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Universidad del País Vasco
Euskal Herriko Unibertsitatea

Departamento de Fisiología
Facultad de Medicina y Enfermería

Cholangiocarcinoma landscape in Europe: diagnostic, prognostic and therapeutic insights from the ENSCCA Registry

Tesis presentada por

LAURA IZQUIERDO SANCHEZ

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instituto de investigación sanitaria

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Laura Izquierdo Sánchez

Para la obtención del título de doctora en

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Tesis dirigida por

Dr. D. Jesús María Bañales Asurmendi

Dr. D. Pedro Miguel Rodrigues

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It's not how much you have,

but what you do

with what you have.

That makes the difference.

Table of content

Introduction

Cholangiocarcinoma (CCA) comprises a heterogenous group of aggressive malignancies arising along the bile ducts.[1–3] It represents the second most common primary liver cancer after hepatocellular carcinoma (HCC), accounting for 10-15% of liver malignancies, 3% of gastrointestinal cancers, and 2% of all cancer-related deaths every year worldwide.[2] CCA commonly emerges from the malignant transformation of the epithelial cells lining the bile ducts, named cholangiocytes, although it is also hypothesized that it may originate from hepatic stem cells, progenitor cells in the peribiliary glands, or even from mature hepatocytes undergoing trans-differentiation.[4,5] CCAs are highly desmoplastic tumors, presenting an extensive stroma, and are extremely heterogeneous at the topographical, morphological and biological levels, as well as in their clinical presentation and evolution.[4] Patients with CCA are in general asymptomatic in early states, resulting in late diagnosis. This together with the chemoresistance nature of CCA tumors strongly limit the accessibility to therapeutic options with curative intent (mainly surgery) resulting in dismal prognosis. Significant international efforts have been done during the last decade to understand the CCA complexity at the molecular, histopathological and clinical levels in order to improve patient's welfare and outcome.

I.1. Anatomical and histopathological classification

I.1.1. Anatomical classification

Considering the potential differences in etiopathogenesis, risk factors, incidence and prognosis, the latest International Classification of Diseases 11th Edition (ICD-11) published by the World Health Organization (WHO) classified CCAs according to their anatomical origin in intrahepatic (iCCA, 2C12), perihilar (pCCA, 2C18) or distal (dCCA, 2C15) (**Fig. I.1.**).[6] iCCAs arise between the bile ductules and the segmental bile ducts and represent 10-20% of all CCAs. pCCAs, historically referred as Klatskin tumors, constitute the most frequent CCA subtype (50-60%), emerging in the right, left or the confluence of both (hilum) hepatic ducts, while dCCAs are located in the common bile duct and account for 20-30% of all CCAs.[2] Noteworthy, previous classification systems have traditionally classified CCAs with a dichotomous code as iCCA or extrahepatic CCA (eCCA), without considering a specific classification for pCCA and dCCA.[1,7] As a result, pCCAs were previously classified as extrahepatic or iCCA depending to the guideline, adding more complexity and generating confusion in the interpretation of the epidemiological trends of CCA subtypes. The use of the term eCCA is now discouraged by the ICD-11 as there are increasing evidence indicating that pCCAs and dCCA (and

also iCCAs) as distinct entities, with different clinicopathological features, management and outcome.

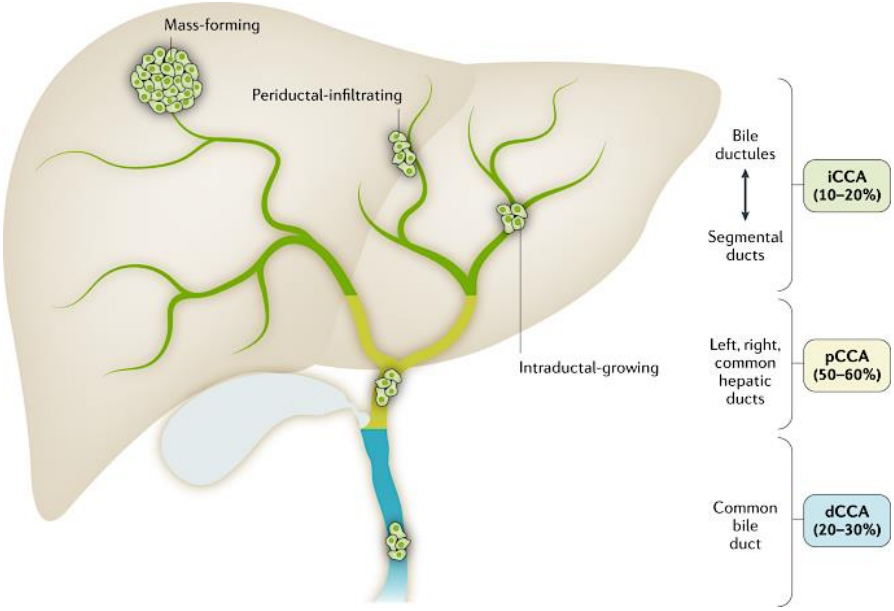


Figure I.1. Anatomical classification of cholangiocarcinoma. According to their anatomical origin, CCAs are classified as intrahepatic (iCCA), perihilar (pCCA) or distal (dCCA). (Obtained from Banales JM, *et al.*, 2020)[2]

I.1.2. Patterns of growth and morphology

According to the pattern of growth iCCAs are categorized into mass-forming (MF), periductal-infiltrating (PI) or intraductal-growing (IG) types (**Fig. I.1.**).[2,8] The MF is the most common iCCA type, accounting for 78% of all cases, and is characterized by a solid mass within the hepatic parenchyma, in the intrahepatic small bile ducts, which can evidence central necrosis or scarring. On the other hand, CCAs arising from the intrahepatic large bile ducts may show either PI (16%) or IG (6% of all iCCAs) growth.[8] PI-iCCAs are characterized by longitudinal spreading along the bile duct, which typically derives in biliary strictures leading to peripheral bile ducts dilation and cholestasis, while, IG-iCCA proliferates within the lumen of the bile duct, sharing features with intraductal papillary neoplasms (**Fig. I.2.**).[8,9] Of note, these three patterns of growth may overlap in a number of combinations within the same tumor. Both pCCA and dCCA display flat or nodular sclerosing tumor features (73% of all p/dCCAs), which are similar to the PI type of iCCAs, or less frequently, present an intraductal papillary growth (27%), which resembles the IG-iCCA cases.[8]

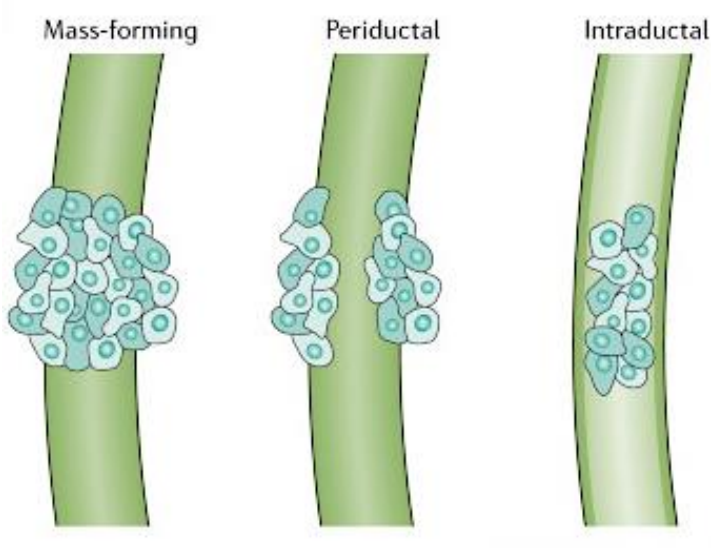


Figure I.2. Patterns of growth of cholangiocarcinoma. Intrahepatic CCAs are cathegorized according to their main pattern of growth in: mass-forming, periductal-infiltrating or intraductal-growing.(Adapted from Banales JM, *et al.*, 2016)[1]

Patients with CCA may also present underlying precursor lesions likely as a result of biliary tract epithelial cell hyperplasia caused by long-standing chronic inflammation of the bile ducts and/or chronic injury of the biliary epithelia. These pre-malignant lesions, when undergoing a multistep process that may result in their growth and transformation, could lead to dysplastic tumor masses and eventually to malignant adenocarcinomas.[10] Two main subtypes of preinvasive lesions are recognized by the WHO Classification of Tumours, including biliary intraepithelial neoplasia (BilIN), mostly leading to the development of flat or nodular sclerosing p/dCCAs or PI-iCCAs, and intraductal papillary neoplasm (IPNB) or tubulopapillary neoplasms (ITPN) of the bile ducts, associated with the appearance of intraductal papillary or tubular growth p/dCCAs and IG-iCCAs.[8] BilIN are characterized by the presence of cuboidal or columnar cells with varying degrees (1-3) of cellular and nuclear atypia. While mucin secretion is usually absent a at ITPN, IPNB are commonly characterized by mucin hypersecretion.[8,10] Notably, no precursor lesions have yet been described for MF-iCCAs.

I.1.3. Histological classification

Histologically, CCAs are well-, moderately-, or poorly-differentiated adenocarcinomas. Additionally, they may be classified as “cholangiolocellular carcinoma”, “conventional” type, or “rare variants”. To this extent, cholangiolocellular carcinoma is believed to arise from progenitors cells in the canals of Hearing and have typical trabecular growth. Conventional type includes iCCAs arising from *i)* small bile ducts, growing as small-sized tubular or acinar adenocarcinomas with nodular growth invading the parenchyma, and with no or minimal mucin secretion or *ii)* large bile ducts, constituted by tall columnar epithelium with mucin production invading into the duct wall and surrounding parenchyma.[8] On the other hand pCCA and dCCA are mainly mucin-producing adenocarcinomas or papillary tumors (**Fig. I.3.**).[2,8]

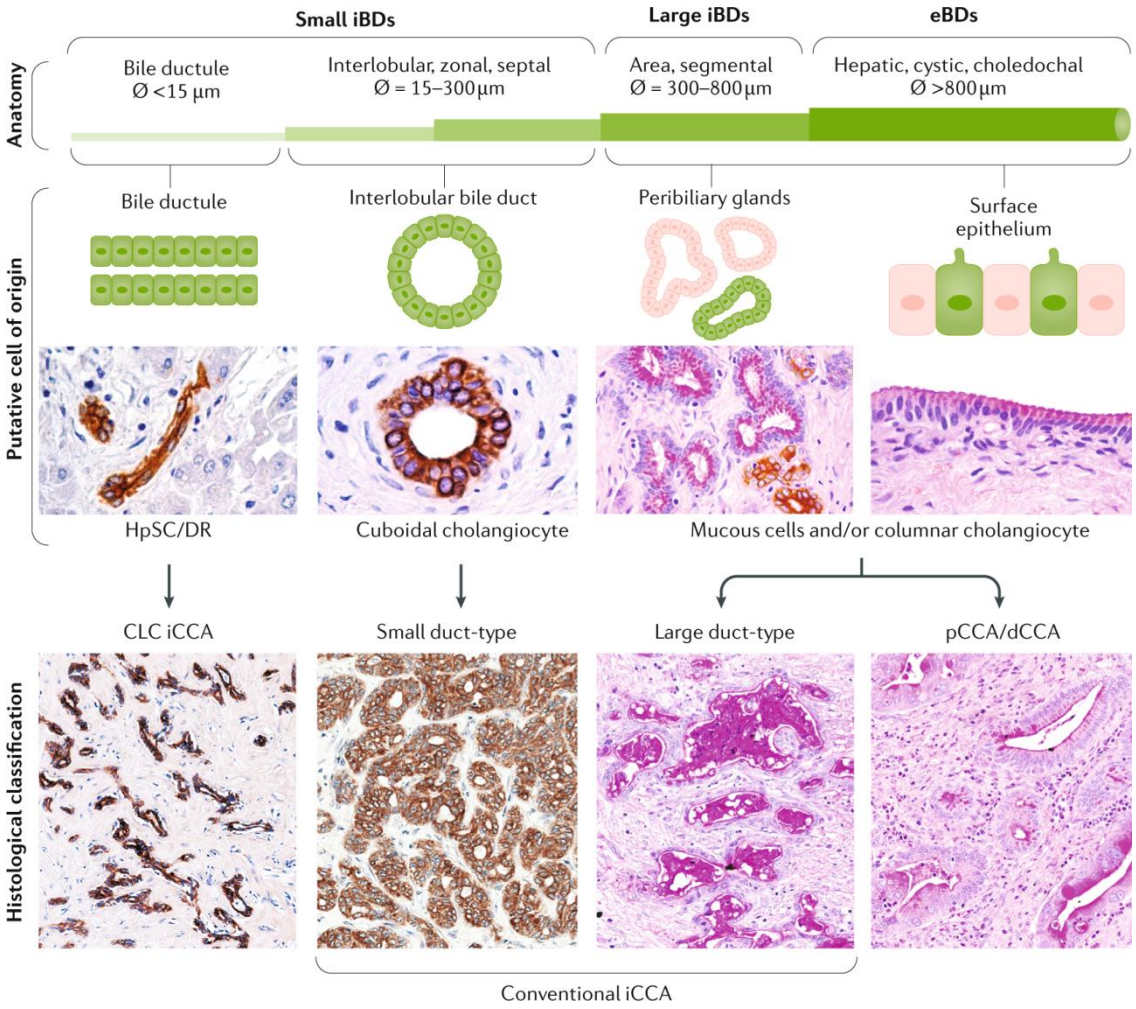


Figure I.3. Histological classification of cholangiocarcinomas. Histological CCA variants mirror the phenotype of the involved duct and the presumed cell of origin. *Abbreviations*: CCA, cholangiocarcinoma; dCCA, distal CCA; eBD, extrahepatic bile duct; iBD, intrahepatic bile duct; iCCA, intrahepatic CCA; pCCA, perihilar CCA. (Obtained from Banales JM, *et al.*, 2020)[2]

I.2. Epidemiology

The epidemiological trends of CCA and its subtypes differ geographically, probably as a result of the different prevalence of certain risk factors and to potential genetic predispositions. Despite being a rare cancer in many areas with less than 6 annual reported cases per 100,000 inhabitants, over the past 20 years, the incidence and mortality rates have been increasing worldwide.[1,2]

The incidence of CCA is low in Western countries (0.3-6 cases per 100,000 inhabitants yearly); however, there are endemic regions on Southeast Asia with significantly higher incidence.[1,11,12] Data referred to the 1971-2009 period reported an age-standardized incidence rate of 85 cases per 100,000 inhabitants in North East Thailand, 14.5 in North Central Thailand, 7.1-8.8 in South Korea, and 7.6 in Shanghai (China).[1] Of note, the higher incidence observed in these regions might be explained, at least in part, by infections with endemic liver flukes (*i.e.*, *Opisthorchis viverrini* and *Clonorchis sinensis*). Overall, a sustained worldwide increase in iCCA has been reported in the last few decades, whereas the rates of pCCA and dCCA seem to be stable or slightly diminishing.[1] Still, these trends should be interpreted with caution as a separate code for pCCA was not considered until 2022, which could have resulted in their registration as either iCCA or dCCA.[13] The potential changes in incidence between CCA subtypes may also be explained by changes on risk factors exposure.

According to data from the WHO and the Pan-American Health Organization (PAHO), global mortality rates are also alarmingly increasing during the last two decades (**Fig. I.4.**). CCA mortality was reported to be superior in men than women, and in Asian countries when compared with the West.[2,14] Hence, Asian population showed the highest age-standardized mortality rates for both iCCA [2.5 per 100,000 men in Hong Kong SAS] and eCCA [2.8 per 100,000 men in Japan].[14] Further, between Western and South American countries, whereas eCCA mortality rates appear to be <1/100,000 men, iCCA shows a heterogeneous topographical distribution; while France, Austria and Spain reported the highest mortality rates [>1.5 per 100,000 men], Latin America, Czech Republic and Lithuania [<0.6 per 100,000 men] showed the lowest.[14]

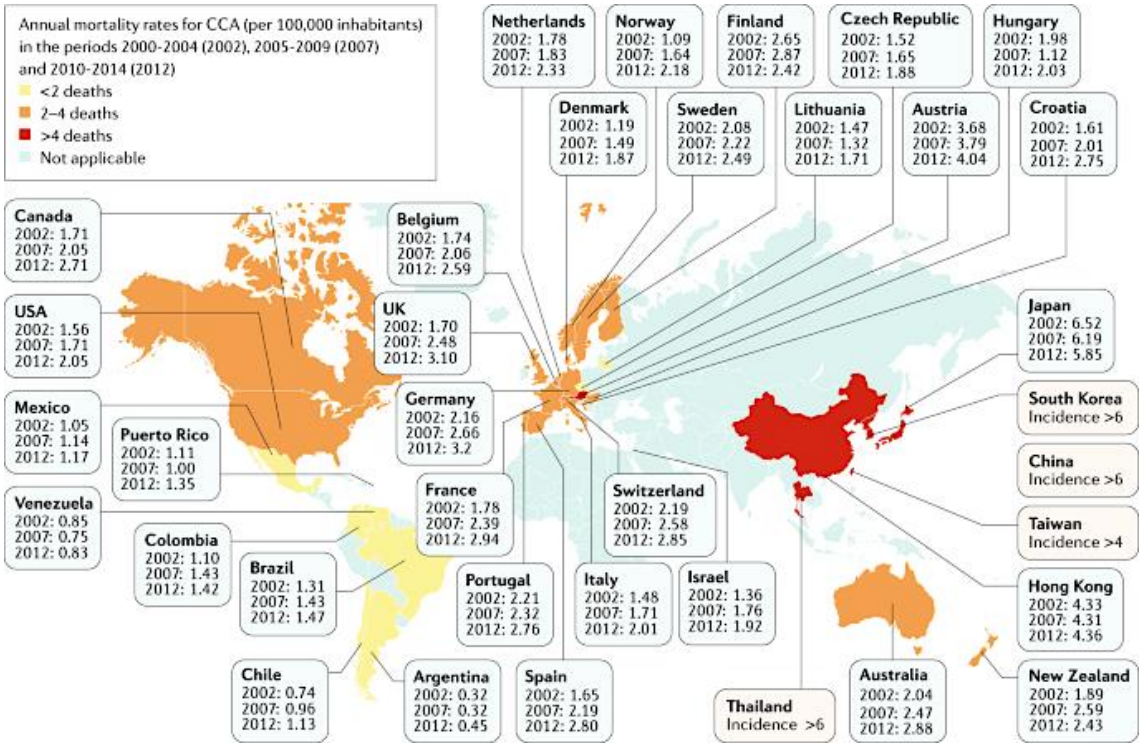


Figure I.4. Annual mortality rates of cholangiocarcinoma. Age-standarized annual mortality rates for CCA in 32 countries during the periods 2000-2004, 2005-2009, and 2010-2014. Incidence is reported in highly prevalent CCA regions where mortality rates were not reported.(Obtained from Banales JM, *et al.*, 2020)[2]

I.3. Risk factors

Different risk factors have been proposed to be involved in cholangiocarcinogenesis.[15–17] Still, in Western countries about 50% of patients remain diagnosed in the absence of identifiable conditions that predispose to the development of CCA. Of note, during the last years, large meta-analyses pointed out several factors that markedly increase the risk of CCA, including the presence of choledochal cysts, gallstones, hepatobiliary diseases (*i.e.*, Caroli´s disease, primary sclerosing cholangitis (PSC), and viral infections), cirrhosis, or liver flukes. In addition, several highly prevalent factors worldwide, such as alcohol-induced liver disease, type II diabetes, tobacco and non-alcoholic fatty liver disease (NAFLD), have been associated with a low risk of biliary carcinogenesis (**Fig. I.5.**). Some of these predisposing conditions are equally related between the different CCA subtypes, while others are more associated to certain subtypes.[15] These risk factors have different geographical distribution, which may be responsible, at least in part, to the different incidence rates worldwide.

Age and sex are related to CCA development, as most the patients are elder males. Moreover, some inherited genetic predispositions have also been described in a small proportion of cases. Stil, it is reasonable to think that there are undefined factors associted to CCA development that could explain the incrising incidence worldwide.

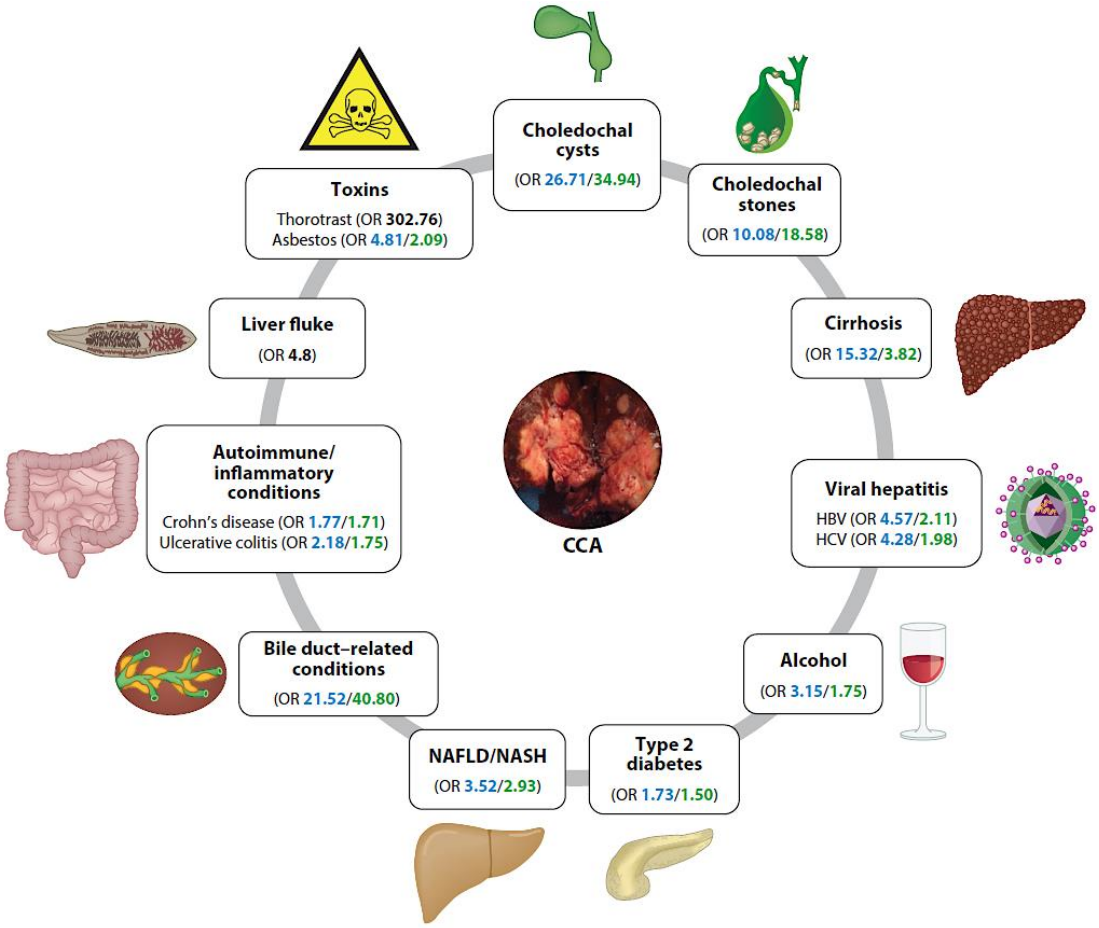


Figure I.5. Risk factors for cholangiocarcinogenesis. Risk factors and associated scores, measured as odds ratio, for the development of CCA (black) and its subtypes [iCCA (blue) and p/dCCA (green)]. *Abbreviations*: CCA, cholangiocarcinoma; dCCA, distal CCA; HBV, hepatitis B viruses; HCV, hepatitis C viruses; iCCA, intrahepatic CCA; NAFLD, nonalcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; OR, odds ratio; pCCA, perihilar CCA (Obtained from Rodrigues PM, *et al.*, 2021)[17]

I.3.1. Bile duct disorders

I.3.1.1. Congenital biliary tract disorders

Choledocal cysts (CC) are congenital enlargements of the bile ducts. It is consider a rare disorder in Western countries, with an incidence of approximately 1 in 100,000-150,000 children in the United States but it is of particular relevance in East Asian populations, with reports of 1 in 13,000 inhabitants.[18] Although CC typically appear within the first decade of life, nearly 20% remain undiagnosed until adulthood.[18] Noteworthy, several

studies have shown increased risk of malignant transformation with age at CC diagnosis. The majority of reported cases of malignant transformation are CCAs (60-80%) developed at a mean age of 32 years, much lower than in general population.[16,18] A recent meta-analysis has shown a strong association between CC and CCA development, with odds ratios (OR) of 26.7 (95% CI 15.8-45.2) for iCCA and 34.9 (95% CI 24.4-50.1) for eCCA, mainly associated with bile flow reflux and chronic biliary inflammation.[19]

Caroli's disease is a rare autosomal recessive inherited disorder characterized by multifocal segmental dilatation of large intrahepatic bile ducts. Caroli's disease has been recognized as one of the strongest risk factors for both iCCA and eCCA, with a 38- and 97-fold greater risk, respectively, and found in individuals above 68 years of age when compared with the general population.[20] Of note, a recent multicenter study reported that 7.1% of patients with Caroli's disease experience biliary malignant transformation, with a median age of 53 years at diagnosis.[21] Accordingly, patients with Caroli's disease seem to be at major risk for CCA with the age.

I.3.1.2. Gallstone disease

Gallstone disease is a chronic condition that preferentially appears between the forties and eighties.[22] It is endemic in certain Hispanic populations with prevalence rates of >50% at 50 years of age. In low-prevalence ethnicities, it is steadily increasing likely as a result of overnutrition and physical inactivity.[23]

Hepatolithiasis refers to the presence of gallstones in the confluence of the right and left hepatic ducts. This bile duct disorder has been reported as a major risk factor for iCCA development.[22] In fact, an OR of 50.0 has been reported in the Korean population, as well as an OR of 6.7 in an Italian case-control study.[16]

Choledocholithiasis is characterized by the appearance of gallstones within the common bile duct while cholelithiasis refers to its presence inside the gallbladder. Conversely to hepatolithiasis, they showed stronger association for eCCA [choledocholithiasis: OR 18.6 (95% CI 11.1-31.2); cholelithiasis: OR 5.9 (95% CI 3.1-11.3)] than for iCCA [choledocholithiasis: OR 10.1 (95% CI 5.5-18.5); cholelithiasis: OR 3.4 (95% CI 1.9-5.9)].[19]

I.3.1.3. Primary sclerosing cholangitis

PSC is a chronic cholestatic and immune-mediated disease affecting the bile ducts. It is characterized by fibro-inflammatory biliary stenosis and the subsequent obstruction of

intrahepatic and/or extrahepatic bile ducts.[24] PSC is a rare disease, affecting <5 per 10,000 inhabitants in the EU (fewer than 250,000 individuals across EU), and less than 200,000 person in the US.[24] Results from the USA Surveillance, Epidemiology and End Results (SEER) registry reported a solid association between PSC and CCA development, with an OR of 21.5 (95% CI 17.2–26.9) for iCCA and 40.8 (95% CI 34.9–47.6) for eCCA.[20] In fact, patients with PSC usually enroll on specific screening programs for early detection of CCA. Alike isolated PSC, PSC-associated CCA displays an ascending incidence gradient from Eastern to Western and from Southern to the Northern countries.[25]

I.3.2. Liver diseases

I.3.2.1. Hemochromatosis

Hemochromatosis is a genetic disorder arising in individuals harboring homozygous mutation in the *C282Y* gene (HFE1 protein), and characterized by the pathological accumulation of iron and secondary tissue damage, particularly in the liver. Overall, individuals with the *C282Y* mutation in homozygosity account for 82-90% of all hemochromatosis diagnosis in northern European descents, with a prevalence of 1 case per 200 inhabitants.[26] Hemochromatosis is a well-established risk factor for HCC development.[26] Results from the USA SEER registry reported a 2.1-fold increase risk [OR 2.1 (95% CI 1.3-3.2)] for iCCA; however, no association was found for eCCA.[27]

I.3.2.2. Viral infections

Hepatitis B (HBV) and C (HCV) virus chronic infections are strong predisposing factors for HCC, but also for CCA development, with a greater association for iCCA.[16] Accordingly, OR of 4.6 (95% CI 3.4-6.1) and 2.1 (95% CI 1.6-2.7) have been reported in patients with iCCA and eCCA infected with HBV and 4.3 (95% CI 3.0-6.2) and 1.5 (95% CI 0.9-2.4) for HCV-infected individuals, respectively. Indeed, the association between viral hepatitis and iCCA incidence showed different geographical distributions. Whilst Western populations showed higher frequency of HCV-related iCCAs, Asian countries are characterized by more prevalent HBV-related iCCAs.[16,28] Of note, the mechanisms by which these hepatotropic viruses cause CCA likely rely not only on the presence of cirrhosis, but also on direct carcinogenic effects of these viruses on cholangiocytes and hepatic progenitor cells.[28]

Epstein-Barr virus (EBV) is a member of the *Herpesviridae* family, which has largely been associated to the development of lymphoid and epithelial neoplasms, such

as Hodgkin's lymphoma, nasopharyngeal and gastric carcinomas.[29] In addition, some case reports and small series have also evidenced the role and impact of EBV in the development of iCCA.[29–31] Recently, a large series including 303 iCCAs has evaluated by *in situ* hybridization the presence of EBV, showcasing a prevalence of 6.6%.[32] Noteworthy, the vast majority presented more frequently lymphoepithelioma-like iCCA, which histologically appears as undifferentiated epithelial cells with lymphoid response.[30,32]

I.3.2.3. Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) includes a spectrum of clinicopathologic lesions, ranging from simple steatosis (NAFL) to non-alcoholic steatohepatitis (NASH), eventually progressing to cirrhosis. NAFLD is highly prevalent in all continents, with a global estimate prevalence of 24%. Specifically, the highest prevalence rates are reported in South America (31%), Asia (32-27%), the USA (24%) and Europe (23%), whereas it is less common in Africa (14%).[33] Accumulating evidence suggest that NAFLD is associated with an increased risk of various cancers, including HCC and CCA, particularly iCCA. According to a meta-analysis including 7 case-control studies, an OR of 2.2 (1.5–3.2) was reported for iCCA, while a milder association was found in eCCAs [OR 1.6 (1.0–2.3)].[34]

I.3.2.1. Liver cirrhosis

Cirrhosis is a clinical manifestation of late-stage liver disease. Cirrhosis of different etiologies has been identified as an underlying condition increasing the odds for primary liver cancers, especially HCC and iCCA. Indeed, iCCA has shown a pooled OR of 15.3 (95% CI 9.3-25.2), while eCCA presented lower association with an OR of 3.8 (95% CI 2.6-5.7).[19] The pathogenic mechanisms behind tumorigenesis in cirrhotic livers is explained, at least in part, by the release of inflammatory cytokines, hepatocellular death, compensatory proliferation and regenerative responses.[35]

I.3.3. Digestive tract diseases

I.3.3.1. Inflammatory bowel disease

Inflammatory bowel disease (IBD) is an umbrella term used to describe disorders that involve non-infectious chronic inflammation of the gastrointestinal tract, namely Crohn's disease and ulcerative colitis (UC). Several meta-analyses have pinpointed IBD as a predisposing factor for CCA, probably due to the underlying bile duct inflammation

observed in some of these individuals. Thus, patients with IBD have been reported to have a pooled OR of 2.7 (95% CI 1.8-4.0) for iCCA and 2.4 (95% CI 1.3-4.2) for eCCA.[19] Noteworthy, since the majority of patients with PSC (70-80%) concomitantly display IBD, particularly UC, the association of IBD and CCA development mainly arise as a results of the marked relationship between PSC and CCA. Nevertheless, the association between IBD and cholangiocarcinogenesis was shown to be regardless the presence of PSC,[36] probably as the result of the gut leakage and subsequent migration of bacterial components into the liver.

