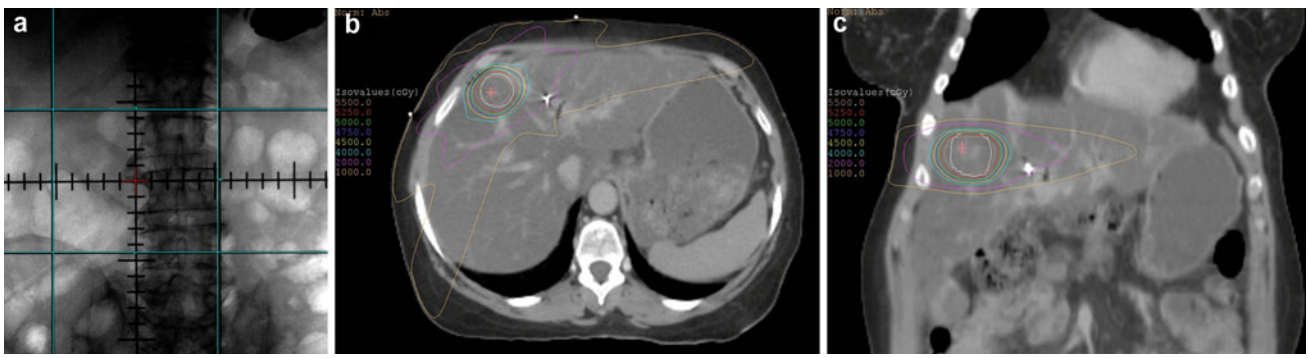


**Fig. 1** Comparison of 2D radiation fields with SBRT fields. **a** Traditional 2D fields relied on bony targets without any adjustment for motion or target localizer such as fiducials. **b** Axial CT scan showing SBRT plan with very high radiation doses using multiple beam angles. **c** Coronal CT from same SBRT plan

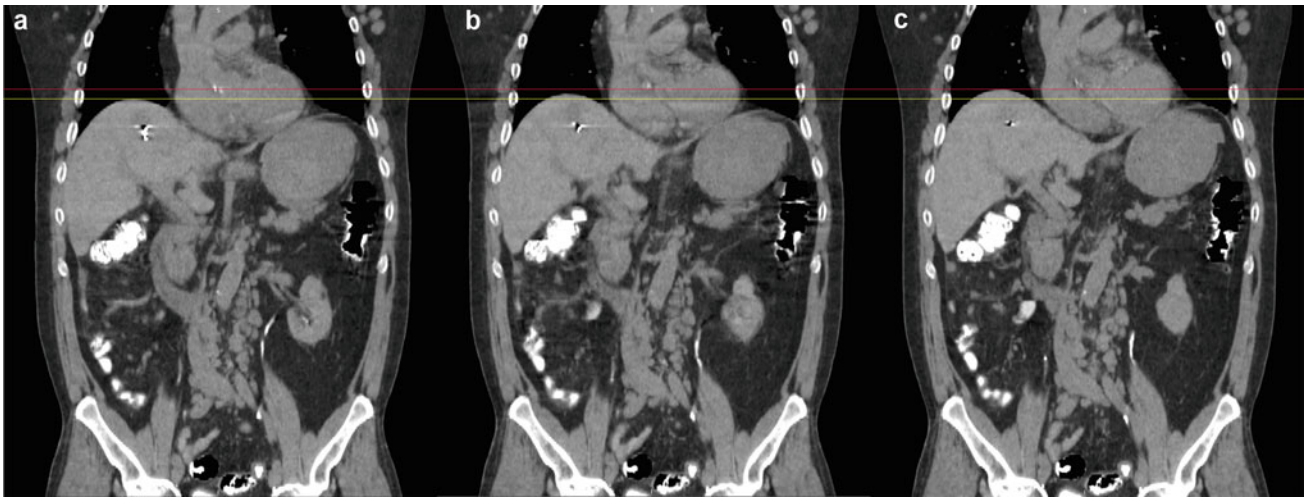


**Fig. 2** Coronal CT demonstrating tumor motion throughout the respiratory cycle in the same patient. **a** End inspiration **b** mid-cycle and **c** end-expiratory. *Red* and *yellow lines* represent same plane at each point and allow for measurement of tumor motion. The artifact within the liver represents 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.