I.3.3.2. Chronic pancreatitis

Chronic pancreatitis is a syndrome involving inflammation, fibrosis, and loss of acinar and islet cells, in which repetitive episodes lead to unrelenting abdominal pain, malnutrition, and exocrine and endocrine pancreatic insufficiency.[37] Notably, 3-23% of patients with chronic pancreatitis develop biliary strictures, leading to an increased risk of CCA. Hence, a positive association between chronic pancreatitis and CCA has been reported, being stronger for eCCA [OR 6.6 (5.2-8.4)] than iCCA [OR 2.7 (1.7-4.1)].[27]

I.3.4. Liver flukes infection

Opisthorchis viverrini and *Clonorchis sinensis* are flatworm parasites (also called flukes) that colonize the bile ducts after human infestation *via* the ingestion of raw, pickled or undercooked infected fish. Despite anti-helminthic treatment, the infection tends to be chronic, leading to long-lasting periportal inflammation (cholangitis). As a consequence, approximately 10% of people infected with liver flukes are likely to develop CCA.[16] A meta-analysis of case-control studies reported a robust association between liver fluke infections (*O. viverrini* or *C. sinensis*) and CCA [OR 4.8 (95% CI 2.8-8.4)].[38]

I.3.5. Metabolic and endocrine disorders

Metabolic syndrome is composed of a bundle of interrelated factors, including type II diabetes, obesity, dyslipidemia and arterial hypertension. During the last decade, several studies have demonstrated that metabolic syndrome is associated with CCA development, presenting an OR of 1.9 (95% CI 1.3-2.7) with stronger association for iCCA than eCCA.[39] Worth mentioning, each one of the single conditions related with the metabolic syndrome have also been independently described to predispose to CCA development.

Type II diabetes has been reported to be positively related to CCA development. The USA SEER registry reported an OR of 1.5 (95% CI 1.4-1.7) for iCCA and 1.5 (95% CI 1.3–1.6) for eCCA.[27] Conversely, a Chinese hospital-based case-control study showed a positive association to iCCA (OR 4.6 (95% CI 2.8-7.6)], while no risk was observed for patients with eCCA [OR 1.0 (95% CI 0.5-1.9)].[39] Overall, a recent meta-analysis shown pooled OR of 1.7 (95% CI 1.5-2.0) and 1.50 (95% CI 1.31-1.71) for iCCA and eCCA, respectively.

On the other hand, the specific role of obesity (regardless NAFLD) in CCA development is still controversial and evidences are not strong enough to reach rock solid conclusions. For instance, a recent meta-analysis consisting of 7 case-control studies did not revealed a relevant association with either iCCA nor eCCA.[19] However, the USA SEER registry reported a positive correlation for iCCA [OR 1.4 (95% CI 1.2–1.7)] but not for eCCA,[27] while another single-center study associates both subtypes with CCA development [OR 2.1 (1.3-3.4) for iCCA; OR 1.8 (1.1-2.8) for eCCA].[39] Similarly, arterial hypertension did not reach statistical significance on a meta-analysis including USA and Asian case-control studies.[19] However, single-site studies stated positive associations with CCA.[27,39] Therefore, further studies, including larger cohorts of patients should be included in order to obtain more robust data and undoubtedly evaluate if obesity and arterial hypertension are actually associated with CCA development.

Overall, whether the potential association of these metabolic disorders and CCA may be direct or due to the co-occurrence between factors remains unclear. Since most of these metabolic conditions (including NAFLD) are interconnected, it is of upmost importance to understand if some of the factors are cooperating in order to promote CCA, or if they represent single risk factors *per se*. Furthermore, considering that obesity is a major public health problem whose prevalence is exponentially growing, especially in Western countries, the study of the potential risk of obesity and its related conditions with CCA deserves future consideration.

I.3.6. Life style and environmental exposure

I.3.6.1. High alcohol consumption

A meta-analysis comprising ten case-control studies reported that high alcohol intake (>80g/day) associates with increased risk of iCCA [OR 2.8 (95% CI 1.5-5.2)].[40] Likewise,

later studies have shown a link between alcohol abuse and CCA development in both subtypes, despite an higher for iCCA [OR of 3.2 (95% CI 2.2-4.4)] than eCCA [OR of 1.8 (95% CI 1.2-2.6)] was observed.[19]

I.3.6.2. Tobacco smoking

Tobacco use has also been postulated as a potential risk factor for CCA, although the data was controversial. The latest meta-analysis showed a slightly positive association of smoking with CCA development, with OR of 1.3 (95% CI 1.1-1.5) and 1.7 (95% CI 1.3-2.2) for iCCA and eCCA, respectively.[19] In addition, no differences on CCA risk rate was found between current smokers and ever smokers.[41]

I.3.6.3. Toxin exposure

Exposure to different environmental carcinogens has been widely described to be associated with cholangiocarcinogenesis, as is the case for Thorotrast, 1,2-dichloropropane and asbestos. Some people have developed CCA decades after administration of Thorotrast, a radiographic contrast agent that has been reported to have an estimated 303-fold increased risk for CCA.[42] However, this compound has been banned since 1969, so the current danger is almost negligible. A retrospective study evaluating the chronic exposure to 1,2-dichloropropane, an organic solvent used in printing, correlated as a causative factor for CCA development with a relative risk of 17.1 (95% CI 3.8-76.2) for highly-exposed individuals.[43] Few case-control studies also suggested that asbestos is probably a causative factor for CCA development. Thereby, a strong risk for iCCA has been reported among subjects occupationally exposed to asbestos for over 30 years with an OR of 4.8 (95% CI 1.7-13.3), although no association was found with eCCA.[44]

I.3.7. Genetic predisposition

Host genetic polymorphisms involved in the metabolism of chemical carcinogens, DNA repair, and inflammation, among others, have been found to increase patients' susceptibility to CCA. Polymorphisms in the glutathione S-transferase (*GST*) gene have been shown to increase CCA susceptibility either alone [*GSTO1**D140: OR of 8.5 (95% CI 2.1-37.9)] or in cooperation with environmental factors.[45,46] Thus, patients harboring deficiency in the *GSTT* gene exhibited upraised risk for CCA development on former alcohol abusers [OR 27.9 (95% CI 1.8-424.6), as well as in those with serological positivity for *O. viverrini* [OR 18.0 (95% CI 3.3-97.4)].[46] On the other hand, the *CYP1A1*

gene, encoding for the enzyme aryl-hydrocarbon hydroxylase, has been found to decrease the odds for CCA among smoker males harboring the *CYP1A2**1A/*1A genotype [OR 0.3 (95% CI 0.1-0.9)].[47] Another study evidenced that 677CC variants in the 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene, combined with polymorphisms in thymidylate synthase enhancer region (*TSER*) increased patients susceptibility for cholangiocarcinogenesis, with an OR of 5.4 (95% CI 1.2-23.6).[48] The multidrug resistance-associated protein 2 (*MRP2/ABCC2*) has been related to HCC, but based on a case series of 60 CCAs and 73 healthy individuals, the common variant c.3972C>T in exon 28 has also been associated to CCA development, resulting in an OR of 1.8 (95% CI 1.1-3.1).[49] Besides, alterations in genes codifying for key proteins in DNA damage repair pathway such as human oxoguanine glycosylase 1 (*hOGG1*) and MutY homolog (*MUTYH, MYH*) have been described to influence CCA development. Indeed, individuals with T/G genotype in *MYH*rs3219476 gene were found to be protected from cholangiocarcinogenesis [OR 0.5 (95% CI 0.2-0.8)].[50] Finally, polymorphisms in the natural killer cell receptor G2D gene (*NKG2D*) were found associated with greater risk of CCA, particularly in patients with PSC [rs11053781, OR 2.1 (95% CI 1.3-3.3); and rs2617167, OR 2.3 (95% CI 1.5-3.7)].[51]

The convenience of next-generation sequencing has revealed not only somatic genetic alterations with therapeutic implications in CCA but also germline mutations useful for potential therapeutic targeting and to implement screening strategies in such individuals as a way to early predict CCA development. In this line, germline mutations, which may predispose to cholangiocarcinogenesis were identified in around 5-10% of patients with CCA. A recent study including 131 patients with biliary tract cancer (BTC), from which 63.4% presented iCCA and 16% eCCA, showed that 9.9% of patients harbored a high/moderate-penetrance cancer predisposition gene, with *BRCA1/2* been the most commonly observed. Indeed, 43% of patients showed biallelic inactivation with loss of heterozygosity in *BRCA1/2* in tumor regions, supporting a role for these germline mutations in tumor etiology.[52] In addition, germline mutations in *ATM* and *BAP1* were also identified. Of note, *ATM* has long been recognized as a susceptibility gene for breast and pancreatic cancer.[53] A single-case report found loss of heterozygosity at the *ATM* region of a 36 years-old woman with iCCA.[52] Last, *BAP1* germline mutations have been related to several cancer types, as mesothelioma, cutaneous melanoma, renal cell carcinoma, and basal cell and squamous cell carcinoma. Latest case control studies showed loss of heterozygosity and lack of nuclear expression in iCCA tumor tissue, suggesting BAP1 functional protein loss in cancer cells.[52,54]

I.4. Clinical presentation and diagnosis

The diagnosis of CCA represents an incidental finding in a significant proportion of cases (25%). However, patients with CCA can develop different unspecific symptoms, like jaundice, fiber, severe weight loss, fatigue and abdominal pain, among others. Clinical manifestations of CCA depend on the anatomical location of the primary tumor and disease status (**Fig. I.6.**). Whilst jaundice is the most common and characteristic symptom of eCCA, only about 10-15% of iCCA cases present this symptom at an early stage, being mainly associated with hilar obstruction due to lymph node compression.[17] Conversely, iCCAs are mostly found to be asymptomatic, or mainly present constitutional symptoms including abdominal pain, asthenia, nausea or weight loss. Thus, it is not surprising that around 20-25% of patients with iCCA have an incidental diagnosis, either through detection of altered liver function tests or by imaging studies for unrelated reasons.[55,56]

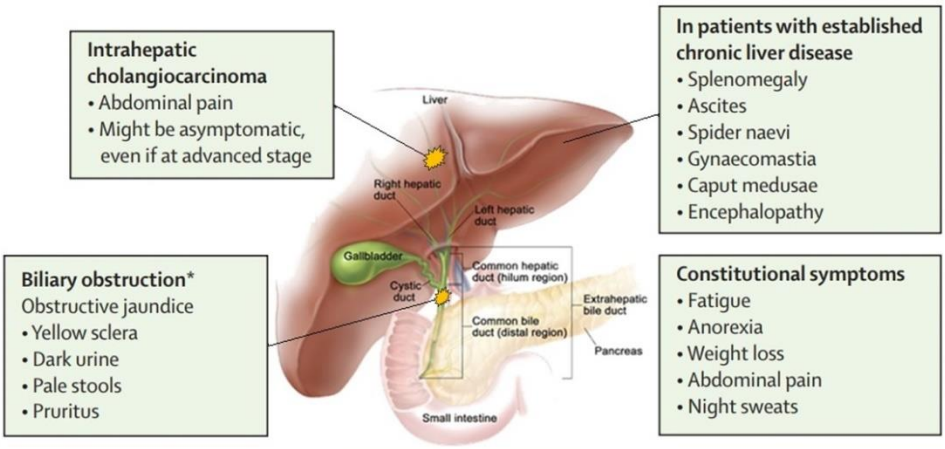


Figure I.6. Clinical presentation of cholangiocarcinoma. Presence and nature of symptoms associated to CCA. *Biliary obstruction can happend due to the tumor itself, or because of lymph node compression at the hilium. (Adapted from Valle JW, *et al.*, 2021)[56,57]

In the presence of the aforementioned symptoms, it is important to perform a careful physical examination including the identification of risk factors and the Eastern Cooperative Oncology Group (ECOG) performance status. All at once, the presence of hepatomegaly, splenomegaly, ascites, abdominal collateral circulation, encephalopathy, and other kind of established signs of chronic liver disease should also be assessed.[55,56]

Following initial physical examination, liver-related biochemical tests are required before imaging or further biopsy and histopathological analysis. An increase in serum levels of bilirubin, alkaline phosphatase (ALP), and gamma-glutamyl transpeptidase

(GGT) are frequently observed in patients with tumor-related obstructive cholestasis.[55] In contrast, liver enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are expected to be normal or minimally elevated, in particular at early tumor stages or in non-cirrhotic patients.[55]

The use of non-specific serum tumor markers is also common on clinical routine. Nonetheless, the carcinoembryonic antigen (CEA) and the carbohydrate antigen 19-9 (CA19-9) seem to have low sensitivity and specificity, particularly at early tumor stages, resulting useless as diagnostic tool for screening in individuals at high risk. This is particularly evident for CA19-9, which can appear elevated in many other benign biliary diseases related to inflammation and cholestasis, including patients with isolated PSC.[58] Conversely, this tumor marker was proposed as a potential tool for assessing prognosis and/or treatment response.[58] For instance, elevated pre-surgical serum levels of CA19-9 may determine the prognosis of patients with CCA before tumor resection. Thus, patients with resectable CCA and high levels of perioperative CA19-9 (≥200 IU/mL) displayed lower post-operative survival rates, with patients exhibiting the best outcome with the tumor marker under 37 IU/mL (3-year survival rate: 51% vs 69%).[59] Moreover, patients undergoing CA19-9 serum level normalization (<37 IU/mL) after surgery had a significant survival benefit compared with those mantaining the marker elevated (3-year survival rate: 73% vs 31%).[60] Regarding response to therapy, decreased serum levels of CA19-9 (<1,000 IU/mL) but not CEA have been associated with a better response to Gem monotherapy, and a succeeding reduction of the tumor marker levels of at least 50-fold during the administration period appeared as an early indicator of good response.[61]

I 4.1. Imaging studies

Cross-sectional imaging, such as ultrasonography (US), computed tomography (CT), magnetic resonance (MRI) and cholangiopancreatography (MRCP), as well as positron emission tomography (PET) are key for the diagnosis, staging, monitoring and assessment of treatment response in patients with CCA.[62] Of note, a multimodality approach is frequently applied, combining the advantages of various imaging modalities and providing accurate data on tumor extent and spread.

As first-line imaging examination, transabdominal US is commonly used to investigate the cause of the suspected bile duct obstruction and to characterize space-occupying lesions within the liver. Importantly, when CCA is suspected in a US-based

observation, further screening is generally performed with a second-level imaging technique that allows for tumor staging.[56,63]

CT scanning is considered the standard imaging modality for diagnosis and staging of BTCs.[62] It provides a comprehensive evaluation of the primary tumor, the extent of tumor invasion into the hepatic artery, portal vein, and hepatic parenchyma, and whole abdomen surveillance to assess for potential metastasis. However, CT scan has failed underestimating or even neglecting longitudinal tumor spread along the bile duct, especially in pCCA.[64] Conventional CT has largely been shown to have a limited ability to estimate tumor resectability,[65,66] though, recent studies have evaluated the preoperative accuracy of high-resolution CT, showcasing negative and positive predictive values of 92% and 85%, respectively.[67]

In recent years, MRI, especially in combination with MRCP, has improved the diagnosis of CCA and the prediction of tumor resectability, constituting now the optimal initial investigation techniques for suspected CCA.[62] MRI can assess local tumor size and extension, vascular patency, lymph node invasion, metastasis and hepatic parenchymal abnormalities. Of note, MRCP allows the evaluation of bile ducts above and below a total obstruction, being of great relevance for CCA diagnosis. Thus, multiparametric imaging with MRI may be of great value in the detection of bile duct invasion and occult intrahepatic metastasis, guiding adequate surgical decisions.[68]

PET, particularly using 18-fluorodeoxyglucose (^{18}FDG), permits the visualization of CCAs as small as 1 cm, and is of value for the differentiation between benign and malignant biliary strictures, as well as for determining lymph node invasion and distant organ metastasis. Hence, after CT or MRI/MRCP imaging, ^{18}FDG-PET may be a useful tool in tumor staging, thus allowing a definite selection of the patients that may benefit from tumor resection.[64,69] Nevertheless, PET has been shown to provide false-positive findings in patients with biliary inflammation, or even false-negative results, particularly resulting in a misdiagnosis of mucinous CCAs.[70]

I 4.2. Endoscopic approaches

In addition to imaging approaches, invasive techniques such as endoscopic retrograde cholangiography (ERCP), endoscopic ultrasound (EUS), or cholangioscopy are also performed for the histological diagnosis or high-spatial-resolution imaging.[71]

ERCP, based on a combination of luminal endoscopy and fluoroscopic imaging, remains the most commonly used method for cytological/histological confirmation of CCA, with a sensitivity and specificity of 74% and 70%, respectively.[72] It enables the

acquisition of histological material by: i) bile aspiration, ii) brush cytology, or iii) biopsy with endobiliary forceps.[71] ERCP brushing is currently the standard method used. A meta-analysis including 1,556 patients showed a pooled diagnostic performance of 42%.[73] Endoluminal forceps biopsy has been shown to yield greater sensitivity for the diagnosis of CCA from biliary strictures (74%),[74] but compared to brushing, is technically more challenging and may require sphincterotomy.[71] It is worth noting that a meta-analysis including 9 studies showed pooled sensitivity values of 45% for brushing, 48% for biopsies, and an improvement of up to 59% with the combination of both methods.[75] In order to improve the sensitivity, different technical alternatives have been investigated. Fluorescence *in situ* hybridization (FISH) is a test employing fluorescently-labelled DNA probes in cytologic samples to identify chromosomal abnormalities in cells. A combination of FISH probes have been shown to identified pancreatobiliary malignancies with a sensitivity from 20% to 43% as compared to routine cytology,[76] whereas when facing indeterminate biliary strictures, it enabled the prediction of malignancy in 62% of patients.[77]

Whilst ERCP is usually favored above percutaneous transhepatic cholangiography (PTC), the latter may be an alternate modality of choice depending on local expertise and anatomical considerations, such as difficult bile duct access.

EUS combines both endoscopy with high-frequency sound waves, making possible the detection of masses, bile duct dilatation and the examination of vascular and nodal involvement.[71] Linear EUS scopes provide the ability to perform fine-needle aspiration (FNA), further improving the diagnostic capability. EUS-FNA has shown sensitivity ranging from 43-89% for discerning eCCA from benign biliary lesions, with higher rates described for dCCA.[78] Some drawbacks should be noted, for instance the low negative predictive value of EUS-FNA, which ranges between 30-65%. In addition, EUS-FNA increases the risk of tumor cell seeding leading to peritoneal metastasis with reported rates up to 80%.[79] As a result, patients who have undergone FNA are illegible for neoadjuvant therapy and liver transplantation.[71]

Digital single operator cholangioscope (DSOC, SpyGlass, Boston Scientific Inc. Massachusetts, USA) is a disposable small caliber scope with an integrated digital sensor and portable processor which enables the visualization of the biliary tract and the ability to perform biopsies with specialized forceps (SpyBite).[80] The sensitivity and specificity for CCA diagnosis are of 90-100% and 76-96%, respectively, whereas biopsy sensitivity was 64%.[81] Despite the currently known results, DSOC should further be evaluated in comparison to other modalities.

I 4.3. Tumor staging

The goal of staging systems is to provide information on the natural history and prognosis of a malignancy, as well as to guide therapeutic decisions. In this sense, malignant tumors are staged according to the extent of the primary tumor (T), regional lymph node infiltration (N), and presence of distant organ metastases (M), being known as the TNM Classification system. The most commonly used TNM system for CCA is the one proposed by the American Joint Committee on Cancer (AJCC).[82] These guidelines refer to two types of classification, *i)* clinical classification (cTNM) based on evidence arising from physical exploration, imaging or endoscopic examination, and essential to select and evaluate therapy; or *ii)* pathological classification (pTNM), based on the postsurgical histopathological classification, more often used to guide adjuvant therapy and estimate prognosis and end-stage results.[82] Afterwards, the assigned TNM categories may be grouped into tumor stages from I to IV, in which the lower the number, the less the tumor spread along lymph nodes to distant organs. Interestingly, iCCA has traditionally been staged using the TNM system for HCC. However, when the 7th Edition was launched (2010), iCCA had, for the first time, a specific staging system, along with separate coding systems for pCCA and dCCA.[83] Currently, the 8th Edition of the AJCC/Union for International Cancer Control (UICC) TNM is the one being used since 2018; however, despite being able to stratify patients according to prognosis and predict survival, it cannot allow to evaluate if the patient is eligible for tumor resection. Therefore, further updated or novel classification systems for CCA are essential.

Concerning pCCA, in 1975, Bismuth and Corlette described a classification criterion for the bile duct involvement on pCCAs, classifying them into four categories (**Fig. I.7.**). This staging can be assessed by both invasive or non-invasive methods, and guides surgical planning for patients thought to have resectable disease.[84]

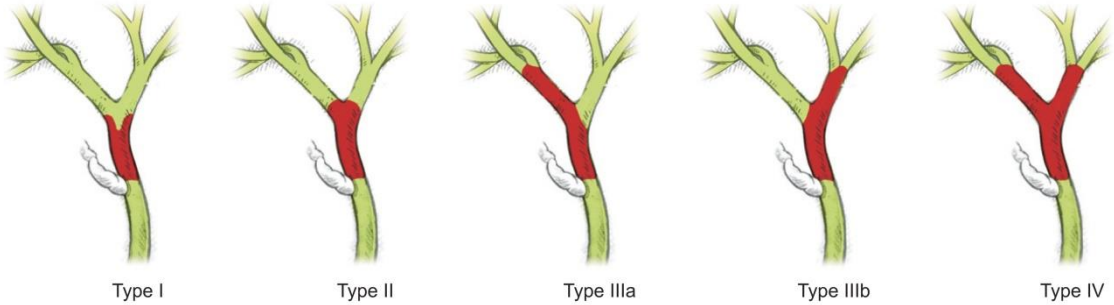


Figure I.7. Bismuth-Corlette classification of perihilar cholangiocarcinomas. Bismuth-Corlette classification of pCCA as *a)* type I, tumor below the confluence of the left and right hepatic ducts; *b)* type II, tumor reaching the confluence; *c)* type III, tumor occluding the common hepatic and right or left hepatic ducts; or *d)* type IV, multicentric or tumors involving the confluence and both right and left hepatic ducts. Red areas are representative of the tumor location, green areas normal bile duct, and white areas the cystic duct. (Obtained from Blechacz B, *et al.*, 2017)[84]

I.5. Therapeutic strategies

Given the complexity of CCA, decisions on the clinical management of patients with CCA should be done based on a multidisciplinary team (MDT), and in view of patient-related factors (i.e., ECOG-PS, comorbidities, and patients' choice), tumor features (i.e., subtype, tumor stage, vascular involvement, and distant metastasis) and medical specialist expertise.[85] Although surgery represents almost the only potentially curative option for patients with CCA, patients are generally diagnosed at advanced stage, when the disease is already widespread, and palliative treatment arises as the only possible approach in a significant proportion of cases.[1,2]

I 5.1. Surgery

Surgical procedures aim to achieve a radical excision of the tumors with histologically negative margins (R0), in addition to lymph node metastasis resection, without compromising post-surgical liver function.[86] Patients' eligibility for surgery is determined by the technical feasibility conditioned to a certain level by primary tumor site and extent, including vascular and/or parenchymal involvement, as well as the assessment of the future liver remnant (FLR), which is particularly altered by the presence of severe cholestasis.[86]

Anatomical resection is usually recommended for iCCA, but due to advanced disease stage at diagnosis, major liver resections such as hemihepatectomy or extended hepatectomy are required in over 50-70% of patients. An additional extrahepatic bile duct resection and reconstruction is required in 20-30% of patients with iCCA invading the ductal bifurcation and/or the main hepatic duct.[62,87,88] Of note, patients with suboptimal predicted liver function (FLR <25% for patients with normal parenchyma, or FLR<40% for patients with chronic liver disease) may benefit from selective portal vein embolization (PVE), which promotes remnant liver proliferation, and, consequently achieves lower incidence of postoperative complications and mortality.[56] Following resection, the

median overall survival (mOS) is around 40 months, with a 5-year survival rate of 25-40% while tumor recurrence occurs in about 50-70% of patients at a median time of 26 months after tumor resection.[89] Lymph node status is found as one of the most important prognostic factor after resection, with up to 45% of patients presenting with nodal involvement. A recent case series study showed that less than 50% of the patients underwent a lymphadenectomy, with a median of 4 harvested lymph nodes, from which over 43% were later diagnosed with metastatic iCCA. Interestingly, the retrieval of ≥3 lymph nodes resulted in improved survival, compared to those with 1-2 nodes surgically removed.[90] Consequently, the AJCC staging system has recently recommended the retrieval of a minimum of 6 lymph nodes to ensure accurate staging and decrease the risk of local recurrence.[82]

Based on the Bismuth-Corlette classification, resection of pCCAs could imply en bloc resection of extrahepatic bile ducts and/or Roux-en-Y hepaticojejunostomy (type I-II), an additional hemihepatectomy (type III) or an extended hepatectomy (type IV). In addition, a lymphadenectomy of the hepatoduodenal ligament and biliary reconstruction may also be necessary.[62] Cholangitis is an important prognostic factor associated with postoperative mortality. Therefore, a correct imaging evaluation of the biliary tree is mandatory before any intervention. Preoperative drainage is indicated for patients with cholangitis, while in the case of biliary obstruction, the decision should be made based on MDT. On the other hand, about 35% of patients are shown to have positive lymph nodes with a 5-year survival rate of 16% compared to 42% in case of negative involvement.[91,92] Similarly to iCCA, the number of retrieved lymph nodes have been shown to impact on survival, probably due to disease understaging.[92]

dCCA cases encompass the highest proportion of resectability when compared to iCCA and pCCA. The intervention for dCCAs generally requires a pancreaticoduodenectomy, thus surgically removing the head of the pancreas, the first part of the small intestine (i.e., duodenum), the gallbladder, and the bile ducts.[93] The 5-year survival rate following curative-intended surgery is between 27-37% for dCCA.[93]

I.5.1.1. Adjuvant chemotherapy

Post-surgical relapse is frequent among patients with CCA, leading to multiple attempts to identify those with increased risk in order to prevent relapse. In this line, three phase III randomized clinical trials have been carry out: *i*) the Bile Duct Cancer Adjuvant Trial (BCAT), using gemcitabine (Gem) in patients with pCCA and dCCA;[94] *ii*) the PRODIGE-12 study (NCT01313377), evaluating the efficacy of gemcitabine and oxaliplatin (GemOx) for all BTCs; and *iii*) the BILCAP study, administering capecitabine for BTCs

(NCT00363584). Among these, Gem-based adjuvant chemotherapy (BCAT and PRODIGE-12 studies) has failed to improve patients' outcome.[94,95] Conversely, the BILCAP study reported longer life expectancy in patients receiving adjuvant capecitabine compared to the observation group, with mOS of 51 and 36 months, respectively [Hazard ratio (HR)=0.7 (95% CI 0.6-0.9)], after adjustment for minimization and prognostic factors (i.e., tumor grade, lymph node invasion, and gender). Moreover, median recurrence-free survival was also longer in the capecitabine group (24 months) compared to placebo (18 months), showcasing an HR 0.8 (95% CI 0.6-0.9).[96] Based on these results, international guidelines published in 2019 recommend the administration of capecitabine as adjuvant therapy for a period of 6 months after curative tumor resection of CCA.[97] Ongoing ACTICCA-1 trial (NCT02170090) is evaluating the effect of the chemotherapeutic combination of gemcitabine and cisplatin (GemCis) as adjuvant therapy compared to surgery followed by capecitabine.[98]

I.5.1.2. Liver transplantation

For a long time, liver transplantation has been contraindicated for unresectable CCAs, as it was associated with high rates of tumor recurrence and low survival (10% for iCCA and 25% for eCCA).[93] Nevertheless, a multicenter study carried out in the USA reported a 5-year disease-free survival rate of 65% in patients with pCCA treated with distinct protocols of neoadjuvant chemoradiation before liver transplantation.[99] Moreover, based on a retrospective multicenter study, liver transplant of unresectable pCCAs was associated with increased survival compared to patients undergoing resection who have not met the criteria for transplantation.[100] Considering the results obtained in pCCA, thanks to the contribution of patient selection criteria and to neoadjuvant chemoradiation protocols, liver transplantation is also being considered as a treatment option for some patients with iCCA. For instance, a 5-year overall survival rate of 65% was reported by the first time for patients with an iCCA single-lesion (≤2 cm). Of note, the outcomes were more disappointing for advanced iCCA, who achieved a 5-year survival rate of 45%.[89] So, liver transplant may be an option for patients with early-stage iCCA who are not eligible for tumor resection due to underlying disease. These results need to be further prospectively validated, thus a multicentric single-arm clinical trial (NCT02878473) is currently ongoing to confirm the effectiveness of liver transplantation for iCCA.