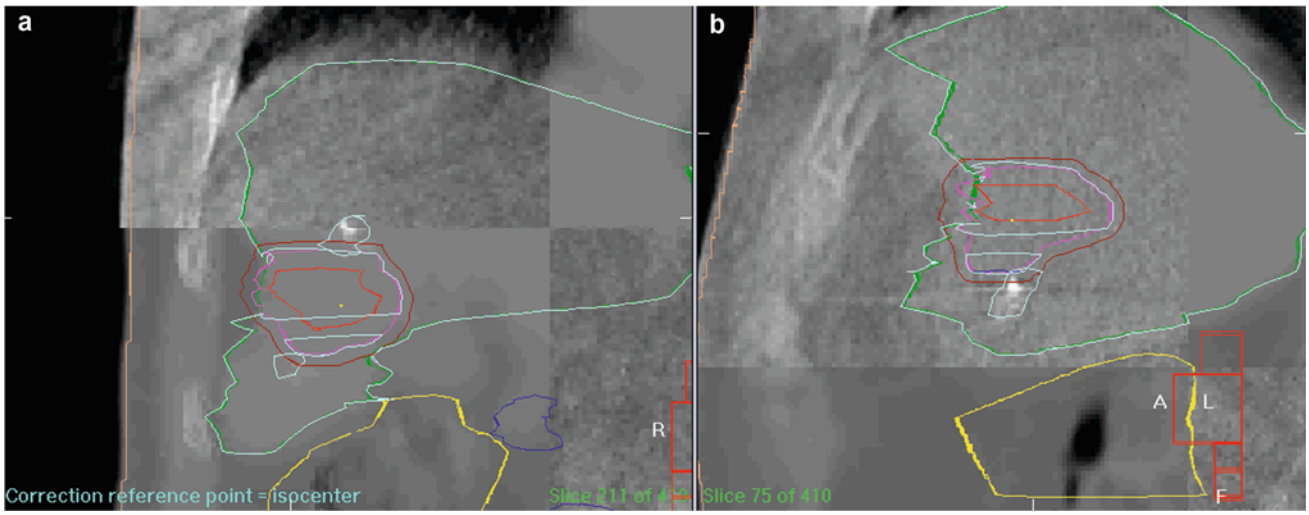


**Fig. 3** **a** Coronal and **b** sagittal cone-beam CT overlays from online imaging prior to a liver SBRT treatment. Quadrants **a** and **c** in each film represent the planning CT scan, while quadrants **b** and **d** represent the cone-beam CT taken at the time of delivery. The liver contour (*light 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

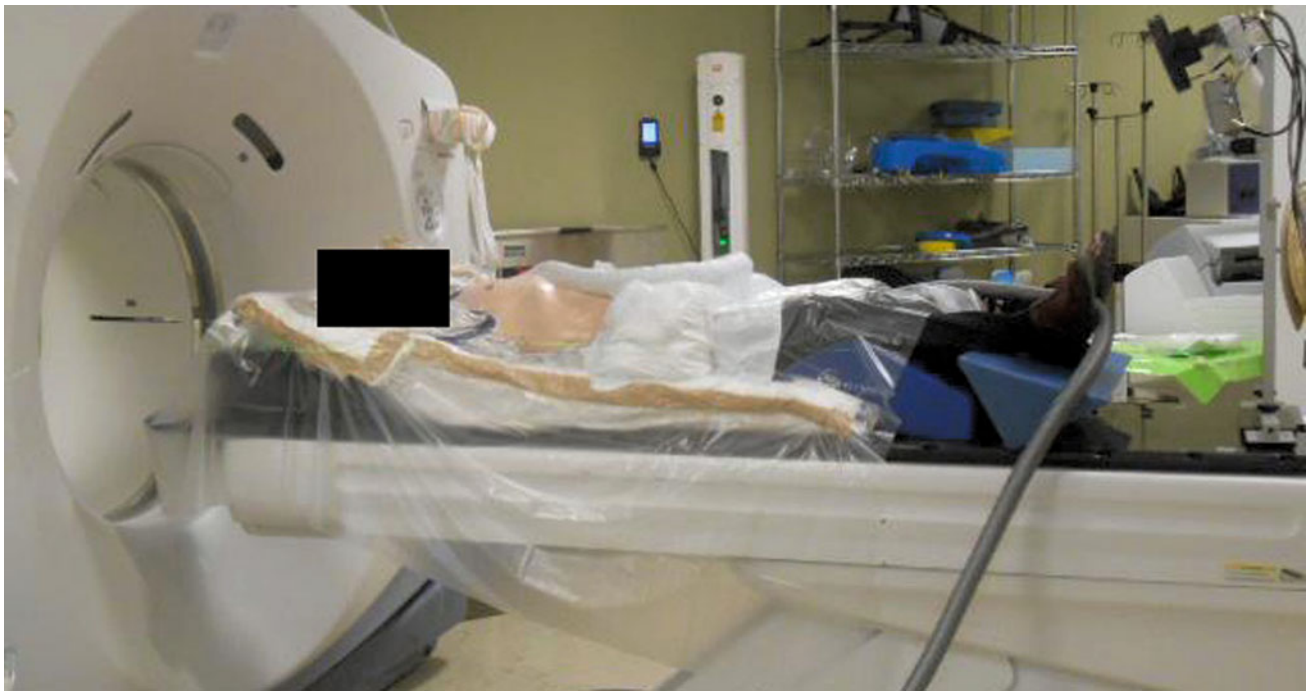

**Fig. 4** Sample patient undergoing radiation planning using a vacuum bag. This bag is placed around the patient and the air removed via vacuum. This both immobilizes the patient and prevents deep inspiration reducing respiratory motion