I 5.2. Chemotherapy

Data from randomized controlled trials, including both treatment and control arms, revealed poor mOS in patients with unresectable advanced BTC, ranging between 2.5-4.5 months.[56] The assessment of patients with CCA for palliative therapy is based on *i)* patients' fitness (patients with ECOG-PS ≥3 are recommended to be managed with best supportive care), *ii*) disease distribution (olygometastatic or liver-only diseases might be suitable for specific liver-targeted therapies), and *iii*) tumor profiling accessibility.[2]

The use of first-line chemotherapy in patients with advanced CCA is well-supported by the data reported from the phase III ABC-02 trial (NCT00262769), based on Gem *vs* GemCis administration.[101] This study revealed a mOS of 11.7 months for GemCis regimen, and 8.1 months for Gem monotherapy [HR 0.6 (95% CI 0.5-0.8)], as well as an improvement in the median progression-free survival (8.0 *vs* 5.0 months) with a HR of 0.6 (95% CI 0.5-0.8). Moreover, the phase II BT22 clinical trial (NCT00380588) validated the results obtained in the Japanese study, with mOS values of 11.2 and 7.7 months for GemCis and Gem, respectively.[102] In some cases, oxaliplatin is replaced by cisplatin, but, this combination has not yet been evaluated in phase III trials.[103] On the other hand, more intensive triple-agent chemotherapy regimens are being tested. Phase II study (NCT02392637) of the combination of GemCis with nab-paclitaxel has shown promising results, showing a mOS of 19.2 months,[104] being now evaluated in a phase III randomized trial in comparison to GemCis alone (SWOG-1815; NCT03768414). In addition, phase II study of GemCis following S-1 (GCS) combination chemotherapy resulted in a mOS of 16.2 months with manageable toxicity,[105] and, upon these results, a randomized phase III trial to investigate the efficacy of this regimen compared to GemCis doublet therapy in patients with advanced BTC is currently being conducted (NCT02182778). Despite the light shed by these studies, results from the recently published phase II-III AMEBICA trial (NCT02591030) did not meet the primary end point for the combination of 5-fluorouracil (5-FU), oxaliplatin, and irinotecan triplet chemotherapy (FOLFIRINOX), showing a mOS of 11.7 months, against the 13.8 months found for GemCis doublet, remaining the latter as the first-line standard regimen in advanced BTC.[106]

Until the past few years, scarce evidence about the benefit of second-line chemotherapy for patients with progressive disease is noticeable. The phase III ABC-06 trial (NCT01926236) randomly assigned patients with BTC who had already progressed after first-line GemCis into active symptom control or treated with the combination of folic acid, 5-FU, and oxaliplatin (FOLFOX group). In this study, the primary end-point was

achieved, resulting in an improvement of patients' outcome with a HR of 0.7 (95% CI 0.5-0.97). While the differences in mOS between study arms were trivial (6.2 *vs* 5.3 months), survival rates for 6 and 12 months were encouraging (35.5% *vs* 50.6%, and 11.4% *vs* 25.9%, respectively).[107]

I 5.3. Locoregional therapy

Liver-directed therapies can be the treatment of choice for selected patients with liver-predominant unresectable disease. Transarterial chemoembolization (TACE) allows intra-arterial injection of chemotherapeutic and embolization agents (cisplatin, doxorubicin, 5-FU, gemcitabine, irinotecan, mitomycin C, and oxaliplatin) into the hepatic artery, blocking tumor's blood supply and increasing drug bioavailability. In retrospective studies, TACE with cisplatin has been shown to achieve tumor regression in 23% of the patients, with mOS of 12.2 months compared to 3.3 months on those under symptomatic treatment.[108] Transarterial radioembolization selectively delivers β-emitting yttrium-90 microspheres (^{90}Y-TARE) through the hepatic vasculature to the target tumor. A systematic review of the surrounding treatment of unresectable iCCAs with TARE reported partial and stable radiological responses in 28% and 54% of cases, respectively.[109] Notably, an average of 10% of patients across the three studies was downstaged to a scenario in which surgical resection was amenable to be used. Based on the pooled analysis, patients with iCCA treated with ^{90}Y-TARE showed a mOS of 15.5 months,[109] similar to the rates found for those under chemotherapy and/or TACE.

Hepatic arterial infusion (HAI) enables the differential infusion of floxuridine (pyrimidine analog) directly into the liver. A single-institution phase II trial with 38 patients with unresectable iCCA treated with HAI in combination with GemOx resulted in a partial radiological response in 58% of patients. Of note, 4 patients (11%) displayed an adequate response allowing them to undergo tumor resection. Patients exhibited a mOS of 25.0 months, together with a median progression-free survival (mPFS) of 11.8 months, with a 6-month PFS rate of 84%, meeting the primary end-point.[110]

Intraductal ablative procedures refer to minimally invasive techniques aimed to restoring or maintaining biliary patency. Studies (mostly retrospective) using radiofrequency ablation for unresectable iCCA have shown prolonged pooled survival rates of 82% at 1-year, 47% at 3-years, and 24% at 5-years.[111] On the other hand, despite showing promising results in small randomized studies, the phase III

PHOTOSTENT-02 trial was early discontinued because patients treated with photodynamic therapy (PDT) had worse outcome than those with stenting alone.[112]

In view of the aforementioned results, future randomized controlled trials comparing the effectiveness of the different locoregional therapies compared to chemotherapeutic regimens alone are essential in order to warrant the optimal treatment modality for unresectable iCCA. In this regard, the ongoing randomized phase II ABC-07 trial (EudraCT 2014-003656-31) is examining whether the addition of stereotactic body radiotherapy (SBRT) to GemCis is able to improve the outcome of patients with unresectable CCA.

I 5.4. Molecular profiling and targeted therapies

Molecular profiling studies have revealed substantial molecular and genetic heterogeneity across CCA subtypes, with additional differences also observed according to disease etiology.[113] In this line, *fibroblast growth factor receptor (FGFR) 2* gene translocations and *isocitrate dehydrogenase-1 (IDH1)* mutations are found nearly exclusively in iCCA, while *receptor tyrosine-protein kinase erb-2 (ERBB2)* amplification is more common in pCCA and dCCA.[2,56,113] The identification of CCA driver genes, noncoding promoter mutations, and structural variants amenable to be therapeutically targeted are currently under evaluation for advanced CCAs.

I.5.4.1. IDH1 mutations

Gain of function mutations in the coding region of the *IDH1* gene are present in about 13% of patients with iCCA. Ivosidenib is an oral inhibitor of the IDH1 enzyme. In a phase I dose-escalation study, 73 patients with *IDH1*-mutant CCAs were enrolled and received ivosidenib. Although only 5% of patients had partial response, the mPFS was 3.8 months, with a 12-month rate of 21.8%, and a mOS of 13.8 months.[114] The subsequent phase III ClarIDHy trial (NCT02989857) evaluated ivosidenib in 185 patients after unsuccessful prior therapy. The primary end-point of PFS was reached, with ivosidenib showing a mPFS of 2.7 months compared to 1.4 months for placebo [HR 0.4 (95% CI 0.3-0.5)].[115] Moreover, mOS was 10.3 months for ivosidenib against 5.1 months with placebo after crossover adjustment [HR 0.5 (95% CI 0.3-0.7)].[116]

I.5.4.2. FGFR translocation

Activating translocations events (fusions or rearrangements) that relieve the *FGFR2* gene occurs in about 10-20% of iCCAs. Promising preliminary results were observed in

the phase II trial evaluating the use of BGJ398 (infigratinib), an orally bioavailable, selective pan-FGFR kinase inhibitor, in patients with CCA who progressed under first-line Gem monotherapy. The overall response rate was 14.8%, with a disease control rate of 75.4% and a mPFS of 5.8 months.[117] Upon these results, a phase III randomized trial (PROOF, NCT03773302) is currently ongoing, comparing infigratinib to the standard of care GemCis in advanced/metastatic CCA with *FGFR2* translocations. On the other side, pemigatinib, another selective inhibitor of FGFR1/2/3, was shown to have a response rate of 35.5%, and a mPFS of 6.9 months in patients with disease progression following at least one previous treatment.[118] The robust and long-lasting activity of FGFR inhibition led to an accelerated approval of pemigatinib for patients with advanced, treatment-refractory CCA harboring *FGFR2* translocations (April 2020). Currently, the phase III FIGHT-302 trial (NCT03656536) is comparing the efficacy and safety of first-line pemigatinib against GemCis doublet regimen in patients with advanced CCA with *FGFR2* rearrangements.

I.5.4.3. Other personalized therapies in clinical trials

The *mitogen-activated protein kinase* (*MAPK*) pathway dysregulation has largely been associated with tumorigenesis, and thus, it appears as a targetable option for CCA. Mutations in the *serine/threonine-protein kinase B-raf (BRAF)*, a component in the *MAPK* pathway, are found in approximately 5% of iCCAs. A phase II basket trial (ROAR, NCT02034110) in patients with *BRAF*-mutated rare cancers, has studied the combination of dabrafenib (BRAF inhibitor) plus trametinib (MEK inhibitor) in patients with BTC. The study showed an overall investigator-reported response of 51%, with a mPFS of 9 months, and a mOS of 14 months.[119] In addition, tumors harboring *ERBB2* genomic alterations have also been found in CCA. In eCCA, *ERBB2* overexpression or gene amplification occurs in up to 15-20% of patients.[120] MyPathway (NCT02091141) a non-randomized, phase II basket study included previously treated patients with metastatic BTC and carrying *ERBB2* amplification and/or overexpression. A total of 39 patients were enrolled and received intravenous pertuzumab plus trastuzumab, achieving an objective response rate of 23%.[121] A second study (SUMMIT, NCT01953926) evaluated the role of neratinib monotherapy in patients with *ERBB2*-mutant cancers of the biliary tract, from which 47% were patients with CCA. The confirmed objective response rate was that of 10.5%, with a clinical benefit of 31.6%, and a mPFS of 1.8 months.[122] Last, the *neurotrophic receptor tyrosine kinase* (*NTRK*) is amenable for fusion events which have been occasionally (<5% of cases) found in BTCs. NTRK inhibitors have demonstrated durable responses in solid tumors with *NTRK*

fusions, but are still under concrete evaluation for BTC, as it is the case for entretinib (STARTRK-2, NCT02568267) and larotrectinib (NAVIGATE, NCT02576431).

I.5.5. Immunotherapy

The efficacy of anti-cancer therapies can be limited by the immunosuppressive microenvironment around the tumor. Furthermore, immune cells present in tumor stroma are known to contribute to tumor growth and metastasis, sustaining tumor progression. Hence, immune-directed therapies have emerged as potential strategies under investigation for patients with CCA. Currently, some ongoing clinical trials are trying to determine the therapeutic potential of adoptive immunotherapies with chimeric antigen receptor (CAR) T-cells to boost immunity in patients with CCA (NCT03633773, NCT03820310, NCT01869166, NCT04660929). In addition, immune checkpoint blockade with human monoclonal antibodies has shown promising results in various solid tumors. In particular, tumors with microsatellite instability, which results in somatic mutations and increased levels of tumor antigen presentation, are more prone to respond to immune-based therapies, leading to significant and durable response rates.[123]

In this regard, the targetable programmed death ligand 1 (PD-L1) has been found to be expressed in tumors of some patients with BTC. The efficacy of pembrolizumab, an anti-PD-1 drug, has been evaluated in the phase II KEYNOTE-158 (NCT02628067) and phase Ib KEYNOTE-028 (NCT02054806) clinical trials, which included patients with BTC. The KEYNOTE-028 study showed an objective response rate of 13%, with a mPFS of 1.8 months, and a mOS of 5.7 months.[124] However, in the KEYNOTE-158 trial, only 59% of the patients expressed PD-L1 and the objective response rate was lower (5.8%), while mPFS and mOS appeared to be greater, with 2.0 and 7.4 months, respectively.[124]

Conversely, the anti-PD-1 nivolumab was conditioned by PD-L1 expression according to a phase II trial (NCT02829918). In these study, patients with advanced refractory BTC presented an investigator-reported objective response rate of 22%, together with a mPFS of 3.7 months and a mOS of 14.2 months.[125]

Bintrafusp alfa (M7824) is a bifunctional fusion protein that can block both the TGF-β and PD-L1 pathways, providing greater power against immune suppression. In a phase I trial, 30 patients with BTC with progressive disease after first-line chemotherapy received M7824 displaying an objective response rate of 20%, with mPFS and mOS of 2.5 months and 12.7 months, respectively. The therapeutic efficacy of M7824 was

observed to be independent on PD-L1 expression levels and microsatellite instability-high status.[126]

Still, the advance of immunotherapy in CCA seems to require the combination of other approaches involving chemotherapy or locoregional therapies. Many phase II clinical trials are evaluating the effect of checkpoint inhibitors alone or in combination with other treatment modalities (e.g., ABC-09 trial, NCT03260712). Moreover, first-line phase III studies aiming to evaluate the combination of immunotherapy with GemCis are underway, including the TOPAZ-1 trial (durvalumab, NCT03875235), KEYNOTE-966 trial (pembrolizumab, NCT04003636), and M7824 (NCT04066491).

I.5.6 Supportive care

Accumulating data show that the introduction of palliative care services at the time of diagnosis of advanced cancer leads to a meaningful improvement in patients.[127] This is the case of the ABC-06 trial, where the Active Symptom Control (ASC) arm consisted of early identification and treatment of biliary-related complications and cancer-related symptoms, showing a mOS greater than expected (5.3 months *vs* previously reported 4 months). Patients with CCA under best supportive care may require biliary drainage, antibiotics, analgesia, steroids, anti-emetics, other palliative treatment for symptom control, palliative radiotherapy (e.g., for painful bone metastases), and transfusion of blood products.[107] Particularly, patients with pCCA are at high risk of developing biliary obstruction and secondary infection and sepsis. Biliary drainage might be beneficial as a palliative treatment for those advanced BTCs, prioritizing endoscopic stenting over percutaneous transhepatic drainage.[128] Both plastic stents and self-expandable metallic stents can be used, but in patients with a life expectancy of >3 months, a metal prosthesis is preferred as it offers higher patency duration.[129] Hence, it is necessary to warrant that patients have full access to palliative care and symptom management.[128]

Hypothesis & Objectives

CCA includes a heterogeneous group of malignancies with increasing incidence worldwide, already representing the second most common primary liver cancer (15%), 3% of all gastrointestinal neoplasias and 2% of all cancer-related deaths yearly. Most of the patients with CCA are asymptomatic in early stages, being commonly diagnosed in late phases when the disease is already found disseminated. [1,2,7] Late diagnosis together with the high chemoresistance of these tumors significantly impact on the efficacy of the available therapeutic options and mostly limit the access to potentially curative approaches (surgery), resulting in dismal prognosis.[1,2] Nonetheless, international collaborative real-word reports on the origin, etiology, pathogenesis, and prognosis of patients with CCA remain elusive, which could provide pivotal clues in the quest for novel targets for therapy and in the development of more effective treatments. Furthermore, according to their anatomical location, the ICD-11 published by the WHO has updated the classification of CCAs into intrahepatic (iCCA), perihilar (pCCA) and distal (dCCA).[6] However, data on similarities and differences between CCA subtypes is scarce and largely awaited.

Taking all the above information into account, and considering that the ENSCCA Registry represents a unique tool and a great opportunity to decipher the jigsaw of CCA, the main aim of this dissertation was to investigate the presentation, management and outcome of patients with CCA in Europe. Moreover, we aimed to provide an in-depth comparison between the distinct CCA subtypes, based on the new ICD-11 classification, in order to provide novel insights at the demographic and clinical levels as well as on their response to current therapeutic approaches.

Hence, the following objectives were postulated:

I. Generation of a European Cholangiocarcinoma Registry of patients.
II. Establishment of a pan-European multicentric clinical data collection network.
III. Revision and harmonization of the data included in the registry.
IV. Analysis of the data:
    a. Determination of the demographics and potential risk factors of patients diagnosed with CCA over the past decade (2010-2019).
    b. Evaluation of the clinical and histomorphological presentation of CCA at diagnosis.
    c. Study the clinical management of patients with CCA and their long-term outcome.

Patients & Methods

M.1. Study design

A multicenter, ambispective, longitudinal, observational cohort study was designed to assess the natural course, management and outcome of patients with CCA in routine clinical practice on several European Health Care Centres. The ENSCCA Registry started in 2016 as a collaborative initiative of the European Network for the Study of Cholangiocarcinoma (ENSCCA), an open, international and multidisciplinary collaborative network of scientists dedicated to study and improve the management of patients with CCA.

The study covered a 10-year period data inclusion, from January 1st 2010 to December 31st 2019. At the start of the registry study in 2016, clinical data of patients diagnosed since 2010 was retrospectively collected; thenceforth, newly diagnosed patients were prospectively annotated. An average of 4-years inclusion period was considered, followed by at least 24 months patient follow-up time (**Fig. M.1.**). Patients recruitment ended-up on February 2020, including 26 referral Healthcare Centers from 11 European countries (**Fig. M.2.**).

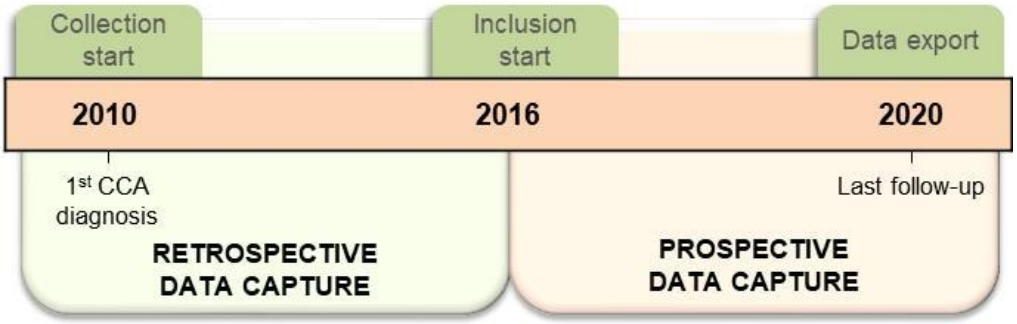


Figure M.1. ENSCCA Registry study period.

The participating centers were the following:

- Kaiser Franz Josef Hospital (Vienna, Austria)
- Hôpitaux Universitaires Pitié Salpêtrière - Sorbonne Université (Paris, France)
- Institute for Pathology – Univ. of Regensburg (Regensburg, Germany)
- Institute of Pathology – Univ. Hospital Heidelberg (Heidelberg, Germany)
- Homburg Univ. Hospital (Homburg, Germany)
- Medical School Hannover (Hannover, Germany)

- Sapienza University (Rome, Italy)
- Ancona Univ. Hospital (Ancona, Italy)
- Padova Univ. Hospital (Padova, Italy)
- Sassari Univ. Hospital (Sassari, Italy)
- Humanitas Clinical and Research Center (Milan, Italy)
- Lithuanian University of Health Sciences (Kaunas, Lithuania)
- Norwegian PSC Research Center (Oslo, Norway)
- Teaching Hospital No 1 (Rzeszów, Poland)
- Octavian Fodor Regional Institute of Gastroenterology and Hepatology (Cluj Napoca, Romania)
- Regional Institute of Oncology Iasi (Iasi, Romania)
- Biodonostia Health Research Institute – Donostia University Hospital (San Sebastian, Spain)
- Hospital Clinic Barcelona (Barcelona, Spain)
- University Hospital of Salamanca (Salamanca, Spain)
- University of Navarra Clinic (Pamplona, Spain)
- "12 de Octubre" University Hospital (Madrid, Spain)
- Amsterdam UMC Locatie AMC (Amsterdam, Netherlands)
- Erasmus MC Hospital (Rotterdam, Netherlands)
- The Christie NHS Foundation Trust (Manchester, United Kingdom)
- Greater Glasgow and Clyde NHS Trust (Glasgow, United Kingdom)
- The Royal Marsden NHS Trust (London, United Kingdom)

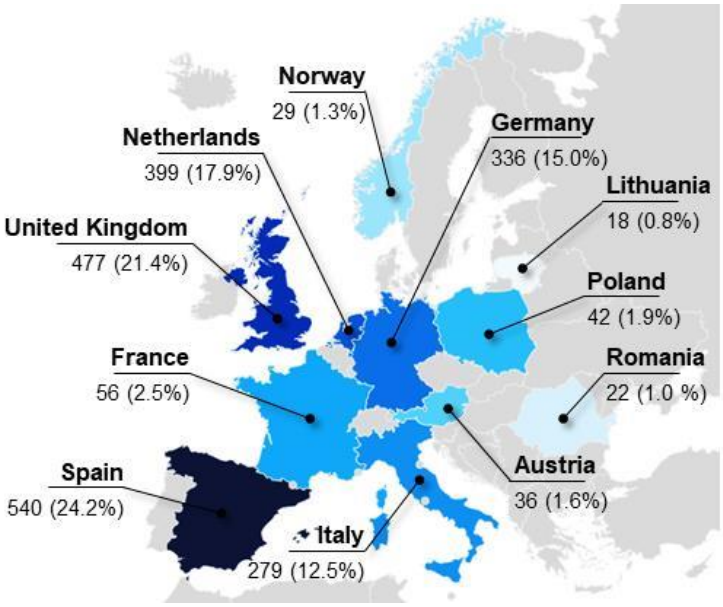


Figure M.1. Patient enrollment. Patients distribution by European countries, shown as number and percentage.

M.1.1. Study population

Patients were recruited from referral centers in Europe, either contacted by the Coordinator of this registry (Prof. Jesús M. Bañales) or by own request to join the initiative. Potential participants were identified within the clinical department (members of the clinical care team) either by reviewing their medical records, or during regularly scheduled medical appointments.

Patients eligibility was based on the following inclusion criteria:

- Diagnosis following the latest ICD-11,[6] in which CCA was categorized as intrahepatic (2C12), perihilar (2C18), or distal (2C15).
- Histological and/or cytological confirmation of the diagnosis.
- Patients of both sexes (male and female) and adults (≥18 years old).

On the other hand, the exclusion criteria were the following:

- Other BTCs different to CCA (i.e., gallbladder cancer or ampullary cancer).
- Patients with suspected CCA but not confirmed by biopsy/citology/histopathology.
- Patients who did not meet the inclusion criteria or who were considered by the investigator to be unsuitable to be included in the study.

M.1.2. Variables

Information on patients' demographics, documented risk factors and medical history, biochemical and clinical parameters, and treatments were included. The most relevant variables (qualitative and quantitavive) considered on the study are listed below, and were recorded in an Electronic Case Report Form (e-CRF) elaborated to collect all the necessary clinical information during the study period (Annex 1).

M.1.2.1. Variables in the initial assessment

- Demographic data: ethnicity, sex, and age.
- Physical exam: height, and weight.
- Medical history: existing diseases/pathologies at the time of CCA diagnosis and/or prior tumor diseases.
- CCA diagnostic methods: radiological methods, and pathological methods.

- Charasteristics of the CCA: type, number, size, location, lymph node invasion, distant metastases.
- ECOG Performance Status
- Laboratory tests: biochemical, haematological, tumor markers
- Family history of tumor diseases.

M.1.4.2. Variables collected in the follow-up

- Therapeutic strategies: surgical approaches, locoregional therapies, and systemic therapies.
- CCA recurrence/progression events
- Survival status

M.1.4.3. Inferred variables

The variables used to assess the clinical evolution of patients were the following:

- **Overall survival (OS)**: time from diagnosis to death. The OS or time to death is defined as the number of months since the date of CCA diagnosis based on histopathology until the date of death by any cause. Patients without information on survival, lost during follow-up or alive at last medical visit were censored at the date of the latest record.
- **After treatment OS**: time from treatment initiation to death. The after treatment OS is defined as the number of months from the date of the first CCA-directed treatment to the date of death by any cause. Patients without information on survival, lost during follow-up or alive at last medical visit were censored at the date of the latest record.
- **Percentage of patients surviving 1, 3 and 5-years after treatment**: number of patients alive at 1, 3 and 5-years from the beginning of the first CCA treatment with respect to the total number of patients under the same treatment group.
- **Relapse-free survival (RFS)**: time from tumor resection to the event of relapse. Deaths during follow-up without evidence of recurrence were censored.

M.2. Data collection and harmonization

Individual patient data was obtained from medical records by the participating hospitals, and were recorded using a de-identified format.

M.2.1. Registry software

Clinical data was collected and managed using the web-based application designed to support data capture for research studies "Research Electronic Data Capture" (REDCap™)[130] hosted at "*Asociación Española de Gastroenterología*" (AEG; www.aegastro.es), a non-profit scientific and medical society focused on gastroenterology research in Spain.

Data from the AEG-REDCap™ platform is hosted on the server provided by the web service provider https://www.guebs.com, which is physically hosted in a secure data center of Amazon Web Services located in the Republic from Ireland. Amazon Web Services data centers comply with the strictest security, availability and service management standards, being certified in ISO/IEC 27001: 2013, 27017: 2015, 27018: 2019 and ISO/IEC 9001: 2015. On the other hand, every 24 hours, a complete backup of the server is made, which includes all the configurations, files and data. At every time, 2 and 4 backup copies of different dates are kept. As part of the disaster recovery strategy, one recent backup is always kept in another Data Processing Center (DPC) of Amazon Web Services located in Germany. Finally, the service uses Centos Linux operating system, which is always updated, along with the rest of the server software and services. In this way, a high-performance, secure, stable and perfectly maintained system for hosting the AEG-REDCap™ platform and its MySQL databases is completed.

M.2.2. Data capture

The clinical data was obtained by the members of the research team of the corresponding Center. These data was included into the Registry in a de-identified form, guaranteeing the privacy of each individual. The Registry was restricted only to members of the study team by using a username and password generated by the study headquarters staff.

In an attempt to guarantee the integrity and reproducibility of data between the different participating institutions, common guidelines were used for data capture, making possible their joint analysis. Thus, patients were classified according to the anatomical location of the primary tumor within the bile ducts (i.e., iCCA, pCCA or dCCA) following the ICD-11[6] criteria and the experience of investigators within MDTs. Findings of positive lymph node invasion and/or tumor metastasis were performed by either histology or imaging techniques, and registered at the time of diagnosis using the 7th edition of the AJCC/UICC TNM cancer staging manual.[83] Likewise, CCA tumor

resectability was determined based on local MDT discussions following widely accepted international guidelines (e.g., from the European Society for Medical Oncology (ESMO) and/or the International Liver Cancer Association (ILCA)),[128,131] and taking into account multiparametric criteria based on the performance status, tumor stage, undelaying diseases, and comorbidities, among others.

M.2.3. Data harmonization

Data was subjected to harmonization and completeness check before the analysis in order to ensure homogenity between centers. Patients were excluded from the study when mandatory epidemiological and/or clinical data (i.e., type of CCA, date of diagnosis, and date of last follow-up or death) were missing. Moreover, patients without tissue-proven CCA (investigator-reported) or with undefined biliary location were also discarded after an internal investigator review process.

M.3. Data analysis

Patients were categorized based on multiple variables in order to describe relevant aspects of the disease. Thus, differences and similarities between the three CCA subtypes (iCCA, pCCA and dCCA) were analyzed in terms of demographics, documented risk factors and medical history, biochemical and clinical parameters, and treatments. Besides, patients were categorized by the disease status at diagnosis, as: (1) local disease (LD), (2) locally advanced disease (LAD), or (3) metastatic disease (MD). LAD was stated as positive regional lymph node tumor invasion measuring above 1.5 cm in diameter (short axis) and classified as N+ (i.e., N1 for iCCA and dCCA; N1 and N2 for pCCA). According to the 7th Edition AJCC/UICC staging guidelines, MD indicated distant involvement (M1), with the exception for liver dissemination of iCCA that is classified as multiple tumors (T2b), and thus, as LD.

Treatments were classified as: (1) surgery [*i.e.*, tumor resection or liver transplantation subdivided into *i)* resection margin R0 (negative margin tumor resection), *ii)* resection margin R1 (microscopic residual disease), and *iii)* resection margin R2 (gross residual disease)], and (2) active palliative treatment (i.e., chemotherapy, hepatic artery-based therapies, radiation therapy, and/or ablation). Patients receiving staging laparoscopy or exploratory laparotomy were classified according to the subsequent therapeutic strategy.