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

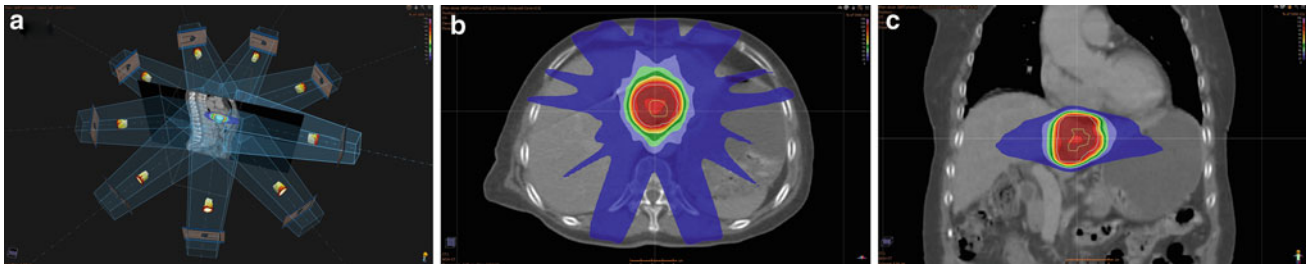


**Fig. 5** Representative SBRT plan for an intrahepatic tumor. A total of 50 Gy was delivered over 5 separate 10 Gy fractions. **a** Radiation planning representation of a nine-beam SBRT plan. **b** Axial CT slice through the isocenter showing the GTV (*yellow unfilled central contour*) being covered 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 ($\leq$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/m$^2$. 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.

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# Symptomatic Management and Palliation

Laura Lambert

## Contents



**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

L. Lambert (✉)
Division of Surgical Oncology and Palliative Medicine, UMass Memorial Medical Center, 55 Lake Avenue North, Worcester, MA, USA
e-mail: laura.lambert@umassmemorial.org

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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 choledochojejunostomy. 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

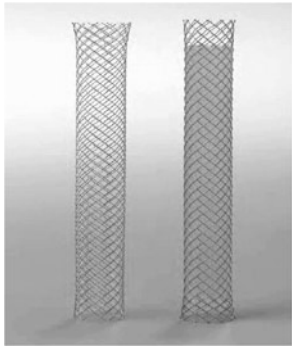

**Fig. 1** Uncovered (*left*) and covered (*right*) self-expandable metal stents (*SEMS*) (from first edition of book—needs permission)

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 actives 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 a 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 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

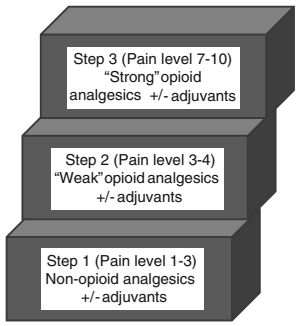


**Fig. 2** Representation of World Health Organization analgesic ladder

**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

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, diamorphine, 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 hypesthsias, 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<br>CTZ | Prokinetics | Metoclopramide, domperidone |
| Intestinal obstruction/peritoneal irritation | D2<br>H1<br>ACHm | CTZ<br>VC<br>Intestine | Phenothiazines<br>Antihistamines<br>Anticholinergics | Prochlorperazine<br>Diphenhydramine<br>Hyoscine |
| Chemotherapy/PONV | 5-HT3 | Intestine<br>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**: *Patients will lose contact with their primary physician*<br>**Truth**: The referring physician (i.e., primary care provider or oncologist) continues to play an important role in the patients' care |
| **Misconception**: *Hospice patients cannot go to the hospital*<br>**Truth**: Hospice patients can be hospitalized as needed for symptoms that cannot be managed at home or at a hospice house |
| **Misconception**: *Patients can use up their hospice eligibility*<br>**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**: *Patients cannot come off hospice if a new treatment comes available*<br>**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**: *Patients enrolling in hospice have to choose not to be resuscitated*<br>**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**: *Patients on hospice cannot receive parenteral nutrition*<br>**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**: *Hospice patients cannot participate in research projects*<br>**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.

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# Future Directions

Joseph M. Herman, Lauren M. Rosati, Timothy M. Pawlik, and Charles R. Thomas Jr.

## Contents



**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.

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

J. M. Herman (✉)
Department of Radiation Oncology and Molecular Radiation Sciences, Johns Hopkins University, Baltimore, MD, USA
e-mail: jherma15@jhmi.edu

L. M. Rosati
Department of Radiation Oncology, Johns Hopkins University, Baltimore, MD, USA

T. M. Pawlik
Department of Surgery, Johns Hopkins Hospital, Baltimore, MD, USA

C. R. Thomas Jr.
Department of Radiation Oncology, Oregon Health and Science University, Portland, OR, USA

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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 interaortocaval (16b1) 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 L1CAM 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-kB 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.

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