M.4. Statistical Analysis

Baseline demographics and risk factors were summarized using descriptive statistics. Continuous data were described as median and interquartile range (IQR), while categorical variables were summarized as number of patients (n) and probability percentage. Probability was calculated excluding cases with unknown information. Shapiro-Wilk test was used to test continuous variables for normal distribution. For multiple comparisons, parametric or non-parametric data were compared using One-Way analysis of variance (ANOVA) or Kruskal-Wallis tests, respectively, and followed by Bonferroni post-hoc test. Pairwise comparisons were calculated using Dunn's method. Pearson's Chi-square (χ^2) test was used to compare categorical variables between the three subgroups. For pairwise comparison between CCA subtypes of categorical data, Fisher's exact test was performed. Logistic regression analysis was carried out in variables previously dichotomized as "normal" *versus* "high" based on the normality threshold to assess the risk of disease dissemination. Survival analysis was performed with the Kaplan-Meier method and Cox regression (univariate an multivariable analysis including variables statistically significant in the univariate analysis, defined as $p<0.05$). The Log-rank test was used for comparisons of survival in Kaplan-Meier curves. Prognostic factors were related to hazard ratio (HR), 95% confidence intervals (CI), and *p* values.

Statistical analyses were performed with IBM SPSS Statistics Version 22.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 6.0 for Microsoft Windows, (GraphPad Software, La Jolla California, USA). All *p* values were obtained in two-tailed tests and $p<0.05$ was considered statistically significant.

M.5. Ethical and regulatory affairs

This research study was performed in agreement with the International Conference on Harmonization—Good Clinical Practice guidelines (CPMP/ICH/135/95)[132] as ethical and scientific quality standards for designing, conducting, recording, and reporting studies that involve the participation of humans; the Declaration of Helsinki-Fortaleza Brazil, October 2013, Oviedo Convention of April 4th, 1997 on human rights and biomedicine, ratified in the Official State Gazette (BOE) of October 20th, 1999, and the Nuremberg Code (1946); the Royal Decree 957/2020, of 3 November, regulating observational studies with medicinal products for human use; the Regulation (EU) 2016/679 of the

European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data (General Data Protection Regulation); and the Organic Law 3/2018, of December 5th, on the Protection of Personal Data and guarantee of digital rights. Compliance with these standards publicly guarantees the protection of the rights, safety and well-being of the subjects participating in the study, and ensures the integrity and credibility of the data obtained.

The ENSCCA Registry Study protocol was approved by the Ethic Committee of Euskadi (Spain), as coordinating Center, on December 19th 2016, and ammended on April 26th 2021 (Annex 2). Additionally, each participating Center obtained a local ethical approval (or equivalent).

Results

R.1. Creation of the ENSCCA Registry

During the study period, between January 2017 (recruitment opening) and February 2020 (recruitment clausure), data capture showed a progressive increase consistent with the number of involved recruiting centers (**Fig. R.1.**). Likely as a result of a marked increase in the involvement of new Health Care Centers since the beginning of the study, a total of 3,039 patients were retrived for the study.

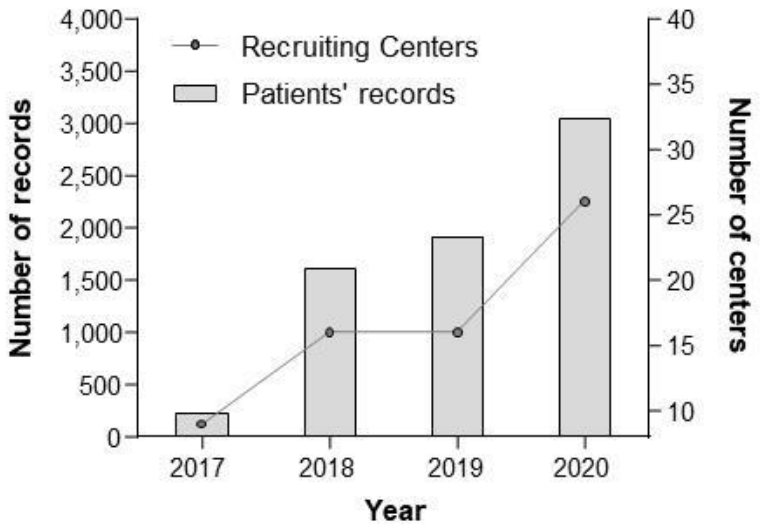


Figure R.1. Number of Recruiting Centers and included patients in the ENSCCA Registry throughout the study period.

R.2. Patients' characteristics and CCA features at diagnosis

From the 3,039 patients initially included in the ENSCCA Registry, 2,234 (73.5%) were selected and further analyzed after fulfilling the inclusion criteria (**Fig. R.2.**), including 1,243 (55.6%) iCCA, 592 (26.5%) pCCA and 399 (17.9%) dCCA.

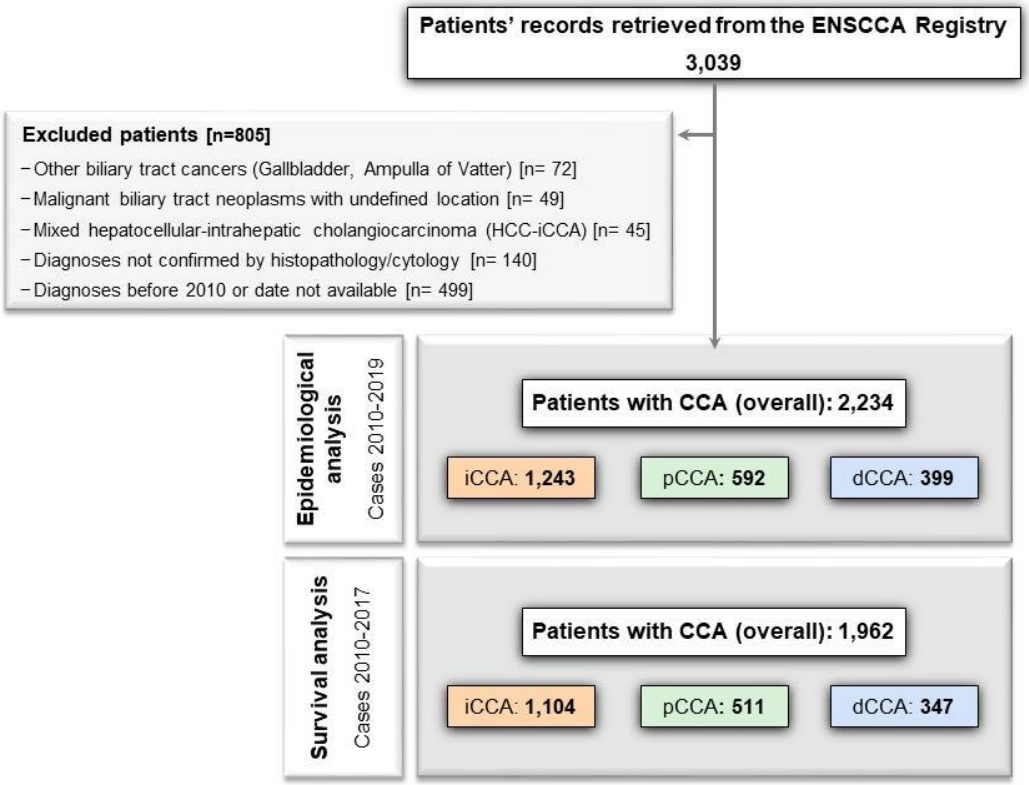


Figure R.2. Study population. CONSORT flow diagram with the detailed exclusion criteria and the final epidemiological and survival analysis study cohorts. *Abbreviations*: CCA, cholangiocarcinoma; dCCA, distal CCA; HCC, hepatocelular carcinoma; iCCA, intrahepatic CCA; pCCA, perihilar CCA.

R.2.1. Patients' clinical characteristics at CCA diagnosis

Baseline patients' characteristics and laboratory tests are listed in **Table R.1**. The majority of patients were Caucasian (96.6%) with a median age at diagnosis of 66 years (IQR 58-73) and slight overrepresentation of males (56.4%). Most patients showed, at diagnosis, increased serum levels of ALT, markers of cholestasis [GGT and ALP] and CA19-9, particularly evident for patients with pCCA or dCCA (**Table R.1.**). No significant abnormalities were observed in specific hematological and metabolic blood test measures (**Table R.1.**).

Table R.1. Baseline patients' characteristics at CCA diagnosis.

	iCCA	pCCA	dCCA	*p value*[a]	CCA (overall)	*p value* (iCCA *vs* pCCA)	*p value* (iCCA *vs* dCCA)	*p value* (pCCA *vs* dCCA)
Age, *median (IQR)*	65 (56 – 72)	66 (59 – 73)	68 (59 – 73)	*<0.01*	66 (58 – 73)	*ns*	*<0.01*	*ns*
range	(24 – 92)	(26 – 87)	(23 – 85)		(23 – 92)			
Sex, *n (%)*								
Males	655 (52.7)	352 (59.5)	252 (63.2)	*<0.001*	1,259 (56.4)	*<0.01*	*<0.001*	*ns*
Females	588 (47.3)	240 (40.5)	147 (36.8)		975 (43.6)			
Caucasian ethnicity, *n (%)* [n = 1,738]	996 (96.6)	319 (96.1)	364 (97.1)	*ns*[b]	1,679 (96.6)			
Liver function tests[c], *median (IQR)*								
ALT [n = 1,598]	32.0 (21 – 61)	99.0 (53 – 199)	66.0 (26 – 149)	*<0.0001*	47.0 (24 – 111)	*<0.0001*	*<0.0001*	*<0.0001*
AST [n = 1,931]	37.0 (25 – 64)	72.0 (41 – 135)	38.0 (25 – 78)	*<0.0001*	43.0 (27 – 86)	*<0.0001*	*ns*	*<0.0001*
GGT [n = 1,946]	160.0 (71 – 419)	497.5 (233 – 945)	159.0 (54 – 482)	*<0.0001*	224.0 (86 – 587)	*<0.0001*	*ns*	*<0.0001*
ALP [n = 1,670]	148.0 (94 – 294)	305.0 (187 – 513)	189.0 (113 – 339)	*<0.0001*	178.5 (103 – 352)	*<0.0001*	*<0.01*	*<0.0001*
Albumin [n = 902]	4.1 (3.6 – 4.4)	3.8 (3.4 – 4.2)	4.0 (3.6 – 4.3)	*<0.0001*	4.0 (3.6 – 4.3)	*<0.0001*	*ns*	*ns*
Bilirubin [n = 1,979]	0.6 (0.4 – 1.1)	3.3 (0.9 – 10.6)	0.8 (0.4 – 3.1)	*<0.0001*	0.8 (0.5 – 2.9)	*<0.0001*	*<0.0001*	*<0.0001*
Tumor markers, *median (IQR)*								
CEA [n = 1,015]	2.5 (1.4 – 5.3)	2.85 (1.6 – 7.0)	3.1 (1.8 – 5.42)	*ns*	2.8 (1.5 – 5.5)	*ns*	*ns*	*ns*
CA19-9 [n = 1,299]	34.7 (9 – 213)	215.7 (37 – 1,069)	78.0 (22 – 310)	*<0.0001*	59.0 (13 – 372)	*<0.0001*	*<0.01*	*<0.01*
AFP [n = 524]	3.5 (2.0 – 7.2)	2.8 (2.1 – 5.1)	2.6 (2.0 - 4.1)	*<0.01*	3.2 (2.0 – 6.1)	*ns*	*ns*	*ns*
Other laboratory tests, *median (IQR)*								
WBC [n = 1,139]	7.8 (6.2 – 11)	7.4 (5.8 – 9.7)	7.5 (6.2 – 9.5)		7.6 (6.1 – 9.9)			
Neutrophils [n = 746]	67.5 (61 – 75)	64.0 (56 – 72)	63.0 (56 – 71)		66.0 (58 – 74)			
Lymphocytes [n = 738]	20.2 (15 – 27)	24.8 (18 – 32)	24.0 (17 – 31)		21.9 (15 – 29)			
Monocytes [n = 700]	8.1 (6.3 – 10)	8.5 (6.8 – 10)	8.1 (6.8 – 10)		8.2 (6.5 – 10)			
Eosinophils [n = 699]	1.6 (0.8 – 2.9)	2.0 (1.1 – 3.1)	2.0 (1.0 – 3.7)		1.8 (0.9 – 3.0)			
Basophils[n = 697]	0.4 (0.2 – 0.7)	0.5 (0.3- 0.9)	0.5 (0.3 – 0.8)		0.4 (0.2 – 0.7)			
RBC [n = 992]	4.5 (4.1 – 4.9)	4.2 (3.8 – 4.5)	4.3 (3.9 – 4.7)	*ns*	4.38 (4.0 – 4.8)	*ns*	*ns*	*ns*
Hematocrit [n = 827]	40.8 (36 – 44)	37.8 (34 – 41)	38.8 (35 – 42)		39.0 (35 – 43)			
Hemoglobin [n = 1,249]	12.7 (11 – 14)	9.2 (7.9 – 12)	12.2 (11 – 14)		12.1 (10 – 14)			
Platelets [n = 1,206]	234 (177 – 298)	272 (193 – 349)	249 (206 – 306)		243 (184 – 306)			
INR [n = 963]	1.06 (1.0 – 1.1)	1.04 (1.0 – 1.1)	1.04 (1.0 – 1.1)		1.05 (1.0 – 1.1)			
Cholesterol [n = 652]	174 (144 – 209)	195 (164 – 257)	190(150 – 240)		181(149 – 222)			
Tryglicerides [n = 586]	107 (81 – 140)	147 (90 – 203)	131 (95 – 188)		116 (87 – 160)			
Glucose [n = 739]	102 (90 – 126)	107 (91 – 122)	106 (92 – 126)		104 (91 – 126)			

[a] Statistics were done comparing the three cholangiocarcinoma subtypes (iCCA *vs* pCCA *vs* dCCA).
[b] Statistical test has been done considering Caucasian *vs* the rest of ethnicities (Asian, African, Hispanic, Caribbean).
[c] Laboratory variables clinical thresholds were the highest reported in the literature: ALT 45 UI/L, AST 40 UI/L, GGT 71 UI/L, ALP 129 UI/L, Albumin 5.2 g/dL, Bilirubin 1.3 mg/dL.
Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CA19.9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; dCCA, distal cholangiocarcinoma; GGT, gamma-glutamyl transferase; iCCA, intrahepatic cholangiocarcinoma; IQR, interquartile range; pCCA, perihilar cholangiocarcinoma.

Considering patients' comorbidities (**Table R.2.**), 55.1% were overweight [body mass index (BMI) 25-30 kg/m^2] (35.7%) or obese [BMI≥30 kg/m^2] (19.4%) at the time of diagnosis, particularly for patients with iCCA (**Table R.2.**); 22.5% had diabetes, observed more frequently in patients with iCCA or dCCA compared to pCCA, and 39.9% presented arterial hypertension. Of note, 15% of the patients with CCA were obese and diabetic. In addition, patients suffered from underlying biliary or liver diseases predisposing CCA development, including primary biliary cholangitis (PBC: 3.3%, mainly iCCA), PSC (4.5%; mainly pCCA), bile duct stones (6.1%; mainly pCCA and dCCA), viral hepatitis (2.8% HCV, 4.6% HBV, and 0.1% concomitant infection; mainly iCCA) and liver cirrhosis (7.8%; mainly iCCA). In this registry cohort there was also a history of smoking or alcohol consumption in 33% and 19.8% of patients, respectively.

Table R.2. Baseline patients' concomitant diseases and conditions.

	iCCA	pCCA	dCCA	*p value*[a]	CCA (overall)	*p value* (iCCA *vs* pCCA)	*p value* (iCCA *vs* dCCA)	*p value* (pCCA *vs* dCCA)
Obesity, *n (%)* [n = 1,973]								
Normal weight (<25)	461 (41.5)	252 (51.1)	172 (46.6)		885 (44.9)			
Overweight (≥25)	393 (35.4)	172 (34.9)	140 (37.9)	*<0.0001*	705 (35.7)	*<0.0001*	*<0.01*	*ns*
Obese (≥30)	257 (23.1)	69 (14.0)	57 (15.4)		383 (19.4)			
Diabetes, *n (%)* [n = 1,904]	257 (25.6)	86 (15.6)	85 (24.3)	*<0.0001*	428 (22.5)	*<0.0001*	*ns*	*<0.01*
Obesity + Diabetes, *n (%)* [n = 1,722]	166 (17.9)	45 (9.6)	47 (14.4)	*<0.001*	258 (15.0)	*<0.0001*	*ns*	*<0.05*
Arterial hypertension, *n (%)* [n = 2,011]	455 (41.8)	198 (36.3)	138 (36.7)	*ns*	791 (39.3)	*ns*	*ns*	*ns*
Metabolic conditions, *n (%)* [n = 2,011]								
Hypertriglyceridemia	41 (3.8)	15 (2.7)	27 (7.2)	*<0.01*	83 (4.1)	*ns*	*<0.01*	*<0.01*
Low HDL cholesterol	16 (1.5)	17 (3.1)	9 (2.4)	*ns*	42 (2.1)	*ns*	*ns*	*ns*
Biliary conditions, *n (%)* [n = 1,569]								
PSC	34 (3.8)	33 (8.8)	4 (1.3)	*<0.0001*	71 (4.5)	*<0.001*	*<0.05*	*<0.0001*
PBC	45 (5.1)	2 (0.5)	4 (1.3)	*<0.0001*	51 (3.3)	*<0.0001*	*<0.01*	*ns*
IBD	28 (3.1)	21 (5.6)	10 (3.3)	*ns*	59 (3.8)	*ns*	*ns*	*ns*
PSC + IBD	12 (1.3)	17 (4.5)	3 (1.0)	*<0.001*	32 (2.0)	*<0.01*	*ns*	*<0.05*
Bile duct stones	35 (3.9)	29 (7.7)	31 (10.3)	*<0.001*	95 (6.1)	*<0.01*	*<0.001*	*ns*
Cholecystitis	14 (1.6)	5 (1.3)	5 (1.7)	*ns*	24 (1.5)	*ns*	*ns*	*ns*
Liver diseases, *n (%)*								
Viral hepatitis [n=1,594]	89 (10.4)	20 (4.4)	11 (3.9)	*<0.0001*	120 (7.5)	*<0.001*	*<0.001*	*ns*
Liver cirrhosis [n=1,568]	112 (12.6)	5 (1.3)	6 (2.0)	*<0.0001*	123 (7.8)	*<0.0001*	*<0.0001*	*ns*
Toxic exposure, *n (%)*[n=1,805]								
Alcohol	206 (19.9)	88 (21.6)	64 (17.7)	*ns*	356 (19.8)	*ns*	*ns*	*ns*
Tobacco	322 (31.1)	160 (39.2)	120 (33.2)	*<0.05*	602 (33.4)	*<0.01*	*ns*	*ns*

[a] Statistics were done comparing the three cholangiocarcinoma subtypes (iCCA *vs* pCCA *vs* dCCA).
Abbreviations: dCCA, distal cholangiocarcinoma; HDL, high density lipoprotein; IBD, inflammatory bowel disease; iCCA, intrahepatic cholangiocarcinoma; PBC, primary biliary cholangitis; pCCA, perihilar cholangiocarcinoma; PSC, primary sclerosing cholangitis.

R.2.2. Patient´s physical fitness and cholangiocarcinoma tumor features at diagnosis

Table R.3. summarizes the patients' fitness measured as ECOG-PS and tumor-related features at diagnosis. The majority of patients with CCA had ECOG-PS of 0 (44.0%) or 1 (39.1%). Regarding tumor size and growth pattern, iCCAs presented frequently as larger lesions (>3 cm or multifocal) with a mass-forming pattern, compared to pCCA and dCCA that in general were small lesions (<3 cm) with periductal infiltration (**Table R.3.**). Moderate grade of tumor differentiation was the most frequently observed in the three CCA subtypes.

Table R.3. Tumor presentation at diagnosis.

	iCCA	pCCA	dCCA	*p value*[a]	CCA (overall)	*p value* (iCCA *vs* pCCA)	*p value* (iCCA *vs* dCCA)	*p value* (pCCA *vs* dCCA)
ECOG Performance Status, *n (%)* [n = 1,984]								
0	564 (51.1)	226 (40.8)	83 (24.4)		873 (44.0)			
1	359 (32.5)	220 (40.7)	196 (57,6)		775 (39.1)			
2	129 (11.7)	74 (13.7)	44 (12,9)	*ns*[b]	247 (12.4)	*ns*	*ns*	*ns*
3	46 (4.2)	20 (3.7)	15 (4.4)		81 (4.1)			
4	5 (0.5)	1 (0.2)	2 (0.6)		8 (0.4)			
Tumor size, *n (%)* [n = 1,268]								
≤ 3 cm	117 (13.4)	147 (56.8)	105 (76.6)		369 (29.1)			
> 3 cm	487 (55.8)	90 (34.7)	23 (16.8)	*<0.0001*	600 (47.3)	*<0.0001*	*<0.0001*	*<0.001*
Multiple lesions	268 (30.7)	22 (8.5)	9 (6.6)		299 (23.6)			
Pattern of growth, *n (%)* [n = 1,108]								
Mass-forming	700 (92.8)	50 (27.0)	57 (33.7)		807 (72.8)			
Periductal infiltrating	21 (2.8)	105 (56.8)	93 (55.0)	*<0.0001*	219 (19.8)	*<0.0001*	*<0.0001*	*ns*
Intraductal growth	8 (1.1)	27 (14.6)	17 (10.1)		52 (4.7)			
Mixed pattern	25 (3.4)	3 (1.6)	2 (1.2)		30 (2.8)			
Differentiation grade, *n (%)* [n = 1,245]								
Not assessed (Gx)	66 (8.9)	15 (5.7)	19 (7.9)		100 (8.0)			
Well (G1)	89 (12.0)	54 (20.5)	60 (24.8)		203 (16.3)			
Moderate (G2)	378 (51.2)	150 (56.8)	108 (44.6)	*<0.0001*	636 (51.1)	*<0.0001*	*<0.0001*	*ns*
Poor (G3)	200 (27.1)	45 (17.0)	55 (22.7)		300 (24.1)			
Undifferentiated (G4)	6 (0.8)	0 (0.0)	0 (0.0)		6 (0.5)			
Lymph node invasion (N+), *n (%)* [n = 1,630]	419 (50.3)	229 (45.3)	145 (49.8)	*ns*	793 (48.7)	*ns*	*ns*	*ns*
Distant metastasis (M1), *n (%)* [n = 2,043]	276 (23.9)	140 (27.3)	78 (20.9)	*ns*	494 (24.2)	*ns*	*ns*	*ns*

[a] Statistics were done comparing the three cholangiocarcinoma subtypes (iCCA *vs* pCCA *vs* dCCA).
[b] Statistical test has been done considering ECOG 0-1 vs ≥2.
Abbreviations: 95% CI, 95% confidence interval; dCCA, distal cholangiocarcinoma; ECOG, eastern cooperative oncology group; iCCA, intrahepatic cholangiocarcinoma; IG, intraductal growth; MF, mass-forming; OS, overall survival; pCCA, perihilar cholangiocarcinoma; PI, periductal infiltrating; PS, performance status.

From the 1,998 patients with available information on imaging, 6.2% had initial tumor staging based on MRI and/or MRCP, 47.4% with CT, and 54.9% with both approaches (**Table R.4.**). Of note, 32.3% of all patients with MRI/MRCP/CT-based staging had an additional US, and 4.0% PET evaluation.

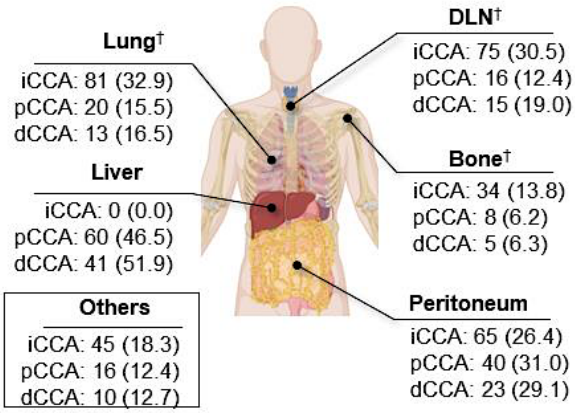
Table R.4. Imaging methods used for the staging of CCA.

	iCCA	pCCA	dCCA	CCA (overall)[a]
DIAGNOSTIC/STAGING IMAGING MODALITY				
CT	471 (46.0)	121 (20.7)	185 (47.4)	777 (38.9)
MRI and/or MRCP	66 (6.4)	34 (5.8)	24 (6.2)	124 (6.2)
CT + MRI and/or MRCP	487 (47.6)	429 (73.5)	181 (46.4)	1,097 (54.9)
ADDITIONAL IMAGING MODALITY				
US	423 (41.3)	137 (23.5)	86 (22.1)	646 (32.3)
PET	71 (6.9)	5 (0.9)	3 (0.8)	79 (4.0)

[a] Total cohort n = 1,998 [iCCA, n = 1,024; pCCA, n = 584; dCCA, n = 390].
Abbreviations: CT, computed tomography; dCCA, distal cholangiocarcinoma; iCCA, cholangiocarcinoma; MRCP, magnetic resonance cholangiopancreatectomy; MRI, magnetic resonance imaging; n, number of patients; pCCA, perihilar cholangiocarcinoma; PET, positron emission tomography; US, ultrasound.

Imaging findings elucidated that regional lymph node invasion and disseminated disease were present in 48.7% and 24.2% of patients, respectively (**Table R.3.**). CCAs preferentially metastasized to lung, liver, distant lymph nodes, bone and peritoneum — including omentum— with significant differences between subtypes. iCCA was mainly found to disseminate into lung, distant lymph nodes, and bone, whereas pCCA and dCCA mainly metastasized into the liver or to the peritoneum (**Fig. R.2A.**). In most patients with MD at presentation, a single site of metastasis was found (80.8%). **Fig. R.2B.** shows the frequency of each metastatic site based on the CCA subtype.

A



B

Nº metastatic sites	Location	iCCA	pCCA	dCCA	CCA (overall)
Single [n=367 (80.8%)]	Liver	-	45 (34.9)	22 (27.8)	67 (14.8)
	Lung	49 (19.9)	10 (7.8)	5 (6.3)	64 (14.1)
	Bone	17 (6.9)	1 (0.8)	2 (2.5)	20 (4.4)
	Peritoneum	53 (21.5)	30 (23.3)	11 (13.9)	94 (20.7)
	DLN	52 (21.1)	11 (8.5)	12 (15.2)	75 (16.5)
	Others	31 (12.6)	11 (8.5)	5 (6.3)	47 (10.4)
Two [n=63 (13.9%)]	Liver + Lung	-	2 (1.6)	2 (2.5)	4 (0.9)
	Liver + Bone	-	1 (0.8)	2 (2.5)	3 (0.7)
	Liver + Peritoneum	-	3 (2.3)	6 (7.6)	9 (2.0)
	Liver + DLN	-	-	2 (2.5)	2 (0.4)
	Liver + Others	-	1 (0.8)	2 (2.5)	3 (0.7)
	Lung + Bone	7 (2.8)	-	-	7 (1.5)
	Lung + Peritoneum	4 (1.6)	1 (0.8)	2 (2.5)	7 (1.5)
	Lung + DLN	13 (5.3)	1 (0.8)	-	14 (3.1)
	Lung + Others	2 (0.8)	-	-	2 (0.4)
	Bone + DLN	3 (1.2)	-	-	3 (0.7)
	Bone + Others	1 (0.4)	-	-	1 (0.2)
	Peritoneum + DLN	1 (0.4)	-	-	1 (0.2)
	Peritoneum + Others	3 (1.2)	1 (0.8)	-	4 (0.9)
	Others (2)	1 (0.4)	2 (1.6)	-	3 (0.7)
≥Three [n=24 (5.3%)]		9 (3.7)	9 (7.0)	6 (7.6)	24 (5.3)

Figure R.2. Preferential metastatic locations of cholangiocarcinoma. (A) Most commonly found metastatic locations stratified by CCA subtype, expressed as number and percentage [n (%)], and (B) classification of patients with disseminated CCA depending on their sites of metastasis, as single, two, three or more sites of metastasis. †Significant Pearson's chi-squared test for Bone (χ^2 *p<0.05*): iCCA *vs* pCCA, *p<0.05*; DLN (χ^2 *p<0.001*): iCCA *vs* pCCA, *p<0.0001*; Lung (χ^2 *p<0.001*): iCCA *vs* pCCA, *p<0.001*, and iCCA *vs* dCCA, *p<0.01*. *Abbreviations:* CCA, cholangiocarcinoma; dCCA, distal CCA; DLN, distant lymph nodes; iCCA, intrahepatic CCA; n, number of patients; pCCA, perihilar CCA.

R.3. Sensitivity of serum CEA and CA19-9 tumor biomarkers

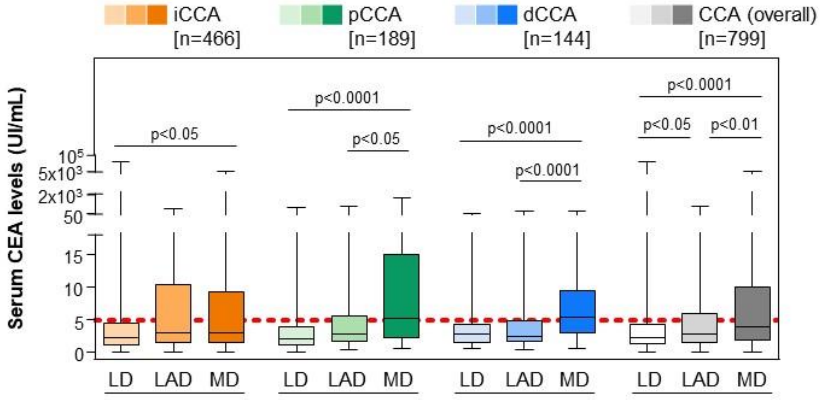
The sensitivity of CEA (cutoff value: 5 IU/mL) and CA19-9 (≥37 IU/mL) was evaluated according to the disease stage.

R.3.1. Carcinoembryonic antigen (CEA)

Serum CEA was above the upper reference limit in 30.9% of patients showing low diagnostic accuracy. This was of greater relevance for early-stage CCAs, with 78.6%, 62.2%, and 54.8% of cases under the upper reference limit for LD, LAD and MD, respectively, correlating with disease severity [OR = 1.71 (95% CI 1.16-2.51) for LAD; OR=3.03 (95% CI 2.11-4.35) for MD] (**Fig. R.3.**).

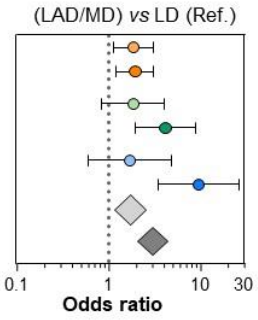
Considering CCA subtypes, CEA presented low diagnostic accuracy for all three at local to locally-advanced disease with the great majority of the patients with values under the established threshold (**Fig. R.3A.**). Of note, only iCCA exhibited increased risk of local spread when CEA levels were uprised compared to local disease with an OR of 1.86 (95% CI 1.13 – 3.07) (**Fig. R.3B.**). In contrast, CEA serum levels were more frequently increased when the disease appeared disseminated, specially for pCCA (53.4%) and dCCA (60.5%) (**Fig. R.3A.**). These resulted on increased risk of CEA tumor marker elevation on MD compared to LD for all three CCA subtypes [OR=1.92 (95% CI 1.19 – 3.09) for iCCA; OR=4.12 (95% CI 1.96 – 8.68) for pCCA; OR=9.40 (95% CI 3.44 – 25.64) for dCCA] (**Fig. R.3B.**).

A



	iCCA	pCCA	dCCA	CCA (overall)
Median (95% CI)	**2.6** (1.4 – 5.5)	**3.0** (1.6 – 7.0)	**3.2** (1.8 – 5.5)	**2.9** (1.5 – 5.8)
Normal / High, n (%)	329 (70.6) / 137 (29.4)	123 (65.1) / 66 (34.9)	100 (69.4) / 44 (30.6)	552 (69.1) / 247 (30.9)
Local disease				
n (%)	232 (49.8)	78 (41.3)	50 (34.7)	360 (45.1)
Median (95% CI)	**2.3** (1.2 – 4.5)	**2.2** (1.3 – 4.0)	**2.8** (1.6 – 4.2)	**2.3** (1.3 – 4.3)
Normal / High, n (%)	179 (77.2) / 53 (22.8)	61 (78.2) / 17 (34.0)	43 (86.0) / 7 (14.0)	283 (78.6) / 77 (21.4)
Locally advanced disease				
n (%)	107 (23.0)	53 (28.0)	51 (35.4)	211 (26.4)
Median (95% CI)	**3.0** (1.5 – 10.4)	**2.8** (1.7 – 5.6)	**2.5** (1.7 – 4.9)	**2.8** (1.6 – 6.0)
Normal / High, n (%)	69 (64.5) / 38 (35.5)	35 (66.0) / 18 (34.0)	40 (78.4) / 11 (21.6)	144 (68.2) / 67 (31.8)
Metastatic disease				
n (%)	127 (27.3)	58 (30.7)	43 (29.9)	228 (28.5)
Median (95% CI)	**3.0** (1.5 – 9.4)	**5.2** (2.3 – 15.0)	**5.5** (3.1 – 9.5)	**3.9** (1.9 – 10.1)
Normal / High, n (%)	81 (63.8) / 46 (36.2)	27 (46.6) / 31 (53.4)	17 (39.5) / 26 (60.5)	125 (54.8) / 103 (45.2)

B



	Locally advanced disease		Metastatic disease	
	OR (95% CI)	*p value*	**OR (95% CI)**	*p value*
iCCA	**1.86** (1.13 - 3.07)	*<0.05*	**1.92** (1.19 – 3.09)	*<0.01*
pCCA	**1.85** (0.84 – 4.03)	*ns*	**4.12** (1.96 – 8.68)	*<0.001*
dCCA	**1.69** (0.60 – 4.78)	*ns*	**9.40** (3.44 – 25.64)	*<0.0001*
CCA (overall)	**1.71** (1.16 – 2.51)	*<0.001*	3.03 (2.11 – 4.35)	*<0.0001*

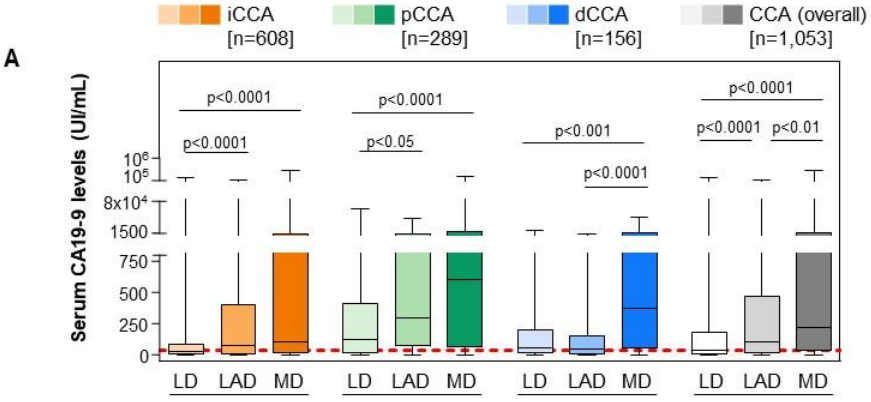
Figure R.3. Serum CEA tumor marker in cholangiocarcinoma. (A) Serum CEA levels depicted for disease stage (i.e., LD, LAD, or MD) for overall CCAs and subtypes. (B) Odds value of CEA as potential tumor biomarker in the identification of tumor spread compared to LD. CEA cutoff value established at 5 IU/mL. *Abbreviations: 95% CI, 95% confidence interval; CCA, cholangiocarcinoma; CEA, carcinoembryonic antigen; dCCA, distal CCA; iCCA, intrahepatic CCA; LAD, locally advanced disease; LD, local disease; MD, metastatic disease; OR, odds ratio; pCCA, perihilar CCA.*

R.3.2. Carbohydrate antigen 19-9 (CA19-9)

Increased CA19-9 was found in 59.1% of cases, particularly in patients with LAD (64.3%) or MD (73.3%) (**Fig. R.4A.**). Of note, CA19-9 showed increased risk of tumor spread when the levels of the biomarker were above the cutoff value [OR=1.99 (95% CI 1.47-2.70) for LAD; OR=3.04 (95% CI 2.21-4.17) for MD] (**Fig. R.4B.**).

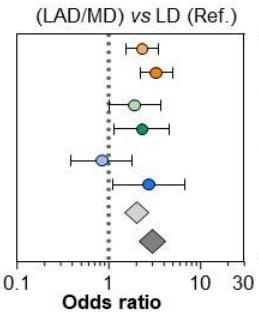
The highest median serum levels of CA19-9 were present in patients with pCCA (239.0 IU/mL) followed by dCCAs (73.9 IU/mL), whereas iCCAs (35.0 IU/mL) showed a median value in the normal range (**Fig. R.4A.**). Notably, CA19-9 levels appeared to augment in association with the disease severity for all three CCA subtypes. In particular, increased CA19-9 serum levels in iCCA showed a 2.33 and 3.30-fold increase on the risk of having LAD and MD, respectively (**Fig. R.4B.**). In addition, although no significant differences were observed betweeen LD and LAD for dCCA [OR=0.84 (95% CI 0.39 – 1.81)], significant increased risk was found between LD and MD [OR=2.73 (95% CI 1.11 – 6.76)] (**Fig. R.4B.**). In contrast, CA19-9 appeared to have good diagnostic accuracy for pCCAs, presenting increased levels in 75.8% of the patients, but showed lower sensitivity distinguishing disease spread [OR=1.93 (95% CI 1.00 – 3.70) for LAD; OR=2.31 (95% CI 1.16 – 4.61) for MD] (**Fig. R.4.**).

A



	iCCA	pCCA	dCCA	CCA (overall)
Median (95% CI)	**35.0** (8.0 – 256.9)	**239.0** (39.0 – 1,179)	**73.9** (19.4 – 313.6)	**66.1** (12.8 – 430.5)
Normal / High, n (%)	307 (50.5) / 301 (49.5)	70 (24.2) / 219 (75.8)	54 (34.6) / 102 (65.4)	431 (40.9) / 622 (59.1)
Local disease				
n (%)	305 (50.2)	121 (41.9)	56 (35.9)	482 (45.8)
Median (95% CI)	**22.3** (4.0 – 84.5)	**117.0** (13.1 – 408.9)	**51.7** (18.5 – 203.4)	**33.6** (8.0 – 175.0)
Normal / High, n (%)	192 (63.0) / 113 (37.0)	39 (32.2) / 82 (67.8)	22 (39.3) / 34 (60.7)	253 (52.5) / 229 (47.5)
Locally advanced disease				
n (%)	147 (24.2)	86 (29.8)	53 (34.0)	286 (27.2)
Median (95% CI)	**73.0** (11.0 – 404.0)	**294.5** (69.4 – 1,060)	**43.7** (9.4 – 152.4)	**103.3** (15.2 – 465.8)
Normal / High, n (%)	62 (42.2) / 85 (57.8)	17 (19.8) / 69 (80.2)	23 (43.4) / 30 (56.6)	102 (35.7) / 184 (64.3)
Metastatic disease				
n (%)	156 (25.7)	82 (28.4)	47 (30.1)	285 (27.1)
Median (95% CI)	**98.75** (17.4 – 856.5)	**600.5** (63.2 – 4,713)	**368.3** (56.8 – 2,055)	**221.2** (30.1 – 2,111)
Normal / High, n (%)	53 (34.0) / 103 (66.0)	14 (17.1) / 68 (82.9)	9 (19.1) / 17 (68.0)	76 (26.7) / 209 (73.3)

B



	Locally advanced disease		Metastatic disease	
	OR (95% CI)	*p value*	**OR (95% CI)**	*p value*
iCCA	**2.33** (1.56 – 3.48)	*<0.0001*	**3.30** (2.20 – 4.95)	*<0.0001*
pCCA	**1.93** (1.00 – 3.70)	*<0.05*	**2.31** (1.16 – 4.61)	*<0.05*
dCCA	**0.84** (0.39 – 1.81)	*ns*	**2.73** (1.11 – 6.76)	*<0.05*
CCA (overall)	**1.99** (1.47 – 2.70)	*<0.0001*	**3.04** (2.21 – 4.17)	*<0.0001*

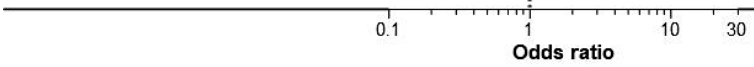
Figure R.4. Serum CA19-9 tumor marker in cholangiocarcinoma. (A) Serum CA19-9 levels depicted for tumor spread stage (i.e., LD, LAD, or MD) for overall CCAs and subtypes. (B) Odds value of CA19-9 as potential tumor biomarker in the identification of tumor spread compared to LD. CA19-9 cutoff value established at 37 IU/mL. *Abbreviations: 95% CI, 95% confidence interval; CA19-9, carbohydrate antigen 19-9; CCA, cholangiocarcinoma; dCCA, distal CCA; iCCA, intrahepatic CCA; LAD, locally advanced disease; LD, local disease; MD, metastatic disease; OR, odds ratio; pCCA, perihilar CCA.*

R.3.3. Serum tumor markers in CCA

The elevation of one single serum marker (i.e., CEA or CA19-9) was slightly associated with LAD [OR=1.72 (95% CI 1.16-2.53)] or MD [OR=2.53 (95% CI 1.56-4.10)] at diagnosis, whereas the concomitant elevation of both considerably increased the odd for LAD [OR=2.16 (95% CI 1.43-3.27)] and for MD [OR=5.86 (95% CI 3.69-9.25)] (**Table. R.5.**).

Table R.5. Serum tumor markers in cholangiocarcinoma. Patients were classified in three comparison groups: (1) with both circulating tumor biomarkers (CA19-9 and CEA) below the established threshold, (2) one of both biomarkers over the cutoff value, or (3) both, CA19-9 and CEA, over the cutoff value. Odds value of the combination of CA19-9 and CEA in the prediction of CCA staging compared to LD. [a]Both tumor markers (CA19-9 and CEA) below the cutoff value and LD as reference groups.

		OR[a] (95% CI)	*p value*
High CEA or CA19-9	LAD	**1.72** (1.16 – 2.53)	*<0.01*
	MD	**2.53** (1.56 – 4.10)	*<0.001*
High CEA & CA19-9	LAD	**2.16** (1.43 – 3.27)	*<0.001*
	MD	**5.88** (3.69 – 9.35)	*<0.0001*



Abbreviations: 95% CI, 95% confidence interval; CA19-9, carbohydrate antigen 19-9; CCA, cholangiocarcinoma; CEA, carcinoembryonic antigen; dCCA, distal CCA; iCCA, intrahepatic CCA; LAD, locally advanced disease; LD, local disease; MD, metastatic disease; OR, odds ratio; pCCA, perihilar CCA.

R.4. Management and outcome of patients with CCA

Patients with CCA often present tumor-mediated biliary obstruction, requiring biliary drainage prior to starting any therapeutic regimen. In particular, 40.3% of the patients received biliary drainage, from whom 42.4% required subsequent stenting, with a median time interval of 1.8 months (**Table R.6.**). This circunstance was specially relevant for pCCA which requiered stenting in 75.7% of the patients, followed by dCCA (57.6%), and lastly, iCCA (16.2%). Noteworthy, from all the pCCA with a primary stent, 56.3% requiered a sencond stenting in a median time interval of 1 month. Nevertheless, only around one forth of all dCCAs and iCCAs needed a second biliary tract dreinage after 4.5 and 5.5 months, respectively (**Table R.6.**).

Table R.6. Imaging methods used for the staging of CCA.

	iCCA	pCCA	dCCA	*p value*	CCA (overall)
BILIARY TRACT DRAINAGE					
Primary stent, *n (%)*	184 (16.2)	436 (75.7)	227 (57.6)	*<0.0001*	847 (40.3)
Secondary stent, *n (%)*	31 (29.0)	144 (56.3)	32 (25.6)	*<0.0001*	207 (42.4)
Time between stentings, *median (95% CI)*	5.5 (2.0 – 10.5)	1.0 (0.3 – 3.0)	4.5 (2.1 – 9.0)	*<0.0001*	1.8 (0.4 – 6.0)

Abbreviations: 95% CI, 95% confidence interval; CCA, cholangiocarcinoma; dCCA, distal CCA; iCCA, intrahepatic CCA; pCCA, perihilar CCA.

Fig. R.5. represents a flow chart summarizing the first therapeutic strategy of patients with CCA following initial diagnosis. Of note, biliary drainage was performed prior to surgery or systemic therapy in 32.2% and 35.0% of patients, respectively (**Fig. R.5.**). Moreover, 61.8% of all patients not receiving anticancer therapy had biliary drainage as part of the best supportive care (BSC). Surgical resection was performed in 50.3% of patients showing an after treatment mOS of 33.4 months. A total of 35.8% of patients had a R0 surgery displaying a mOS of 45.1 months and 1, 3, 5-year survival rates of 84.5%, 56.3% and 43.3%, respectively (**Fig. R.5.**).

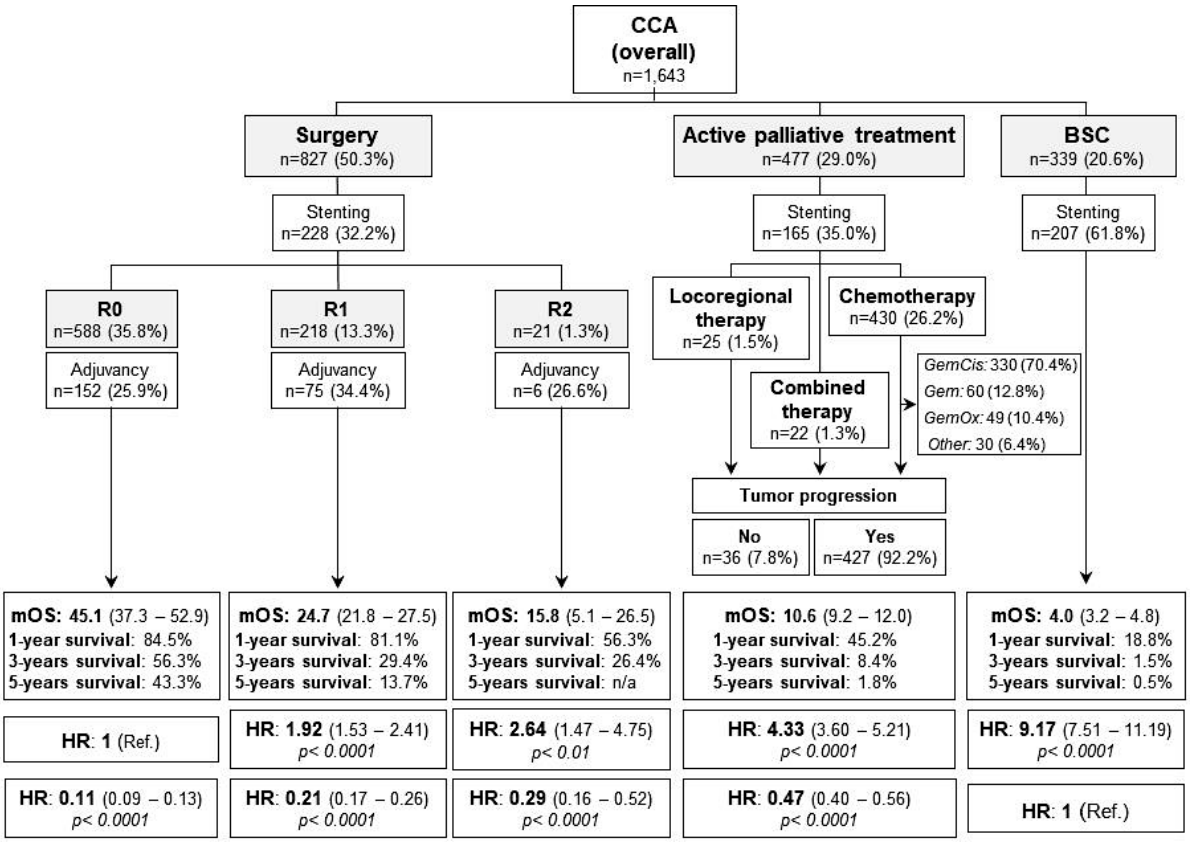
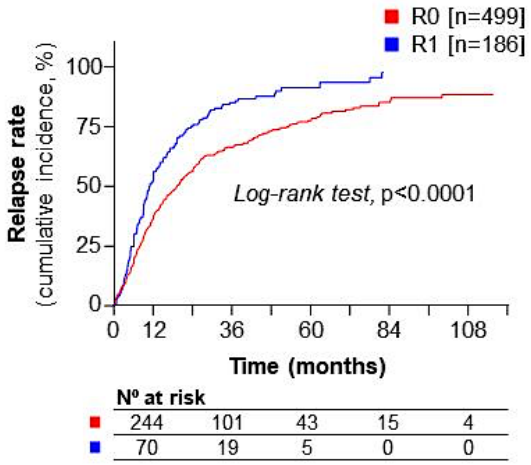


Figure R.5. Cholangiocarcinoma clinical management and outcome. Diagram of classification of patients with CCA divided by type of treatment strategy [i.e., surgery, active palliative treatment or BSC] together with the corresponding median overall survival and

Cox regression analysis between groups. Kaplan-Meier analysis and multivariable Cox regression models for the assessment of long-term outcome of patients with CCA after tumor resection. *Abbreviations: BSC, best supportive care; CCA, cholangiocarcinoma; Cis, cisplatin; dCCA, distal CCA; Gem, gemcitabine; HR, hazard ratio; iCCA, intrahepatic CCA; mOS, median overall survival; n, number of patients; Ox, oxaliplatin; pCCA, perihilar CCA; R0, null margin tumor resection; R1, microscopic residual disease tumor resection; R2, gross residual disease tumor resection; Ref, reference value.*

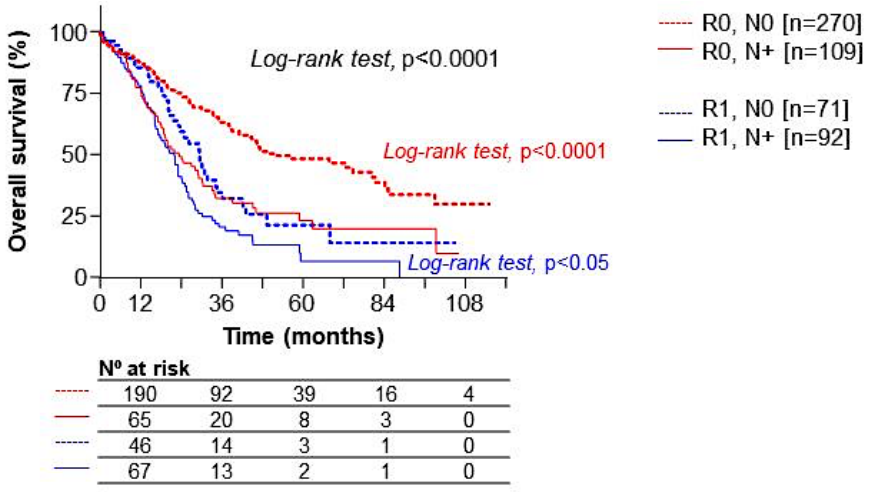
Patients with R1 tumor resection showed increased risk of relapse compared to R0 resection [HR=1.65 (95% CI 1.35-2.02)], with a mRFS of 10.7 and 19.1 months, respectively (**Fig. R.6.**). Moreover, R1 surgery achieved a mOS of 24.7 months and 1, 3, 5-year survival rates of 81.1%, 29.4% and 13.7%, respectively (**Fig. R.5.**). Patients with R1 after surgery showed increased risk of death compared to patients who had R0 resection [HR=1.92 (95% CI 1.53-2.41)] (**Fig. R.5.**), despite not showing survival differences compared to R2 surgery [HR=1.37 (95% CI 0.76-2.48)].



	R0	R1
Relapse, *n (%)*	299 (59.9)	144 (77.4)
mRFS, *months (95% CI)*	**19.1** (15.8 – 22.3)	**10.7** (8.9 – 12.6)
HR (95% CI), *p value*	**1** (Ref.)	**1.65** (1.35 – 2.02) *p<0.0001*

Figure R.6. Cholangiocarcinoma relapse after tumor resection. Kaplan-Meier analysis and multivariable Cox regression models for the assessment of tumor relapse as primary end-point of patients after tumor resection. *Abbreviations: 95% CI, 95% confidence interval; HR, hazard ratio; mRFS, median relapse-free survival; n, number of patients; R0, null margin tumor resection; R1, microscopic residual disease tumor resection; Ref, reference value.*

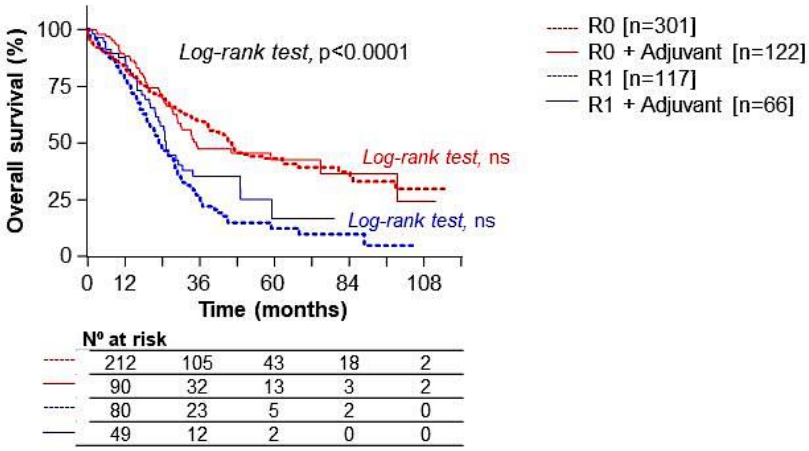
Lymph node invasion (N+) also compromised the OS of patients after resection (**Fig. R.7.**). Worse outcome was found in patients with N+ compared to N0, both after R0 or R1 tumor resections [HR=2.13 (95% CI 1.55-2.94), and HR=1.61 (95% CI 1.08-2.38), respectively]. These differences were also observed in post-surgical mOS: 52.2 months for R0/N0, 23.3 months for R0/N+, and 29.3 months and 21.8 months for R1/N0 and R1/N+, respectively (**Fig. R.7.**).



	R0		R1	
	N0	**N+**	**N0**	**N+**
Deaths, *n (%)*	98 (36.3)	61 (56.0)	36 (50.7)	73 (79.3)
mOS, *months (95% CI)*	**52.2** (33.5 – 71.0)	**23.3** (15.5 – 31.0)	**29.3** (23.1 – 35.5)	**21.8** (17.9 – 25.8)
HR (95% CI), *p value*	**1** (Ref.)	**2.13** (1.55 – 2.94) *p<0.0001*	**1.88** (1.28 – 2.76) *p<0.001*	**3.02** (2.22 – 4.11) *p<0.0001*
HR (95% CI), *p value*	**0.33** (0.24 – 0.45) *p<0.0001*	**0.71** (0.50 – 0.99) *p<0.05*	**0.62** (0.42 – 0.93) *p<0.05*	**1** (Ref.)

Figure R.7. Lymph node invasion influence on long-term outcome after tumor resection. Kaplan-Meier analysis and multivariable Cox regression models for the assessment of lymph node invasion-associated mortality for patients under tumor resection. Abbreviations: 95% CI, 95% confidence interval; HR, hazard ratio; mOS, median overall survival; N, lymph node invasion; n, number of patients; R0, null margin tumor resection; R1, microscopic residual disease tumor resection.

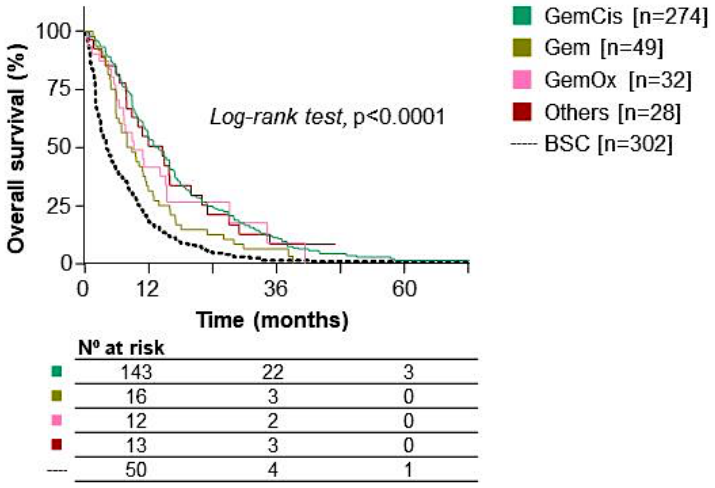
Noteworthy, 25.9% of R0 and 34.4% of R1 resected patients received adjuvant treatment, which did not improve the mOS when compared to patients not receiving any adjuvant therapy (**Fig. R.8.**).



	R0		R1	
	–	Adjuvant	–	Adjuvant
Deaths, *n (%)*	126 (41.9)	50 (41.0)	84 (70.9)	36 (54.5)
mOS, *months* *(95% CI)*	**46.3** (38.9 – 53.6)	**35.0** (11.9 – 58.1)	**23.0** (18.8 – 27.2)	**25.4** (23.7 – 27.1)
HR (95% CI), *p value*	**1** (Ref.)	**1.01** (0.73 – 1.40) *ns*	**1** (Ref.)	**0.77** (0.52 – 1.13) *ns*

Figure R.8. Kaplan-Meier survival curves for the analysis of the adjuvant treatment in resected CCAs. Kaplan-Meier analyses and Multivariable Cox regression models for the assessment of long-term outcome of patients with resected CCA (R0 or R1) after adjuvant therapy. *Abbreviations: 95% CI, 95% confidence interval; HR, hazard ratio; mOS, median overall survival; n, number of patients; R0, null margin tumor resection; R1, microscopic residual disease tumor resection; Ref, reference value.*

Out of the 816 (49.6%) patients with unresectable disease at diagnosis, the majority (477; 29.0%) received active palliative therapy (i.e., chemotherapy (26.2% of whole cohort), locoregional therapy (1.5%) and combined chemo- and locoregional therapies (1.3%)), with mOS and 1 and 3-year survival rates from time of treatment initiation of 10.6 months, and 45.2% and 8.4%, respectively (**Fig. R.5.**). In total, 92.2% of patients under palliative treatment showed tumor progression before death (**Fig. R.5.**). In patients receiving palliative chemotherapy, gemcitabine plus cisplatin (GemCis) was the most common regimen used (70.4%) (**Fig. R.5.**). Notably, GemCis showed significant reduced risk of death compared to BSC [HR=2.24 (95% CI 1.87-2.67)] or Gem alone [HR=1.66 (95% CI 1.22-2.28)] (**Fig. R.9.**).



	BSC	GemCis	Gem	GemOx	Others
Deaths, *n (%)*	269 (89.1)	243 (88.7)	47 (95.9)	24 (75.0)	23 (82.1)
mOS, *months* *(95% CI)*	**4.0** (3.2 – 4.85)	**12.0** (10.3 – 13.7)	**7.0** (3.1 – 10.9)	**8.6** (5.0 – 12.3)	**11.0** (9.5 – 11.9)
HR (95% CI), *p value*	-	**1** (Ref.)	**1.76** (1.28 – 2.41) *p<0.0001*	**1.21** (0.80 – 1.85) *ns*	**0.99** (0.65 – 1.52) *ns*
HR (95% CI), *p value*	**1** (Ref.)	**0.45** (0.37 – 0.53) *p<0.0001*	**0.74** (0.55 – 1.02) *ns*	**0.52** (0.34 – 0.79) *p<0.01*	**0.44** (0.28 – 0.67) *p<0.0001*

Figure R.9. Kaplan-Meier survival curves of the different chemotherapeutic regimens for CCA. Kaplan-Meier analyses and Multivariable Cox regression models for the assessment of long-term outcome of patients with the most commonly used chemotherapeutic regimens. *Abbreviations: 95% CI, 95% confidence interval; Cis, cisplatin; Gem, gemcitabine; HR, hazard ratio; mOS, median overall survival; n, number of patients; Ox, oxaliplatin; Ref, reference value.*

Patients under active palliative treatment showed shorter mOS when compared to curative-intended surgery (R0/R1) [HR=4.33 (95% CI 3.60-5.21) for R0; HR=2.25 (95% CI 1.82-2.77) for R1] (**Fig. R.5.**). No significant survival differences were found between active palliative therapy and R2 tumor resection [HR=1.62 (95% CI 0.91-2.89)]. Of note, 20.6% of patients received only BSC resulting in a mOS of 4.0 months (**Fig. R.5.**).

This comparative analysis of patients' management and outcome raised certain differences between the three CCA subtypes (**Fig. R.10.**). In particular, significant differences in survival were observed between CCA subtypes receiving BSC (**Fig. R.10D.**). Patients with pCCA received BSC more often (37.3%); however, patients with iCCA showed the poorest prognosis with mOS of 2.8 months over the 7.0 and 7.7 months found in pCCA and dCCA, respectively (**Table R.7.**). On the other hand, comparable mOS were obtained between CCA subtypes either undergoing tumor resection or active palliative treatment (**Fig. R.10., Table R.7.**).

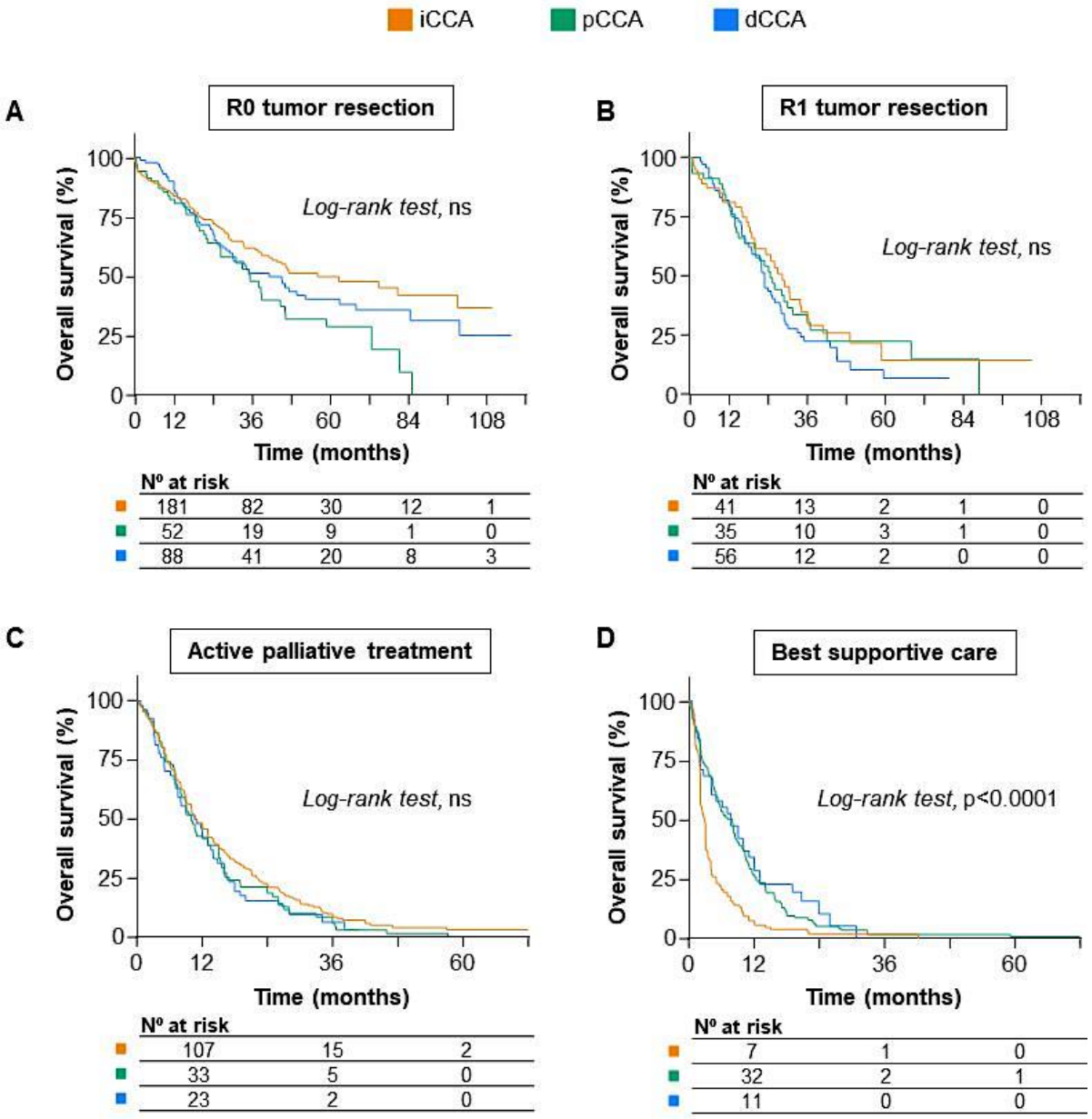


Figure R.10. Therapeutic approaches and outcomes in cholangiocarcinoma subtypes. Kaplan-Meier analysis for the assessment of long-term outcome of patients with CCA after (A) R0 tumor resection, (B) R1 tumor resection, (C) active palliative treatment, and (D) best supportive care. *Abbreviations: dCCA, distal cholangiocarcinoma; iCCA, intrahepatic cholangiocarcinoma; pCCA, perihilar cholangiocarcinoma; R0, null margin tumor resection; R1, microscopic residual disease tumor resection.*

Table R.7. Study of the clinical management and outcome for iCCA, pCCA and dCCA. Classification of patients with the International Classification of Diseases 11th version (ICD-11) coded CCA subtypes divided by type of treatment strategy [i.e., surgery, active palliative treatment or BSC] together with the corresponding median overall survival and 1-, 3- and 5-year survival proportions.

	R0 tumor resection	R1 tumor resection	Active palliative treatment	BSC
iCCA				
Number of cases, n (%)	314 (41.2)	68 (8.9)	250 (32.8)	125 (16.4)
Deaths, n (%)	93 (29.6)	34 (50.0)	213 (85.2)	115 (92.0)
mOS (95% CI)	**56.0** (33.5 – 78.5)	**28.5** (22.3 – 34.7)	**10.9** (9.0 – 12.8)	**2.8** (2.0 – 3.5)
1-year survival (%)	84.0	81.2	46.6	6.4
3-year survival (%)	62.0	34.9	9.2	1.8
5-year survival (%)	50.0	14.5	3.0	0.0
HR (95%CI)	**1** (Ref.)	**1.88** (1.27 – 2.79) *p<0.01*	**4.45** (3.45 – 5.73) *p<0.0001*	**15.66** (11.59 – 21.17) *p<0.0001*
p value	**0.06** (0.05 – 0.09) *p<0.0001*	**0.12** (0.08 – 0.18) *p<0.0001*	**0.28** (0.22 – 0.36) *p<0.0001*	**1** (Ref.)
pCCA				
Number of cases, n (%)	90 (24.3)	53 (14.3)	84 (22.7)	138 (37.3)
Deaths, n (%)	41 (45.6)	33 (62.3)	77 (91.7)	120 (87.0)
mOS (95% CI)	**35.5** (26.2 – 44.7)	**24.7** (19.1 – 30.2)	**10.0** (8.4 – 11.5)	**7.0** (5.1 – 9.0)
1-year survival (%)	81.5	79.8	42.7	26.6
3-year survival (%)	47.5	34.0	7.1	1.7
5-year survival (%)	28.5	22.6	0.0	0.9
HR (95%CI)	**1** (Ref.)	**1.28** (0.81 – 2.03) *ns*	**3.55** (2.41 – 5.24) *p<0.0001*	**5.32** (3.67 – 7.72) *p<0.0001*
p value	**0.19** (0.13 – 0.27) *p<0.0001*	**0.24** (0.16 – 0.36) *p<0.0001*	**0.67** (0.50 – 0.89) *p<0.01*	**1** (Ref.)
dCCA				
Number of cases, n (%)	110 (39.9)	74 (26.1)	55 (19.4)	39 (13.8)
Deaths, n (%)	57 (51.8)	56 (75.7)	50 (90.9)	34 (87.2)
mOS (95% CI)	**41.1** (28.6 – 53.6)	**23.0** (20.9 – 25.1)	**11.0** (6.3 – 15.6)	**7.7** (3.2 – 12.2)
1-year survival (%)	88.1	82.0	42.6	31.4
3-year survival (%)	51.4	22.8	6.6	0.0
5-year survival (%)	40.3	7.2	n/a	0.0
HR (95%CI)	**1** (Ref.)	**2.17** (1.48 – 3.17) *p<0.0001*	**5.37** (3.57 – 8.08) *p<0.0001*	**7.53** (4.76 – 11.92) *p<0.0001*
p value	**0.13** (0.08 – 0.21) *p<0.0001*	**0.29** (0.19 – 0.45) *p<0.0001*	**0.71** (0.46 – 1.11) *ns*	**1** (Ref.)

Abbreviations: BSC, best supportive care; 95% CI, 95% confidence interval; CCA, cholangiocarcinoma; dCCA, distal cholangiocarcinoma; HR, hazard ratio; iCCA, intrahepatic cholangiocarcinoma; mOS, median overall survival; n, number of patients; pCCA, perihilar cholangiocarcinoma; R0, null margin tumor resection; R1, microscopic residual disease tumor resection.

R.5. Prognostic factors

The univariate analysis between clinical and demographic variables at diagnosis and OS showed significant associations for CCA subtype, age, sex, ECOG-PS, disease status, and the serum levels of CA19-9, CEA, ALT, AST, GGT, ALP, albumin, or bilirubin (**Table R.8.**). However, a stepwise multivariate Cox regression analysis indicated that ECOG-PS, MD, and elevated CA19-9 levels were independent factors of prognosis (HR=1.52, 4.03, 2.79, respectively). Thus, patients' outcome based on these three independent variables was further depicted.

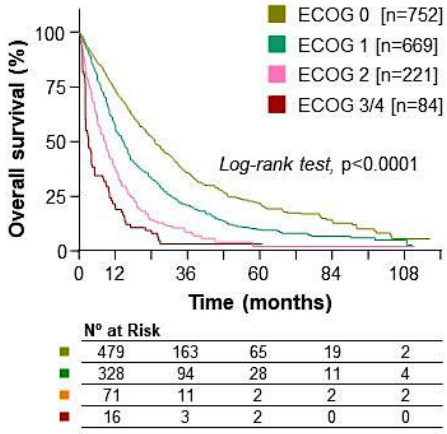
Table R.8. Univariate and multivariate Cox regression analysis of variables at diagnosis.

		UNIVARIATE			MULTIVARIATE[a]		
COVARIABLES	**Deaths,** n(%)	**HR**	**95% CI**	*p value*	**HR**	**95% CI**	*p value*
Subtype of CCA, *(vs pCCA)*							
iCCA	1,348 (68.7)	**0.74**	0.65 – 0.84	*<0.0001*	**1.48**	0.74 – 2.97	*ns*
dCCA		**0.67**	0.57 – 0.78	*<0.0001*	**1.31**	0.50 – 3.44	*ns*
Age, *≥65 (vs <65)*	1,348 (68.7)	**1.28**	1.15 – 1.42	*<0.0001*	**1.24**	0.70 – 2.22	*ns*
Sex, *male (vs female)*	1,348 (68.7)	**1.12**	1.00 – 1.24	*<0.05*	**0.99**	0.58 – 1.70	*ns*
ECOG-PS, *(continuous)*	1,247 (72.2)	**1.66**	1.56 – 1.78	*<0.0001*	**1.52**	1.01 – 2.31	*<0.05*
Disease status, *(vs local disease)*							
locally advanced disease	1,098 (72.9)	**1.91**	1.65 – 2.22	*<0.0001*	**1.68**	0.87 – 3.25	*ns*
metastatic disease		**3.46**	2.98 – 4.02	*<0.0001*	**4.03**	1.82 – 8.92	*<0.01*
CEA, *≥5 (vs <5)*	487 (62.0)	**2.02**	1.67 – 2.43	*<0.0001*	**1.19**	0.65 – 2.19	*ns*
CA19-9, *≥37 (vs <37)*	660 (61.1)	**2.02**	1.70 – 2.37	*<0.0001*	**2.79**	1.46 – 5.33	*<0.01*
ALT, *≥45 (vs <45)*	853 (63.5)	**1.15**	1.00 – 1.31	*<0.05*	**1.26**	0.62 – 2.59	*ns*
AST, *≥40 (vs <40)*	1,180 (69.8)	**1.43**	1.27 – 1.61	*<0.0001*	**0.48**	0.21 – 1.09	*ns*
GGT, *≥71 (vs <71)*	1,189 (70.1)	**1.96**	1.68 – 2.28	*<0.0001*	**1.51**	0.69 – 3.31	*ns*
ALP, *≥129 (vs <129)*	1,014 (70.2)	**1.80**	1.57 – 2.06	*<0.0001*	**1.24**	0.57 – 2.71	*ns*
Albumin, *<5.2 (vs ≥5.2)*	556 (71.5)	**0.26**	0.08 – 0.82	*<0.05*	**0.28**	0.03 – 2.64	*ns*
Bilirubin, *≥1.3 (vs <1.3)*	1,209 (70.0)	**1.41**	1.26 – 1.58	*<0.0001*	**0.98**	0.49 – 1.95	*ns*

[a] Multivariate analysis, number of events, n=66 (63.5%)

Abbreviations: 95% CI, 95% confidence interval; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CA19.9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; dCCA, distal cholangiocarcinoma; ECOG, eastern cooperative oncology group; GGT, gamma-glutamyl transferase; iCCA, intrahepatic cholangiocarcinoma; IQR, interquartile range; pCCA, perihilar cholangiocarcinoma; PS, performance status.

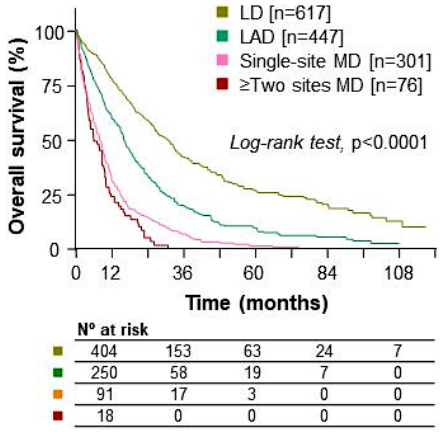
Fig. R.11. represents the OS for patients stratified according to ECOG-PS scores (0, 1, 2, 3-4) at diagnosis with pronounced differences between groups and with a mOS of 25.2 months [reference], 14.8 months [HR=1.57 (95% CI 1.38-1.78), 8.7 months [HR=2.76 (95% CI 2.32-3.28)] and 3.0 months (HR=4.65 (95% CI 3.64-5.95)] respectively.



	ECOG 0	ECOG 1	ECOG 2	ECOG 3/4
Deaths, *n (%)*	477 (63.4)	512 (76.5)	182 (82.4)	76 (90.5)
mOS, *months* *(95% CI)*	**25.2** (22.7 – 27.7)	**14.8** (13.2 – 16.3)	**8.7** (7.0 – 10.4)	**3.0** (1.6 – 4.4)
HR *(95% CI), p value*	**1** (Ref.)	**1.57** (1.38 – 1.78) *<0.0001*	**2.76** (2.32 – 3.28) *<0.0001*	**4.65** (3.64 – 5.95) *<0.0001*
HR *(95% CI), p value*	**0.22** (0.17 – 0.28) *p<0.0001*	**0.34** (0.26 – 0.43) *p<0.0001*	**0.59** (0.45 – 0.78) *p<0.001*	**1** (Ref.)

Figure R.11. Independent prognostic value of ECOG-PS in CCA. Kaplan-Meier analysis and multivariable Cox regression models for the assessment of long-term outcome of patients with CCA, with all-cause mortality as primary end-points for ECOG. *Abbreviations: 95% CI, 95% confidence interval; ECOG, eastern cooperative oncology group; HR, hazard ratio; mOS, median overall survival; n, number of patients.*

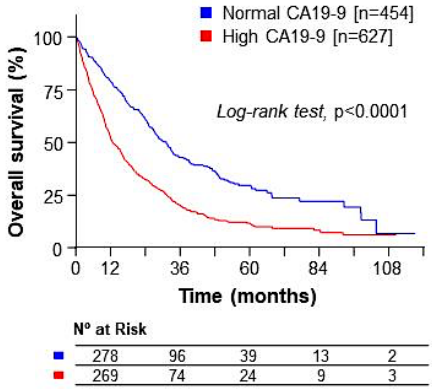
Besides, the disease stage [LAD, single-site MD, two or more sites MD] progressively increased the risk of death compared to patients with LD [(mOS=30.9 months), reference], with mOS of 16.2 months [HR=1.94 (95% CI 1.67-2.26)] for LAD, 8.1 months [HR=3.75 (95% CI 3.18-4.42)] for one single site MD, and 6.1 months [HR=5.02 (95% CI 3.86-6.54)] for two or more sites MD (**Fig. R.12.**).



	LD	LAD	MD	
			Single-site	≥Two sites
Deaths, *n (%)*	343 (55.6)	355 (79.4)	273 (90.7)	71 (93.4)
mOS, *months* *(95% CI)*	**30.9** (27.7 – 34.0)	**16.2** (14.8 – 17.5)	**8.1** (6.9 – 9.4)	**6.1** (3.7 – 8.6)
HR *(95% CI), p value*	**1** (Ref.)	**1.94** (1.67 – 2.26) *<0.0001*	**3.75** (3.18 – 4.42) *<0.0001*	**5.02** (3.86 – 6.54) *<0.0001*
HR *(95% CI), p value*	**0.20** (0.15 – 0.26) *<0.0001*	**0.39** (0.30 – 0.50) *<0.0001*	**0.75** (0.57 – 0.97) *<0.05*	**1** (Ref.)

Figure R.12. Independent prognostic value of tumor dissemination in CCA. Kaplan-Meier analysis and multivariable Cox regression models for the assessment of long-term outcome of patients with CCA, with all-cause mortality as primary end-points for disease status. *Abbreviations: 95% CI, 95% confidence interval; HR, hazard ratio; LAD, locally advanced disease; LD, local disease; MD, metastatic disease; mOS, median overall survival; n, number of patients.*

Moreover, CA19-9 had intrinsic prognostic value [HR=2.02 (95% CI 1.71-2.37)], with mOS of 31.0 and 12.7 months for normal or elevated (>37 IU/mL) CA19-9 levels, respectively (**Fig. R.13.**).



	Normal CA19-9	High CA19-9
Deaths, *n (%)*	218 (48.0)	442 (70.5)
mOS, *months (95% CI)*	**31.0** (27.5 – 34.5)	**12.7** (10.8 – 14.5)
HR *(95% CI), p value*	**1** (Ref.)	**2.02** (1.71 – 2.37) *p<0.0001*

Figure R.13. Independent prognostic value of CA19-9 in CCA. Kaplan-Meier analysis and multivariable Cox regression models for the assessment of long-term outcome of patients with CCA, with all-cause mortality as primary end-points for CA19-9 serum tumor biomarker. *Abbreviations: 95% CI, 95% confidence interval; CA19-9, carbohydrate antigen 19-9; HR, hazard ratio; mOS, median overall survival; n, number of patients.*

Discussion

The constant and exponential growth of the human population combined with the increase in life expectancy has escalated the medical burden of chronic liver diseases on the health systems. By the beginning of last decade, approximately 29 million EU citizens were suffering from at least one chronic liver disease.[133] Since the 1970s, chronic liver disease-related mortality rates have increased 400% in the United Kingdom, and now rank third in the most common causes of premature death.[134] The burden of chronic liver diseases results not only from its high mortality rates but also from a large network of associated components. Patients experience higher rates of comorbidities, lower quality of life and employment rates, more disability and higher healthcare costs.[135] In 2015, the average healthcare expenses in chronic liver disease patients was approximately $14.000 per patient, driven not only by the prescription of drugs, but also by a higher use of medical devices, evidencing the high economic impact worldwide.[135] The increase in chronic liver injury prevalence is markedly contributing to an alarming rise in the incidence of liver cancer, particularly in bile duct cancer. Consequently, liver cancer is now considered the sixth most common cancer worldwide.[136] Of note, due to its raising incidence and very poor prognosis due to late diagnosis, liver cancer (including both HCC and BTCs) is predicted to become the third-leading cause of cancer-related deaths in the Western world by 2040, thus surpassing the mortality rates of breast, prostate and colorectal cancers.[137].

CCA has traditionally remained as an orphan cancer, with scarce available information, and insufficient basic and clinical research behind. Consequently, different non-profit initiatives have been developed during the last years in order to improve the knowledge and management of this cancer, including the ENSCCA Registry. The ENSCCA Registry is an open, international and collaborative project promoted by the ENSCCA consortia with the main objective of collecting demographic and epidemiological information on patients with CCA in Europe. This international collaborative effort involves multidisciplinary groups, including hepatologists, gastroenterologists, oncologists, surgeons, radiologists and basic scientists, among others. Since 2016, the ENSCCA Registry has grown exponentially, both in the number of affiliated centres and in the proportion of patients included. This allowed obtaining a cohort of more than 2,000 patients, whose data have been herein analysed for the first time, giving an overview of the natural course, tumour presentation, management and outcome of patients with CCA in Europe, together with a comparison of the similarities and differences between the three recently WHO-classified CCA subtypes.

We herein provide evidence that, although a significant proportion of CCAs arises within an apparently healthy liver, population-based studies have identified different risk

factors very much associated with chronic liver injury.[1,2,19] Herein, we show that more than 50% of patients with CCA were overweight/obese and 20% were diabetic at diagnosis. Evidence suggests obesity, and particularly the metabolic syndrome, as a major risk factor for all cancers, but also evident for CCA.[19] In fact, the obesity pandemic in the adult population has experienced a rapid growth since 1970s, which preceded the increased incidence of iCCA observed since 1980s.[138–140] Moreover, recent studies suggest that NAFLD, a condition related with the metabolic syndrome, might be a major risk factor, alone or in association with obesity, for cancer, and in particular for HCC and CCA.[20,141,142] Based on our results and on a meta-analysis of twenty-four studies showing a pooled prevalence of 77.9% NAFLD in diabetic patients with obesity,[143] we could expect a considerable prevalence of NAFLD in our patients with CCA.

Other pathologic conditions that have traditionally been associated with CCA development seem to have a subtype-dependent impact. For instance, HBV and HCV infections inferred greater risk for iCCA than p/dCCA. Of note, during the last decades the prevalence of viral hepatitis display decreasing trends due to vaccination programs or new effective treatments,[144] while others like the aforemention metabolic-associated conditions are altering the epidemiological scenario for CCA. This raises the need for the involvement of primary care to develop and implement awareness policies based on lifestyle changes. Furthermore, 12.6% of the patients with iCCAs concomitantly presented liver cirrhosis, most probably in association with the previously reported viral infection, alcohol consumption, or NAFLD. Interestingly, and according to our data, although end-stage liver cirrhosis in patients with PBC is a well-known risk factor for the development of HCC, it may also predispose to iCCA development. This confirms previous data from the SEER program of the National Cancer Institute (NCI) from United States, in which PBC was associated to iCCA, but not to extrahepatic CCA.[145] Moreover, PSC, the most accepted risk factor for CCA, resulted more associated to pCCA in our dataset, being in accordance with previous reports.[20] Up to 15-20% of patients with PSC might develop CCA during lifetime, commonly within the first year after PSC diagnosis.[146] Besides, CCA accounts for more than 30% of all PSC-related deaths,[147] constituting an important health and social problem. For this reason, attempts have been made to implement detection strategies for the diagnosis of CCA in patients with PSC, although none of the screening tests have a satisfying accuracy. As shown in our results, serum CA19-9 levels are generally not elevated in early stages of CCA. This finding, together with the serum biomarker elevation in ~30% of patients with isolated PSC and the inability to express CA19-9 in up to 7% of the general population due to *FUT3* deficiency,[148] strongly compromises the capability of this biomarker as an early indicator

of CCA development. On the other hand, non-invasive imaging modalities, including US, CT, and MRI/MRCP, represent valuable techniques for CCA detection, but abnormalities seen with these tools may be confusing indicators of both CCA and benign strictures commonly present in patients PSC.[148]

CCAs are usually diagnosed by a sequential protocol comprising imaging approaches and assessment of non-specific tumor biomarkers in serum, followed by biopsy or cytology, when feasible.[2] Serum levels of CA19-9 and CEA are frequently determined in the clinical practice when CCA is suspected.[149,150] Nevertheless, the diagnostic accuracy of both tumor markers is controversial, but limited in most of the cases, particularly in early-stage tumors.[151,152] Our data showed that CEA and CA19-9 appear more often elevated as the disease progresses, supporting previous reports in which preoperative elevation of serum CA19-9 appears as a predictor of nodal invasion and worse post-surgical survival.[59,153] Moreover, increased CA19-9 but not CEA, has also been related to a poorer response to chemotherapy.[61] Consequently, future prospective studies should determine the potential utility of CEA and CA19-9 to identify patients who would benefit from a more detailed staging, using, for instance, ^{18}FDG-PET. Our results highlight the underuse of FDG-PET for the staging of CCA, even though it may help in the identification of occult nodal and distant metastatic status.[154] Of note, the multivariate Cox regression analysis revealed that elevation of CA19-9, but not of CEA, is an independent prognostic factor for CCA, a finding of translational relevance for patient stratification and design of clinical trials. Indeed, our findings showed independent prognostic value not only for CA19-9, but also for both the ECOG-PS, and disease status. These results share some similarities with a previous work that proposed a new clinical-based staging system for pCCA that includes ECOG-PS, tumor size and number, vascular encasement, tumor dissemination and CA19-9 as stratification factors.[155]

Alltogether, there is an unmet clinical need for a consensus on specific CCA screening programs in high-risk populations [*i.e.*, choledochal cysts, gallstones, cirrhosis, biliary diseases (Caroli's, PSC), and viral infections (HBV, HCV)],[19] and advocates the need to increase awareness of CCA to address those lifestyle factors [*i.e.*, obesity, alcohol abuse, smoking]. Regarding the former, in recent years new innovative studies have been carried out focused on the search for precise biomarkers for the early diagnosis of CCA. These strategies include "omics" approaches in blood, bile, urine, extracellular vesicles, and tissues, and have resulted in promising candidates, such as circulating tumor cells, miRNAs, proteins, and metabolites, which could allow early detection of general CCA, and also when associated with specific risk factors.[149]

Beyond the features shared by all CCAs, increasing evidence indicate that CCA subtypes might differ in clinical presentation, etiology, natural history, management, and prognosis and thus, should be regarded as distinct entities.[1,156] This study suggests that perihilar and distal tumors have an earlier detection than intrahepatic lesions, mainly because they usually cause obstructive jaundice in an early stage. Consequently, iCCAs appeared as larger or multifocal lesions, and predominantly as moderate-to-poorly differentiated tumors. Most iCCAs showed a mass-forming growth pattern, whereas pCCA and dCCA were mostly flat or periductal infiltrating, and less frequently intraductal, supporting previous observations.[8] Nonetheless, no differences in disease stage at diagnosis were found between CCA subtypes. This may be, at least in part, because hepatic dissemination of iCCA is not formally considered metastasis according to the current AJCC guidelines. In this regard, we have recently shown that patients with iCCA and cancer spread within the liver, with or without lymph node invasion, have worse prognosis than patients with local iCCA, strongly encouraging the establishment of a new specific coding for these kind of patients as M1.[157]

In our dataset, one out of five patients did not receive active palliative treatment (just BSC), probably due to late diagnosis and deterioration of patients' ECOG-PS. However, our cohort study confirmed longer survival in patients who received some form of anti-cancer treatment for unresectable disease,[101,131,158] highlighting the need to consider these therapies when performance status is suitable.[131,159] Despite the standard GemCis regimen showed the longest median survival, no statistical difference was observed compared to GemOx. These results are consistent with data obtained in a previous systematic review;[103] however, the superiority of one platinum over the other has never been directly compared in a clinical trial. Actually, no triple-agent chemotherapeutic regimens were evaluated in this analysis, which deserves future attention based on the promising results coming from the lattest clinical trials. Into the bargain, future studies should also compare locoregional —with current limited experience in CCA— *versus* systemic therapies for the treatment of unresectable CCAs, along with immunotherapy. In this regard, immune checkpoint modulation has emerged as a potential strategy in CCA, which in combination with the standard of care chemotherapy have shown a relevant therapeutic value as first-line therapy.[160]

According to our data, tumor resection is the best therapeutic option. However, the decision to perform tumor resection is a difficult trade-off between short-term risk (i.e., post-surgical mortality) and potential long-term benefit. Both resection margin involvement and lymph node invasion are important determinants of clinical outcome. Moreover, postopeative relapse is frequent in patients undergoing curative-intended

tumor resection, with surgical margins being important factors of disadvantage. Our data did not show any survival benefit when adjuvant chemotherapy was used, although this result should be carefully addressed as patients included in the analysis were those diagnosed before 2017, and thus, before the recommendation of capecitabine as adjuvant therapy after curative tumor resection.[161]

According to the CCA subtypes, dCCA has the highest resectability rate, but the proportion of surgeries with positive margins is considerable. Although they show a remarkable number of resectable tumors, iCCA are characterized by higher rate of palliative active therapy compared to p/dCCA; this could be due, at least in part, to the presence of underlying liver diseases, which make these patients more often inelegible for tumor resection. On the other hand, patients with pCCA, probably due to compromised patient phycal stutus related to the higher risk of developing biliary obstruction, are not frequent candidates for any anti-cancer treatment other than BSC, and presenting the lowest rate of tumor resection. Despite the proportions to each therapeutic strategy, similar outcomes were evidenced for all three CCA subtypes under active palliative treatment and tumor resection. Still, patients with iCCA under BSC showed the worst overall survival, probably due to the progression of associated chronic liver diseases (e.g., cirrhosis, viral hepatitis, NAFLD). Altogether, these data reinforce the need of adequate investment in early and accurate diagnosis of CCA, the shortening of time to surgery, and the systemic treatment of advanced disease when feasible, as the best strategies to improve the outcome of patients. Furthermore, molecular profiling has shown that approximately 45% of patients with iCCA have actionable alterations,[162] paving the path for new opportunities for these patients to be treated with targeted therapies. However, actionable alterations in eCCA are rare,[162] and targeted therapies are urgently needed for patients with these malignancies. Hence, the high prevalence of these targetable alterations underscores the importance of performing next-generation sequencing for patients with CCA in order to fully depict the genomic landscape of these tumors. However, even with the initial success of targeting various molecular subtypes, further results based on the ongoing phase III

clinical trials with ivodesinib (IDH1 inhibitor), infigratinib or pemigatinib (FGFR inhibitors), are needed in order to assess the real benefit of these therapies over the current standard GemCis. Still, the identification of additional pharmacological alterations remains an important goal for iCCA, and particularly for p/dCCA.

This study has several limitations. It shows novel data on the course of CCAs in European reference centers, but it cannot be interpreted as a demographical study; therefore, caution is required when extrapolating the results. Patients' selection bias related to clinical specialties of participating centers (hepatologists, gastroenterologists, medical oncologists, surgeons) could explain the differences between CCA subtypes and disease stages at diagnosis. Besides, the diagnosis and classification by CCA subtypes were based on investigator-reported data following a data harmonization. Even though no external audit was performed, and internal review was conducted by each center in order to double-check the included data. Nevertheless, the absence of a central reading should not have a major impact on the conclusions drawn from this work. In fact, the expected number of cases with undistinguishable location would be very low as they are retrieved by investigators affiliated to referral hospitals with large experience in the management of CCA. Clinical approaches related to the diagnostic work-up and disease monitoring programs may diverge between hospitals and specific departments of specialization. In addition, differences in terms of disease phenotype and incidence of risk conditions for CCA may exist between countries. In a separate matter, the percentage of patients receiving BSC is probably underestimated as invasive methods for histological/cytological disease confirmation required patients to be eligible for this study, and thus, are often not performed to those unfit for anti-cancer treatment.

In conclusion, to our knowledge, this study constitutes the largest and most comprehensive international analysis, including more than 2,200 patients with CCA from eleven European countries, providing a comprehensive analysis of diagnostic, prognostic and therapeutic aspects of the complex CCA landscape. Our results show that CCA is still diagnosed at an advanced stage, a significant proportion of patients fail to receive any cancer-specific therapy, and therefore, the prognosis is dismal. Accordingly, the promotion of awareness campaigns and education programs aimed to prevent life-style related risk factors and the implementation of surveillance for early detection of CCA in high-risk populations are urgently required in order to decrease cancer-related mortality. In this regard, this study represents valuable knowledge for future comparisons with new targeted therapies and for the design of next generation personalized clinical trials.

Conclusions

1- Patients with CCA are more commonly men and are diagnosed at an advanced age and presenting good performance status.

2- The majority of patients with CCA were overweight or obese at diagnosis, which may suggest obesity as an important contributing factor during cholangiocarcinogenesis.

3- There is a subtype-dependent association with underlying diseases, with iCCA arising more often in a cirrhotic background, pCCA in patients with biliary diseases such as PSC, and dCCA being related to gallstone disease.

4- CCA subtypes differ in size and growth pattern, with iCCA being larger or multifocal MF lesions, and p/dCCAs smaller and mostly PI tumors.

5- Most CCAs are diagnosed at advanced stage, when the tumor is spread to regional lymph nodes and/or distant organs, with the peritoneum and the liver as commonly preferred sites of metastasis.

6- The site of metastasis in patients with CCA differs between subtypes, with iCCA spreading more frequently into distant lymph nodes, lung, and bone, compared to p/dCCAs.

7- Serum CA19-9 exhibits low sensitivity in the early stages of the disease, however, its levels progressively increase with regional lymph node invasion, and especially, at metastatic disease.

8- Tumor resection is the best therapeutic strategy displaying the greatest survival rates, but tumor margins and lymph node invasion compromises survival after surgery.

9- Patients with margin involvement after tumor resection are at higher risk of relapse compared to those with complete resection.

10- For patients with unresectable disease, active palliative treatment improves survival, with GemCis showing the best survival response, compared to those receiving only best supportive care.

11- Patients with iCCA present shorter survival rates under best supportive care than those with pCCAs or dCCAs.

12- The multivariate survival model indicates that the ECOG performance status, disease stage, and CA19-9 levels are independent prognostic factors for cholangiocarcinoma.

Summary in spanish

Resumen en español

INTRODUCCIÓN

El colangiocarcinoma (CCA) agrupa un conjunto heterogéneo de tumores malignos que pueden surgir en cualquier localización a lo largo de los conductos biliares. A pesar de ser una enfermedad rara, en los últimos 20 años, su incidencia y tasa de mortalidad han aumentado a nivel mundial (0,3-6 casos por 100.000 habitantes al año en los países occidentales, y >6 casos en algunas regiones de Asia oriental).[2] Pese a la identificación de diversos factores de riesgo, en países occidentales cerca de un 50% de los pacientes continúan siendo diagnosticados en ausencia de condiciones reconocibles que predispongan al desarrollo de CCA.[19] Por ello, y en ausencia de programas de vigilancia de pacientes en alto riesgo, se encuentra limitada la capacidad de detección temprana del CCA. Cabe destacar la naturaleza asintomática del CCA, especialmente en sus etapas más tempranas, así como su alta agresividad y resistencia a tratamientos quimioterápicos que comprometen las posibilidades de supervivencia de los pacientes.[2] Según el origen anatómico, el CCA se ha clasificado recientemente (ICD-11) en iCCA, pCCA y dCCA.[2,163] Algunos trabajos han reportado la existencia de diferencias en términos de factores de riesgo, presentación clínica, histomorfología, manejo clínico y pronóstico entre los subtipos de CCA;[2] sin embargo, estudios de cohorte con grandes conjuntos de datos que puedan justificar estas diferencias son limitados.[164] Por todo ello, resulta fundamental la realización de un estudio multicéntrico, internacional para mejorar la granularidad de la situación clínica mundial del CCA, a fin de mejorar el conocimiento sobre el curso de la enfermedad, definir las similitudes y/o diferencias entre los subtipos de CCA, describir los resultados tras los tratamientos, e identificar nuevas cuestiones clínicas para futuros análisis prospectivos.

HIPÓTESIS Y OBJETIVOS

El Registro ENSCCA representa una herramienta única para conocer en detalle el curso del CCA, con el objetivo principal de investigar la presentación, manejo clínico y evolución de los pacientes para mejorar la asistencia sanitaria. En segundo lugar, se espera mejorar la clasificación de los subtipos de CCA, proporcionar nuevos conocimientos sobre su patogenia y su respuesta a las terapias actuales.

Por lo tanto, es este estudio se proponen evaluar los siguientes objetivos:

I. Generación de un registro europeo de pacientes con colangiocarcinoma.
II. Establecimiento de una red pan-europea multicéntrica de recopilación de datos clínicos.
III. Revisión y armonización de los datos incluidos en el registro.
IV. Análisis de los datos:
    a. Determinación de la demografía y los posibles factores de riesgo de los pacientes diagnosticados con CCA a lo largo de una década (2010-2019).
    b. Evaluación de la presentación clínica e histomorfológica del CCA al diagnóstico.
    c. Estudio sobre el manejo clínico de pacientes con CCA y su evolución a largo plazo.

MÉTODOS

Diseño del estudio y tratamiento de los datos

El Registro ENSCCA es un estudio observacional multicéntrico de pacientes con CCA, en el que participaron un total de 26 Centros Sanitarios de referencia de 11 países europeos (Alemania, Austria, España, Francia, Italia, Lituania, Noruega, Países Bajos, Polonia, Reino Unido y Rumanía). El estudio incluyó pacientes diagnosticados con CCA durante un período de 10 años (1 de enero de 2010 a 31 de diciembre de 2019), siendo los criterios de inclusión los siguientes: *i*) diagnóstico de CCA según el *International Classification of Diseases* 11ª Edición (ICD-11),[6] en intrahepático (2C12), perihiliar (2C18), o distal (2C15); y *ii*) confirmación diagnóstica por histológia y/o citológia. La obtención de datos de pacientes se llevó a cabo a partir de las historias clínicas de los hospitales participantes, contando con información demográfica, factores de riesgo documentados, antecedentes médicos previos, parámetros bioquímicos y clínicos, y tratamientos frente al CCA.

En el Registro ENSCCA, alojado en la aplicación web *Research Electronic Data Capture* (REDCap™) de la Asociación Española de Gastroenterología (AEG; www.aegastro.es), la recogida de se lleva a cabo en formato no identificado y cumpliendo con los reglamentos y disposiciones aplicables a los estudios observacionales. El protocolo de estudio del Registro ENSCCA fue aprobado por el Comité de Ética de Euskadi, España (Código: PI2016137), como Centro coordinador,

así mismo, cada Centro participante obtuvo una aprobación local para avalar su participación.

La exportación de los datos para el análisis se realizó en febrero de 2020. Se excluyeron del estudio todos aquellos casos que no cumplían con los criterios de inclusión y/o que estaban incompletos en cuanto a datos epidemiológicos y/o clínicos obligatorios (*i.e.*, tipo de CCA, fecha de diagnóstico y fecha de la última visita médica o muerte). Por otro lado, para el análisis de supervivencia, se consideraron pacientes diagnosticados entre 2010 y 2017 (n=1.962), garantizando un mínimo de 2 años de seguimiento. Los pacientes fueron clasificados de acuerdo a la localización anatómica del tumor primario en iCCA, pCCA o dCCA. Por otro lado, también fueron clasificados en base a la etapa de la enfermedad al momento del diagnóstico, como: *i*) enfermedad local (LD), *ii*) enfermedad localmente avanzada (LAD), o *iii*) enfermedad metastásica (MD). De acuerdo a las pautas de estadificación de la AJCC, el término LAD hace referencia a aquellos casos clasificados como N+ (*i.e.*, N1 para iCCA y dCCA; N1 y N2 para pCCA), y MD a aquellos con afección en distales (M1), con la excepción de la diseminación hepática de tumores intrahepáticos que se consideran múltiples tumores (T2b), y por lo tanto, LD. La resectabilidad de los tumores fue determinada por el equipo multidisciplinario local en base a pautas internacionales publicadas, tales como las de ESMO e ILCA,[128,131] así como considerando criterios multiparamétricos basados en el estado funcional, el estadio del tumor, enfermedades subyacentes y comorbilidades, entre otras. De este modo, los tratamientos se clasificaron en: 1) cirugía (*i.e.*, resección tumoral o trasplante hepático subdividido en i) R0 [resección tumoral de margen negativo], ii) R1 [enfermedad residual microscópica], y iii) R2 [enfermedad residual macroscópica]), y 2) tratamiento paliativo activo (*i.e.*, quimioterapia y terapias locoregionales).

Análisis estadístico

Los datos demográficos y los factores de riesgo se resumieron utilizando estadísticos descriptivos. Los datos continuos se describieron como mediana y rango inter-cuartílico (RIC), mientras que las variables categóricas se resumieron como numero y porcentaje. La probabilidad se calculó excluyendo los casos con información desconocida. Se utilizó la prueba de Shapiro-Wilk para evaluar la distribución normal en variables continuas. Comparaciones múltiples con datos paramétricos o no paramétricos se llevaron a cabo mediante ANOVA o pruebas de Kruskal-Wallis, respectivamente. Las comparaciones por pares se realizaron usando el método de Dunn. La prueba $\chi 2$ de Pearson se realizó

para comparar variables categóricas entre los 3 subgrupos, mientras que fue la prueba exacta de Fisher la de elección para comparaciones entre 2 grupos. La supervivencia global (SG) se evaluó como el tiempo desde el diagnóstico hasta muerte o última visita médica, mientras que la supervivencia posterior al tratamiento consideró la fecha de inicio del tratamiento. La supervivencia libre de recaídas fue calculada como el tiempo desde la resección del tumor hasta el evento de recaída o la muerte. Pacientes sin información sobre supervivencia, perdidos durante el seguimiento o vivos en la última visita médica fueron censurados en la fecha de último registro. El análisis de supervivencia se realizó con los métodos Kaplan- Meier y regresión de Cox.

Los análisis estadísticos se realizaron con IBM SPSS Statistics Versión 22.0 (IBM Corp., Armonk, NY, EE.UU.) y GraphPad Prism versión 6.0 para Microsoft Windows, (Software GraphPad, La Jolla California, EE.UU). Todos los valores de *p* se obtuvieron en pruebas de 2 colas y $p<0,05$ se consideró estadísticamente significativo.

RESULTADOS Y DISCUSION

Características clínicas de los pacientes al diagnóstico

De los 3.039 pacientes incluidos inicialmente en el Registro ENSCCA, cumpliendo con los criterios de inclusión, 2.234 (73,5%) fueron seleccionados y analizados. En total, se analizaron 1.243 (55,6 %) pacientes con iCCA, 592 (26,5%) con pCCA y 399 (17,9%) con dCCA. La mayor parte de los pacientes eran caucásicos (96,6%) con una mediana de edad al diagnóstico de 66 años (RIC 58-73) y una ligera sobrerrepresentación de hombres (56,4%). Gran parte de los pacientes mostraron aumento de los niveles séricos de alanina aminotransferasa (ALT), marcadores de colestasis (gamma-glutamiltransferasa [GGT] y fosfatasa alcalina [ALP]) y antígeno carbohidrato 19-9 (CA19-9), particularmente pacientes con pCCA o dCCA.

En relación con las comorbilidades, el 55,1% de los pacientes presentaban sobrepeso/obesidad (IMC 25-30 kg/m2 [35,7 %] o IMC ≥30 kg/m2 [19,4%]) en el momento del diagnóstico, siendo esta característica más frecuente en pacientes con iCCA. Un 22,5% tenía diabetes, observándose con mayor frecuencia en pacientes con iCCA o dCCA en comparación con pCCA, y el 39,9% tenía hipertensión arterial. Estos resultados concuerdan con evidencias previas que señalan la obesidad, y en general el síndrome metabólico, como un factor de riesgo importante para el desarrollo de cáncer en general, pero también de CCA.[19] De hecho, la pandemia de obesidad en la población

adulta de EE.UU. ha experimentado un rápido crecimiento desde la década de 1970, precediendo al aumento de la incidencia de iCCA observado en la década de 1980.[138,139] Por otro lado, se detectó una asociación dependiente del subtipo con enfermedades biliares o hepáticas subyacentes, tales como la colangitis biliar primaria (PBC: 3,3%, principalmente iCCA), colangitis esclerosante primaria (PSC: 4,5%; principalmente pCCA), cálculos en las vías biliares (6,1%; principalmente pCCA y dCCA), hepatitis viral (2,8% virus de la hepatitis C, 4,6% virus de la hepatitis B y 0,1% de infección concomitante; principalmente iCCA) y cirrosis (7,8%; principalmente iCCA). Además, se observó la presencia de antecedentes de tabaquismo o consumo de alcohol en el 33% y 19,8% de los pacientes, respectivamente. El hecho de que un 12,6% de los pacientes con iCCA tuvieran antecedentes cirróticos, es muy probable que esté asociado con la presencia de infecciones virales, el consumo de alcohol o la enfermedad de hígado graso, entre otras. En general, los datos del presente estudio concuerdan con lo ya descrito en relación a los factores de riesgo.[19,27] Resulta relativamente novedosa la importancia que toma la PBC como comorbilidad en pacientes con iCCA, apoyando datos previos del programa *Surveillance, Epidemiology and End Results* (SEER) del Instituto Nacional del Cáncer (NCI) de Estados Unidos, donde ya relacionaron la PBC con el iCCA y no al CCA extrahepático.[145] Por lo tanto, nuestros resultados apoyan la necesidad de programas de cribado específicos de CCA en pacientes con factores de alto riesgo para la detección precoz de este cáncer.

Descripción macroscópica del CCA al diagnóstico

Los pacientes con CCA son generalmente asintomáticos al diagnóstico. Por lo tanto, no sorprende que la mayor parte de los pacientes presentaran un estado funcional adecuado en base a la escala ECOG, siendo de 0 en el 44,0% de los casos, y de 1 en el 39,1%. En cuanto a las características del tumor, en términos de tamaño y patrón de crecimiento, los iCCA eran con frecuencia lesiones mayores de >3 cm o multifocales con un patrón de formación de masa, mientras que los pCCA y dCCA en general, fueron lesiones más pequeñas (<3 cm) con infiltración periductal. Por lo tanto, más allá de las características compartidas por los CCAs en su conjunto, las crecientes evidencias indica que los subtipos de CCA (i.e., iCCA, pCCA y dCCA) difieren entre otras en su etiología y presentación del tumor.[1,156] Así, nuestros resultados respaldan que los pacientes con iCCA comúnmente se diagnostican en un estadio más avanzado de la enfermedad, mientras la colestasis obstructiva, ictericia y/o niveles elevados de ALP frecuentemente aparentes en los pCCA y dCCA favorecen su detección más temprana.[165]

Los hallazgos por imágenes revelaron presencia de invasión de los ganglios linfáticos regionales y enfermedad diseminada en el 48,7% y el 24,2% de los pacientes, respectivamente. La localización preferente de metástasis en el CCA fue pulmón, hígado, ganglios linfáticos distantes, hueso y peritoneo. No se encontraron diferencias en el estadio de la enfermedad entre los subtipos de CCA. Sin embargo, esto puede deberse, al menos en parte, al hecho de que la diseminación hepática del iCCA no se considera formalmente metástasis según las pautas actuales de la AJCC. En este sentido, recientemente se ha demostrado que los pacientes con iCCA y cáncer diseminado dentro del hígado, con o sin invasión de ganglios linfáticos, tienen un peor pronóstico que los pacientes con iCCA local, haciendo latente la necesidad del establecimiento de una nueva pauta de codificación específica para este tipo de pacientes.[157]

Sensibilidad de los marcadores tumorales séricos: CEA y CA19-9

La sensibilidad del antígeno carcinoembriogénico (CEA) (valor de corte: 5 UI/ml) y el CA19-9 (≥37 UI/ml) se evaluó según el estadio de la enfermedad. Los valores séricos del CEA estaban por encima del límite superior de referencia en el 30,9% de los pacientes, correlacionando con la gravedad de la enfermedad (para LAD: OR 1,71; IC 95% 1,16-2,51; para MD: OR 3.03; IC 95% 2.11-4.35). Así mismo, se encontraron valores aumentados de CA19-9 en el 59,1% de los casos, particularmente con LAD o MD. Niveles de CA19-9 por encima del valor de corte se asociaron con un mayor riesgo de diseminación tumoral, con un OR 1,99 (IC 95% 1,47-2,70) para LAD y de 3.04 (IC 95% 2.21-4.17) para MD. En base a estos resultados, futuros estudios prospectivos deberían determinar la potencial utilidad del CEA y el CA19-9 para identificar pacientes que se beneficiarían de una estadificación más detallada, utilizando, por ejemplo, tomografía por emisión de positrones con ^{18}F-fluorodesoxiglucosa (FDG-PET), que se ha descrito como técnica complementaria en la identificación de diseminación y recaída tumoral.[154]

Manejo clínico y evolución de los pacientes con CCA

Los pacientes con CCA a menudo presentan obstrucción de la vía biliar y colestasis, lo que requiere de drenaje biliar antes de iniciar cualquier régimen terapéutico. En concreto, a un 40,3% de los pacientes se les insertó un *stent* biliar, de los cuales el 42,4% requirió de una segunda intervención, con un intervalo de tiempo de 1,8 meses entre ambos drenajes. Cabe destacar que el drenaje biliar estuvo presente en un 32,2% y el 35,0% de los pacientes previo a la cirugía y el tratamiento sistémico

respectivamente. Sin embargo, hasta un 61,8% de todos los pacientes que no reciben tratamiento contra el cáncer son intervenidos para la colocación del *stent* como parte de los cuidados paliativos para el adecuado control de los síntomas.

El 50,3% de los pacientes fueron candidatos a resección tumoral, con un total de 35,8% mostrando márgenes negativos tras la cirugía. Estos pacientes ostentaron el mejor pronóstico, con una mSG de 45,1 meses y una tasa de supervivencia a los 5 años del 43,3%. Sin embargo, la enfermedad residual microscópica (R1) después de la resección del tumor se asoció con un mayor riesgo de recaída en comparación con la cirugía R0 (HR 1,65; IC 95%: 1,35-2,02), mostrando una mediana de supervivencia libre de recaídas de 10,7 y 19,1 meses, respectivamente. Además, la cirugía R1 exhibió una mSG de 24,7 meses y una tasa de supervivencia a los 3 y 5 años de 29,4% y 13,7%, respectivamente. Así pues, pacientes con cirugía R1 asumieron un mayor riesgo de muerte en comparación con aquellos con resección R0 (HR 1.92; IC 95% 1.53-2.41), y no mostraron diferencias frente a cirugías R2 (HR 1,37; IC 95% 0,76-2,48). Asimismo, la invasión de los ganglios linfáticos (N+) también comprometió la SG de los pacientes después de la resección, tanto en cirugías R0 como R1 [HR 2,13 (IC 95% 1,55-2,94) para R0 y HR 1,61 (IC 95% 1,08-2,38) para R1).

De los 816 (49,6%) pacientes con enfermedad irresecable, el 29,0% recibieron tratamiento paliativo activo terapia, i.e., quimioterapia (26,2% de toda la cohorte), locorregional terapia (1,5%) y terapias combinadas (1,3%). El tratamiento anti-cancer mostró una mSG de 10,6 meses desde el inicio del tratamiento, con tasas de supervivencia a 1 y 3 años de 45,2% y 8,4%, respectivamente. La combinación de gemcitabina y cisplatino resultó el régimen más utilizado (70,4%), mostrando una reducción significativa del riesgo de muerte en comparación con los cuidados paliativos (HR 2,24; IC 95% 1,87-2,67) o la gemcitabina en monoterapia (HR 1,66; IC 95% 1,22-2,28). Pacientes sometidos a tratamiento anti-cáncer mostraron una supervivencia menor en comparación con aquellos sometidos a cirugía con intención curativa (i.e., R1) mostrando un HR de 2,25 (IC 95% 1,82-2,77). Sin embargo, no se detectaron diferencias significativas entre la terapia paliativa activa y resección tumoral R2 (HR 1,62; IC 95% 0,91-2,89). Es de destacar que el 20,6% de los pacientes recibieron solo cuidado de apoyo, resultando en una mSG de 4,0 meses.

El análisis comparativo del manejo clínico entre los 3 subtipos de CCA reveló diferencias significativas en la supervivencia de los pacientes no tratados frente al

cáncer. En este sentido, los pacientes con pCCA fueron el grupo mayoritario (37.3% de todos los pCCA) bajo cuidados paliativos; sin embargo, fueron los pacientes con iCCA los que mostraron el peor pronóstico (mSG de 2,8 meses frente a 7,0 y 7,7 meses para pCCA y dCCA, respectivamente).

En resumen, el CCA generalmente se diagnostica en etapas avanzadas, lo que implica que una proporción relevante de pacientes no sea elegible para la cirugía, y en algunos casos, ni siquiera para alguna terapia específica contra el cáncer. Así, la detección temprana del CCA y la reducción del intervalo entre el diagnóstico y la cirugía, que es la única opción potencial curativa, podría ser una solución clínica.

Factores pronósticos en CCA

El modelo univariable de regresión de Cox incluyendo variables clínicas y demográficas en el momento del diagnóstico mostró asociaciones significativas para el subtipo de CCA, la edad, el sexo, el estado funcional, el estadío de la enfermedad y los niveles séricos de CA19-9, CEA, ALT, AST, GGT, ALP, albúmina, y bilirrubina. Sin embargo, el modelo multivariable indicó que solo el estado funcional del paciente, la presencia de metástasis y los niveles elevados de CA19-9 fueron factores pronósticos independientes (HR 1.52, 4.03, 2,79, respectivamente). Estos resultados comparten algunas similitudes con un trabajo anterior que propuso un nuevo sistema de estadificación basado en la evaluación clínica para pacientes con pCCA que incluye el ECOG-PS, tamaño y número de tumores, revestimiento vascular, diseminación del tumor y CA19-9 como elementos de estratificación.[155]

CONCLUSIONES

1- Los pacientes con CCA son comúnmente hombres y diagnosticados a una edad avanzada, presentando un buen estado funcional.

2- La mayoría de los pacientes con CCA tenían sobrepeso u obesidad en el momento del diagnóstico, lo que puede sugerir que la obesidad es un factor relevante durante la colangiocarcinogénesis.

3- Existe una asociación dependiente del subtipo de CCA con enfermedades subyacentes, el iCCA que surge más a menudo en presencia de antecedentes cirróticos, el pCCA en pacientes con enfermedades biliares como PSC y el dCCA que está más relacionado con la enfermedad de cálculos biliares.

4- Los subtipos de CCA difieren en tamaño y patrón de crecimiento, con el iCCA presentándose frecuentemente como lesiones más grandes o multifocales y con un patrón MF, y los p/dCCAs siendo más pequeños y en su mayoría tumores PI.

5- La mayoría de los CCA se diagnostican en estadios avanzados, cuando el tumor se disemina a los ganglios linfáticos regionales y/u órganos distales, siendo el peritoneo y el hígado los sitios de metástasis principales.

6- El sitio de metástasis en pacientes con CCA difiere entre los subtipos, con el iCCA extendiéndose con mayor frecuencia a ganglios linfáticos distales, pulmón y hueso, en comparación con p/dCCA.

7- El CA19-9 sérico presenta baja sensibilidad en las primeras etapas de la enfermedad; sin embargo, sus niveles aumentan progresivamente con la invasión de los ganglios linfáticos regionales y, especialmente, con la enfermedad metastásica.

8- La resección tumoral es la mejor estrategia terapéutica, mostrando las mayores tasas de supervivencia, pero los márgenes tumorales y la invasión ganglionar comprometen el pronóstico tras la cirugía.

9- Los pacientes con afectación de márgenes tras la resección tumoral tienen mayor riesgo de recaída en comparación con aquellos con resección completa.

10- Para los pacientes con enfermedad irresecable, el tratamiento paliativo activo mejora la supervivencia, con GemCis mostrando la mejor respuesta en términos de supervivencia, en comparación con aquellos que reciben únicamente cuidados paliativos.

11- En ausencia de terapias anti-cáncer, los pacientes con iCCA presentan tasas de supervivencia más cortas con los que aquellos con pCCA o dCCA.

12- El modelo de supervivencia multivariante indica que el estado funcional, así como el estadio de la enfermedad y los niveles de CA19-9 son factores pronósticos independientes para el colangiocarcinoma.

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Annexes

ANNEX 1. Electronic Case Report Form (e-CRF)

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International Cholangiocarcinoma (INT-CCA) Registry
Page 1

General Data

Record ID	_____
Record inclusion date	_____ (YYYY-MM-DD)
Signed inform consent	◯ Yes ◯ No
DEMOGRAPHIC DATA	
Gender	◯ Woman ◯ Man
Ethnic group	◯ Not available ◯ Caucasian ◯ Hispanic ◯ African ◯ Asian ◯ Others
Ethnic group: Specification	_____
Birthdate	_____ (YYYY-MM-DD)
Birthplace	_____ (City, Country)
MEDICAL HISTORY AND RISK FACTORS	
Height	_____ (Centimeters (cm))
Weight	_____ (Kilograms (kg))
Body Mass Index	_____
Obesity	◯ Yes ◯ No ◯ Not available (Obesity ≥ 30 BMI)

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Diabetes mellitus	◯ Yes ◯ No ◯ Not available
Diabetes mellitus: Date of diagnosis	_____ (YYYY-MM-DD)
Diabetes mellitus: Treatment	☐ Oral hypoglycemic agent ☐ Insulin ☐ Not treated ☐ Not available (Multiple choice)
Non-alcoholic fatty liver disease (NAFLD)	◯ Yes ◯ No ◯ Not available
NAFLD: Specification	◯ Non-alcoholic fatty liver (NAFL) ◯ Non-alcoholic steatohepatitis (NASH)
Metabolic factors	☐ Not available ☐ None
	☐ Hyperglycemia ☐ Arterial Hypertension ☐ Hypertriglyceridemia ☐ Low HDL cholesterol ☐ Hypercholesterolemia ☐ Others (Multiple choice)
Metabolic factors: Specification	_____
Drugs, toxins and chemicals	☐ Not available ☐ None
	☐ Smoking ☐ Alcohol ☐ Thorotrast ☐ Dioxin ☐ Vinyl Chloride ☐ Nitrosamines ☐ Asbestos ☐ Oral contraceptive pills ☐ Isoniazid ☐ Others (Multiple choice)
Drugs, toxins and chemicals: Specification	_____
Drugs, toxins and chemicals: Smoking	
Smoking status	◯ Smoker ◯ Former smoker

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Smoking consumption	_____ (Cigarettes/day)
Date of smoking abstention	_____ (YYYY-MM-DD)
Drugs, toxins and chemicals: Alcohol	
Alcohol status	◯ Regular drinker ◯ Ex-drinker
Alcohol consumption	_____ (grams/day)
Date of alcohol abstention	_____ (YYYY-MM-DD)
Inflammatory diseases and/or conditions	☐ Not available ☐ None
	☐ Primary sclerosing cholangitis (PSC) ☐ Primary biliary cholangitis (PBC) ☐ Inflammatory bowel disease ☐ Cholecystitis ☐ Hepatolithiasis ☐ Biliary tract stone disease ☐ Liver cirrhosis ☐ Others (Multiple choice)
Inflammatory diseases: Specification	_____
Inflammatory disease: PSC	
PSC diagnosis date	_____ (YYYY-MM-DD)
History and frequency of cholangitis episodes (prior to CCA diagnosis)	_____
Inflammatory disease: PBC	
PBC diagnosis date	_____ (YYYY-MM-DD)
Inflammatory disease: Inflammatory bowel disease	

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Type of Inflammatory bowel disease	☐ Ulcerative colitis (UC) ☐ Chron's disease ☐ Others (Multiple choice)
Inflammatory bowel disease: Specification	_____
UC diagnosis date	_____ (YYYY-MM-DD)
Chron's disease diagnosis date	_____ (YYYY-MM-DD)
Inflammatory disease: Cholecystitis	
Cholecystitis diagnosis date	_____ (YYYY-MM-DD)
Inflammatory disease: Hepatolithiasis	
Hepatolithiasis diagnosis date	_____ (YYYY-MM-DD)
Inflammatory disease: Biliary tract stone disease	
Biliary tract stone disease diagnosis date	_____ (YYYY-MM-DD)
Inflammatory disease: Liver cirrhosis	
Liver cirrhosis diagnosis date	_____ (YYYY-MM-DD)
Infectious diseases	☐ Not available ☐ None
	☐ Opisthorchis viverrini ☐ Clonorchis sinensis ☐ Helicobacter Pylori ☐ Hepatitis C ☐ Hepatitis B ☐ HIV infection ☐ Others (Multiple choice)
Infectious diseases: Specification	_____
Infectious disease: Hepatitis C	

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Hepatitis C viral load	_____
Hepatitis C treatment date	_____ (YYYY-MM-DD)
Hepatitis C treatment	_____
Infectious disease: Hepatitis B	
Type of Hepatitis B	◯ Not assesed ◯ Anti-core positive ◯ Australian antigen positive
Hepatitis B viral load	_____
Hepatitis B treatment date	_____ (YYYY-MM-DD)
Hepatitis B treatment	_____
Congenital conditions	☐ Not available ☐ None
	☐ Biliary/choledocal cysts ☐ Caroli's disease ☐ Congenital hepatic fibrosis ☐ Alpha-1 antitrypsin deficiency ☐ Hemochromatosis ☐ Lynch symdrome ☐ Others (Multiple choice)
Congenital conditions: Specification	_____
Other risk factors	☐ Not available ☐ None
	☐ Biliary-enteric drainage procedures ☐ Cholecystectomy ☐ Others
Other risk factors: Specification	_____
Other risk factors: Biliary-enteric drainage procedures	
Date of the Biliary-enteric drainage procedure	_____ (YYYY-MM-DD)

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Other risk factors: Cholecystectomy

Date of the cholecystectomy

_____
(YYYY-MM-DD)

Comments on general data

_____

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Diagnosis & Tumor Features

Record ID	_____
DIAGNOSIS	
Type of CCA	◯ iCCA ◯ pCCA ◯ dCCA ◯ Undefined CCA
Intrahepatic cholangiocarcinoma subtype	◯ Pure intrahepatic cholangiocarcinoma ◯ Combined hepatocellular-cholangiocarcinoma
Diagnosis date Use date of biopsy/cytology. If diagnosis is based only on radiology, with no pathology confirmation, please provide date of imaging confirming diagnosis.	_____ (YYYY-MM-DD)
Age at diagnosis	_____
Basis of diagnosis	◯ Radiological imaging ◯ Tumor resection ◯ Tumor biopsy/cytology ◯ Other
Basis of diagnosis: Specification	_____
ECOG performance status at diagnosis time	◯ 0 ◯ 1 ◯ 2 ◯ 3 ◯ 4
Genetic alterations	◯ Yes ◯ No ◯ Not available
Genetic alterations: Specification	_____
Epigenetic alterations	◯ Yes ◯ No ◯ Not available
Epigenetic alterations: Specification	_____

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RADIOLOGICAL STUDY OF THE TUMOR	
Radiological study	◯ Yes ◯ No ◯ Not available
Radiological study method	☐ Magnetic resonance imaging ☐ Computed tomography ☐ Ultrasonography ☐ Cholangiopancreatography ☐ Other (Multiple choice)
Radiological method: Specification	_____
Mass lesion based on radiology	
AJCC 7th edition staging	◯ Unicentric (≤ 3 cm) ◯ Unicentric (> 3 cm) ◯ Multicentric ◯ Not applicable
AJCC 8th edition staging	◯ Unicentric (≤ 5 cm) ◯ Unicentric (> 5 cm) ◯ Multicentric ◯ Not applicable
Vascular encasement based on radiology	◯ Yes ◯ No ◯ Not available
Regional lymph nodes metastases based on radiology	◯ Yes ◯ No ◯ Not available
Regional lymph nodes metastases: Location	_____
Distant metastasis based on radiology	◯ Yes ◯ No ◯ Not available
Location of distant metastases Referred only to the moment of diagnosis	☐ Liver ☐ Lung ☐ Bone ☐ Peritoneum ☐ Distant lymph nodes ☐ Other (Multiple choice)
Distant metastasis: Specification	_____
Distant lymph node metastasis: Location	_____

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PATHOLOGIC STUDY OF THE TUMOR	
Pathologic study	◯ Yes ◯ No ◯ Not available
Pathologic study method	☐ Tumor resection ☐ EUS or FNA Endoscopic Ultrasonography (EUS) or Fine needle aspiration (FNA) ☐ ERCP Endoscopic retrograde cholangiopancreatography (ERCP) ☐ US/CT guided biopsy Ultrasound (US)/Computed tomography (CT) ☐ Other (Multiple choice)
Pathologic study method: Specification	_____
TUMOR MACROSCOPIC PATTERN	
Pattern of growth	☐ Not available ☐ Mass-forming ☐ Periductal infiltrating ☐ Intraductular growth (Multiple choice)
Size of tumor (bidimensional) If resected	_____ (mm2)
Size of tumor (maximum axial dimension) If resected	_____ (cm)
Intrahepatic cholangiocarcinoma tumor location	☐ Peripheral small ducts ☐ Perihilar large ducts (Multiple choice)
Perihilar cholangiocarcinoma tumor location Bismuth-Corlette classification	☐ Tumor involves common hepatic duct (Type I) ☐ Tumor involves bifurcation of the common hepatic duct (Type II) ☐ Tumor involves the right hepatic duct (Type IIIa) ☐ Tumor involves the left hepatic duct (Type IIIb) ☐ Tumor involves both right and left hepatic ducts (Type IV) (Multiple choice)
Distal cholangiocarcinoma tumor location	☐ Choledocus ☐ Intrapancreatic bile duct ☐ Ampulla of Vater ☐ Other (Multiple choice)
Tumor location: Specification	_____
Tumor location	_____

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Number of tumors	_____
TUMOR HISTOLOGICAL PATTERN	
Histological tumor classification	◯ Not available ◯ Adenocarcinoma ◯ Other carcinomas
Adenocarcinoma: Specific subtype	_____ (i.e., small bile duct type adenocarcinoma, large bile duct type adenocarcinoma, intestinal type adenocarcinoma, clear cell adenocarcinoma...)
Other carcinomas: Specific subtype	_____ (i.e., carcinoma in situ, mucinous carcinoma, squamous cell carcinoma, signet ring cell carcinoma, small cell carcinoma...)
Histological grade	◯ Not available ◯ Grade cannot be assessed (Gx) ◯ Well differentiated (G1) ◯ Moderately differentiated (G2) ◯ Poorly differentiated (G3) ◯ Undifferentiated (G4)
Histological tumor heterogeneity	☐ Not available ☐ Pure mucin producing areas ☐ Cholangiolo-like (ductular) areas ☐ Hepatocytic differentiation areas (Multiple choice)
Immunohistochemistry	◯ Yes ◯ No ◯ Not available
Immunohistochemistry markers	☐ Citokeratin 7 (CK-7) ☐ Citokeratin 19 (CK-19) ☐ Ki-67 ☐ α-smooth muscle actin (α-SMA) ☐ Epithelial cell adhesion molecule (EpCAM) ☐ CD68 ☐ von Willebrand Factor (vWF) ☐ Other (Multiple choice)
Immunohistochemistry: Specification	_____ (Protein 1 nomenclature (result); Protein 2 nomenclature (result); ...)
CK-7: Result	◯ Positive ◯ Negative
CK-19: Result	◯ Positive ◯ Negative

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Ki-67: Proliferative index	_____ (%)
α-SMA: Result	◯ Positive ◯ Negative
EpCAM: Result	◯ Positive ◯ Negative
CD68: Result	◯ Positive ◯ Negative
Macrophage infiltration	_____ (Number macrophages per mm2)
vWF: Result	◯ Positive ◯ Negative
Microvessel density	_____ (Area (%))
SURRONDING TUMOR HISTOLOGICAL PATTERN	
Steatosis	◯ Yes ◯ No ◯ Not available
Fibrosis	◯ Yes ◯ No ◯ Not available
Cirrhosis	◯ Yes ◯ No ◯ Not available
Cirrhosis: Stage	◯ Child A ◯ Child B ◯ Child C
Presence of pre-neoplastic lesions	◯ Yes ◯ No ◯ Not available
Type of precursor lesion	☐ Biliary intraepithelial neoplasia (BilIN) ☐ Intraductal papillary neoplasm of the bile duct (IPNB) ☐ Intraductal tubulo-papillary neoplasm (ITNB) ☐ Hepatobiliary mucinous cystic neoplasms (BMCN) ☐ Others
Type of precursor lesion: Specification	_____

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Ductular reaction	◯ Yes ◯ No ◯ Not available
Ductular reaction: Extension	_____
Ductular reaction: Proliferation	_____
Peribiliary gland	◯ Yes ◯ No ◯ Not available
Peribiliary gland: Volume	_____
Peribiliary gland: Proliferation	_____

TUMOR STAGING

Stage
The X category for T and N should be used only when absolutely necessary.

	◯ Clinical staging ◯ Pathologic staging
Primary Tumor (T): AJCC 7th edition staging	◯ Tx ◯ T0 ◯ Tis ◯ T1 ◯ T2a ◯ T2b ◯ T3 ◯ T4
Regional Lymph Nodes (N): AJCC 7th edition staging	◯ Nx ◯ N0 ◯ N1
Distant Metastasis (M): AJCC 7th edition staging	◯ M0 ◯ M1
Primary Tumor (T): AJCC 7th edition staging	◯ Tx ◯ T0 ◯ Tis ◯ T1 ◯ T2a ◯ T2b ◯ T3 ◯ T4
Regional Lymph Nodes (N): AJCC 7th edition staging	◯ Nx ◯ N0 ◯ N1 ◯ N2

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Distant Metastasis (M): AJCC 7th edition staging	◯ M0 ◯ M1
Primary Tumor (T): AJCC 7th edition staging	◯ Tx ◯ T0 ◯ Tis ◯ T1 ◯ T2 ◯ T3 ◯ T4
Regional Lymph Nodes (N): AJCC 7th edition staging	◯ Nx ◯ N0 ◯ N1
Distant Metastasis (M): AJCC 7th edition staging	◯ M0 ◯ M1
Primary Tumor (T): AJCC 8th edition staging	◯ Tx ◯ T0 ◯ Tis ◯ T1a ◯ T1b ◯ T2 ◯ T3 ◯ T4
Regional Lymph Nodes (N): AJCC 8th edition staging	◯ Nx ◯ N0 ◯ N1
Distant Metastasis (M): AJCC 8th edition staging	◯ M0 ◯ M1
Primary Tumor (T): AJCC 8th edition staging	◯ Tx ◯ T0 ◯ Tis ◯ T1 ◯ T2a ◯ T2b ◯ T3 ◯ T4
Regional Lymph Nodes (N): AJCC 8th edition staging	◯ Nx ◯ N0 ◯ N1 ◯ N2
Distant Metastasis (M): AJCC 8th edition staging	◯ M0 ◯ M1
Primary Tumor (T): AJCC 8th edition staging	◯ Tx ◯ Tis ◯ T1 ◯ T2 ◯ T3 ◯ T4

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Regional Lymph Nodes (N): AJCC 8th edition staging	◯ Nx ◯ N0 ◯ N1 ◯ N2
Distant Metastasis (M): AJCC 8th edition staging	◯ M0 ◯ M1
Primary Tumor (T)	◯ Tx ◯ Tis ◯ T1 ◯ T2 ◯ T3 ◯ T4
Regional Lymph Nodes (N)	◯ Nx ◯ N0 ◯ N1 ◯ N2
Distant Metastasis (M)	◯ M0 ◯ M1

Intrahepatic bile ducts carcinoma - 7th Edition AJCC Cancer Staging Manual

Primary Tumor (T)

TX	Primary tumor cannot be assesed
T0	No evidence of primary tumor
Tis	Carcinoma *in situ* (intraductular tumor)
T1	Solitary tumor without vascular invasion
T2a	Solitary tumor with vascular invasion
T2b	Multiple tumors, with or without vascular invasion
T3	Tumor perforating the visceral peritoneum or involving the local extra hepatic structures by direct invasion
T4	Tumor with periductal invasion

Regional Lymph Nodes (N)

NX	Regional lymph nodes cannot be assesed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis present

Distant Metastasis (M)

M0	No distant metastasis
M1	Distant metastasis present

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Perihilar bile ducts carcinoma - 7th Edition AJCC Cancer Staging Manual

Primary Tumor (T)

TX	Primary tumor cannot be assesed
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 surrounding 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	Regional lymph nodes cannot be assesed
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 mesenteric artery, and/or celiac artery lymph nodes

Distant Metastasis (M)

M0	No distant metastasis
M1	Distant metastasis present

Distal bile duct carcinoma - 7th Edition AJCC Cancer Staging Manual

Primary Tumor (T)

TX	Primary tumor cannot be assesed
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 meseneric artery

Regional Lymph Nodes (N)

NX	Regional lymph nodes cannot be assesed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis present

Distant Metastasis (M)

M0	No distant metastasis
M1	Distant metastasis present

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[Attachment: "Intrahepatic CCA_AJCC 8th.jpg"]

Perihilar bile ducts carcinoma - 8th Edition AJCC Cancer Staging Manual

Primary Tumor (T)

TX	Primary tumor cannot be assesed
T0	No evidence of primary tumor
Tis	Carcinoma *in situ*/high-grade dysplasia
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 surrounding 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	Regional lymph nodes cannot be assesed
N0	No regional lymph node metastasis
N1	One to three positive lymph nodes typically involving the hilar, cystic duct, common bile duct, hepatic artery, posterior pancreatoduodenal, and portal vein lymph nodes
N2	Four or more positive lymph nodes from the sites described for N1

Distant Metastasis (M)

M0	No distant metastasis
M1	Distant metastasis present

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Distal bile duct carcinoma - 8th Edition AJCC Cancer Staging Manual

Primary Tumor (T)

TX	Primary tumor cannot be assesed
Tis	Carcinoma *in situ*/high-grade dysplasia
T1	Tumor invades the bile duct wall with a depth less than 5 mm
T2	Tumor invades the bile duct wall with a depth of 5-12 mm
T3	Tumor invades the bile duct wall with a depth greater than 12 mm
T4	Tumor involves the celiac axis, superior meseneric artery, and/or common hepatic artery

Regional Lymph Nodes (N)

NX	Regional lymph nodes cannot be assesed
N0	No regional lymph node metastasis
N1	Metastasis in one to three regional lymph nodes
N2	Metastasis in four or more regional lymph nodes

Distant Metastasis (M)

M0	No distant metastasis
M1	Distant metastasis present

Comments on diagnosis and tumor features

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Clinical and Laboratory Parameters

Record ID _____

BIOCHEMICAL ANALYSIS AT DIAGNOSIS

Same date for every biochemical parameter?
◯ No (date requested for each parameter)
◯ Yes

Date of biochemical analysis _____ (YYYY-MM-DD)

NOTE: Please, use "-1" value for those parameters which are unknown or not available.

Biochemistry & Immunology

Alanine Aminotransferase (ALT) _____ (UI/L)

ALT: Date _____ (YYYY-MM-DD)

Aspartate Aminotransferase (AST) _____ (UI/L)

AST: Date _____ (YYYY-MM-DD)

Gamma Glutamyl Transferase (GGT) _____ (UI/L)

GGT: Date _____ (YYYY-MM-DD)

Alkaline Phosphatase (ALP) _____ (UI/L)

ALP: Date _____ (YYYY-MM-DD)

Total Bilirubin _____ (mg/dL)

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Bilirubin: Date

_____
(YYYY-MM-DD)

Total Cholesterol

_____
(mg/dL)

Total Cholesterol: Date

_____
(YYYY-MM-DD)

HDL Cholesterol

_____
(mg/dL)

HDL Cholesterol: Date

_____
(YYYY-MM-DD)

LDL Cholesterol

_____
(mg/dL)

LDL Cholesterol: Date

_____
(YYYY-MM-DD)

Tryglicerides

_____
(mg/dL)

Tryglicerides: Date

_____
(YYYY-MM-DD)

Alpha-Fetoprotein (AFP)

_____
(ng/mL)

Alpha-Fetoprotein: Date

_____
(YYYY-MM-DD)

Carcinoembryonic Antigen (CEA)

_____
(ng/ml)

CEA: Date

_____
(YYYY-MM-DD)

Carbohydrate antigen 19-9 (CA19-9)

_____
(Ul/ml)

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CA19-9: Date

_____ (YYYY-MM-DD)

Immunoglobulin G4 (IgG4)

_____ (mg/dL)

IgG4: Date

_____ (YYYY-MM-DD)

Glucose

_____ (mg/dL)

Glucose: Date

_____ (YYYY-MM-DD)

Glycated Hemoglobin (HbA1c)

_____ (%)

HbA1c: Date

_____ (YYYY-MM-DD)

Albumin

_____ (g/dL)

Albumin: Date

_____ (YYYY-MM-DD)

Hematology

White Blood Cell Count (WBC)

_____ (per microliter)

Total leucocytes count: Date

_____ (YYYY-MM-DD)

Neutrophils

_____ (%)

Lymphocytes

_____ (%)

Monocytes

_____ (%)

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Eosinophils

_____ (%)

Basophils

_____ (%)

Immature granulocytes

_____ (%)

Red Blood Cell Count (RBC)

_____ (per microliter)

Red Blood Cell Count: Date

_____ (YYYY-MM-DD)

Hematocrit Red Blood Cell Volume (HCT)

_____ (%)

Hemoglobin Concentration (Hb)

_____ (g/dL)

Platelet Count

_____ (per microliter)

Platelet Count: Date

_____ (YYYY-MM-DD)

Prothrombin Time (PT)

_____ (seconds)

PT: Date

_____ (YYYY-MM-DD)

International Normalized Ratio (INR)

_____

INR: Date

_____ (YYYY-MM-DD)

Comments on clinical and laboratory parameters

_____

Confidential

Therapeutic Strategy

Record ID	_____
TREATMENT	
Biliary stent	◯ Yes ◯ No ◯ Not available
Biliary stent: Date of first biliary stent	_____ (YYYY-MM-DD)
Biliary stent: Subsequent stenting	◯ Yes ◯ No
Biliary stent: Date of subsequent stenting	_____ (YYYY-MM-DD)
Treatment plan following diagnosis	◯ Not available ◯ Resection with curative aim ◯ Palliative treatment due to inoperable/unresectable disease
Participation in clinical trials	◯ Yes ◯ No ◯ Not available
Clinical trials: Specification	_____ (Trial identifier; Agent)
NOTE: Please, complete the whole information that appears below about the administered treatments, not only in relation to the treatment at the time of diagnosis.	
Surgery	
	◯ Yes ◯ No
Neoadjuvant therapy Treatment given prior to a definitive surgical procedure in order to shrink the tumor.	◯ Yes ◯ No ◯ Not available
Neoadjuvant therapy: Type of treatment	_____ (Agent)
Neoadjuvant therapy: Date of start	_____ (YYYY-MM-DD)

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Neoadjuvant therapy: Completion date	_____ (YYYY-MM-DD)
Neoadjuvant therapy: Number of cycles	_____
Neoadjuvant therapy: Best Radiological response (RECIST 1.1)	◯ Not assesed ◯ No measurable disease ◯ Complete pathologic response ◯ Complete radiological response ◯ Partial response ◯ Stable disease ◯ Progressive disease
Date of surgery	_____ (YYYY-MM-DD)
Type of surgery	◯ Tumor resection ◯ Liver transplantation ◯ Other
Type of surgery: Specification	_____
Resection margin	◯ R0 Complete resection with grossly and microscopically negative margins of resection. ◯ R1 Grossly negative but microscopically positive margins of resection. ◯ R2 Grossly and microscopically positive margins of resection.
Affected lymph nodes	◯ Yes ◯ No
Number of affected lymph nodes	_____
Location of affected lymph nodes	_____
Number of resected lymph nodes	_____
Adjuvant therapy Treatment given after the primary therapy in order to lower the risk of tumor progression.	◯ Yes ◯ No ◯ Not available
Adjuvant therapy: Type of treatment	_____ (Agent)
Adjuvant therapy: Date of start	_____ (YYYY-MM-DD)

Confidential

Adjuvant therapy: Completion date	_____ (YYYY-MM-DD)
Adjuvant therapy: Number of cycles	_____
Tumor recurrence after surgery	◯ Yes ◯ No ◯ Not available
Tumor recurrence: Date of recurrence	_____ (YYYY-MM-DD)
Tumor recurrence: Tumor location	☐ Local relapse ☐ Liver ☐ Lung ☐ Bone ☐ Peritoneum ☐ Other (Multiple choice)
Tumor recurrence location: Specification	_____
Tumor recurrence: Treatment	_____
Relapse-free survival	_____ (months)
Locoregional therapy	
	◯ Yes ◯ No
Date of first locoregional treatment	_____ (YYYY-MM-DD)
Type of locoregional treatment	◯ Not available ◯ Stereotactic body radiation therapy (SBRT) ◯ Radioembolisation/Selective internal radiation therapy (SIRT) ◯ Transarterial embolization (TAE) ◯ Transarterial chemoembolization (TACE) ◯ Microwave ablation (MWA) ◯ Radiofrequency ablation (RFA) ◯ Other
Locoregional therapy: Specification	_____

Confidential

Tumor progression after locoregional therapy	◯ Yes ◯ No ◯ Not available
Tumor progression: Date of progression	_____ (YYYY-MM-DD)
Tumor progression: Treatment	_____
Progression-free survival	_____ (months)
Systemic therapy (excluding adjuvant/neoadjuvant)	
	◯ Yes ◯ No
Systemic therapy: First line treatment	
Date of first line systemic treatment	_____ (YYYY-MM-DD)
Type of first line systemic treatment	◯ Chemotherapy ◯ Immunotherapy ◯ Targeted therapy
First line treatment: Chemotherapy	◯ Gemcitabine ◯ Gemcitabine + Cisplatin ◯ Gemcitabine + Oxaliplatin ◯ Other
Chemotherapy: Specification	_____
First line treatment: Immunotherapy	_____
First line treatment: Targeted therapy	_____
Best Radiological response to first systemic treatment (RECIST 1.1)	◯ Not assesed ◯ No measurable disease ◯ Complete pathologic response ◯ Complete radiological response ◯ Partial response ◯ Stable disease ◯ Progressive disease
Progression after first line systemic treatment	◯ Yes ◯ No ◯ Not available

Confidential

First line progression: Date of progression	_____ (YYYY-MM-DD)
Progression-free survival	_____ (months)
Systemic therapy: Second line treatment	
	◯ Yes ◯ No
Date of second line systemic treatment	_____ (YYYY-MM-DD)
Type of second line systemic treatment	◯ Chemotherapy ◯ Immunotherapy ◯ Targeted therapy
Second line treatment: Chemotherapy	◯ Gemcitabine ◯ Gemcitabine + Cisplatin ◯ Gemcitabine + Oxaliplatin ◯ Other
Chemotherapy: Specification	_____
Second line treatment: Immunotherapy	_____
Second line treatment: Targeted therapy	_____
Best Radiological response to second systemic treatment (RECIST 1.1)	◯ Not assesed ◯ No measurable disease ◯ Complete pathologic response ◯ Complete radiological response ◯ Partial response ◯ Stable disease ◯ Progressive disease
Progression after second line systemic treatment	◯ Yes ◯ No ◯ Not available
Second line progression: Date of progression	_____ (YYYY-MM-DD)
Progression-free survival	_____ (months)
Systemic therapy: Third/fourth line treatment	

Confidential

◯ Yes
◯ No

Third/fourth line systemic treatment

_____
(Please, briefly describe as above)

Comments on treatment

_____

Confidential

Other Tumors

Record ID	_____
OTHER TUMORS	
Non-CCA tumoral event	◯ Yes ◯ No ◯ Not available
Number of non-CCA tumoral events	◯ 1 ◯ 2 ◯ 3
Diagnosis & Treatment	
First non-CCA tumoral event	
Date of diagnosis	_____ (YYYY-MM-DD)
Type of malignancy	◯ Not available ◯ Prior malignancy ◯ Synchronous malignancy
Histology	_____
Treatment	◯ Not available ◯ No/Best Supportive Care (BSC) ◯ Yes
Treatment strategy	☐ Systemic therapy ☐ Locoregional therapy ☐ Surgery
Systemic therapy: Specification	_____ (Date, type (agent) [e.g., Chemotherapy (Erlotinib)]...)
Locoregional therapy: Specification	_____ (Date, type [e.g., Radiotherapy]...)
Surgery: Specification	_____ (Date, type [e.g., Transplant]...)
Second non-CCA tumoral event	

Confidential

Date of diagnosis	_____ (YYYY-MM-DD)
Type of malignancy	◯ Not available ◯ Prior malignancy ◯ Synchronous malignancy
Histology	_____
Treatment	◯ Not available ◯ No/Best Supportive Care (BSC) ◯ Yes
Treatment strategy	☐ Systemic therapy ☐ Locoregional therapy ☐ Surgery
Systemic therapy: Specification	_____ (Date, type (agent) [e.g., Chemotherapy (Erlotinib)]...)
Locoregional therapy: Specification	_____ (Date, type [e.g., Radiotherapy]...)
Surgery: Specification	_____ (Date, type [e.g., Transplant]...)
Third non-CCA tumoral event	
Date of diagnosis	_____ (YYYY-MM-DD)
Type of malignancy	◯ Not available ◯ Prior malignancy ◯ Synchronous malignancy
Histology	_____
Treatment	◯ Not available ◯ No/Best Supportive Care (BSC) ◯ Yes
Treatment strategy	☐ Systemic therapy ☐ Locoregional therapy ☐ Surgery

Confidential

Systemic therapy: Specification	_____ (Date, type (agent) [e.g., Chemotherapy (Erlotinib)]...)
Locoregional therapy: Specification	_____ (Date, type [e.g., Radiotherapy]...)
Surgery: Specification	_____ (Date, type [e.g., Transplant]...)

FAMILY TUMORS

Family tumors	◯ Yes ◯ No ◯ Not available
Family tumors: Specification	_____
Comments on other tumors	_____

Confidential

Follow-up & Survival

Record ID	_____
FOLLOW-UP	
Date of follow-up data collection If the results vary throughout the follow-up time, update to the date of last data collection.	_____ (YYYY-MM-DD)
New sites of distant metastases after initial diagnosis or relapse	◯ Yes ◯ No
Location of distant metastases	☐ Liver ☐ Lung ☐ Bone ☐ Peritoneum ☐ Distant lymph nodes ☐ Other (Multiple choice)
Distant metastasis: Specification	_____
Distant lymph node metastasis: Location	_____
New CCA event after treatment	◯ Yes ◯ No
New CCA tumor event: Date of diagnosis	_____ (YYYY-MM-DD)
New CCA primary tumor: Specification	_____
Last medical visit	_____ (YYYY-MM-DD)
Disease status at last medical visit	◯ Evidence of disease ◯ No evidence of disease ◯ Lost

Confidential

SURVIVAL	
Deceased	◯ Yes ◯ No
Deceased: Date of death	_____ (YYYY-MM-DD)
Deceased: Cause of death	◯ Related to cholangiocarcinoma ◯ Not-related to cholangiocarcinoma
Cause of death: Specification	_____
Overall survival	_____ (months)
Comments on follow-up	_____

ANNEX 2. Ethical Committee aproval

CEIC E
Euskadiko Ikerketa Klinikoetarako Batzorde Etikoa
Comité Ético de Investigación Clínica de Euskadi

EUSKO JAURLARITZA
GOBIERNO VASCO
OSASUN SAILA
DEPARTAMENTO DE SALUD

INFORME DEL COMITE ETICO DE INVESTIGACION CLINICA DE EUSKADI (CEIC-E)

Dª. Idoia Camaron Echeandia como Secretaria del CEIC de la Comunidad Autónoma del País Vasco (CEIC-E)

CERTIFICA

Que este Comité, de acuerdo a la ley 14/2007 de Investigación Biomédica, Principios éticos de la declaración de Helsinki y resto de principios éticos aplicables, ha evaluado el proyecto de investigación, titulado **CHOLANGIOCARCINOMA (CCA) REGISTRY,** Código interno: PI2016137

Versión del Protocolo: v 0.1 16 septiembre 2016
Versión de la HIP: GENERAL / 02/12/16 V. 0.1

Y que este Comité reunido el día 23/11/2016 (recogido en acta Reunión Noviembre CEIC de Euskadi) ha decidido emitir **informe favorable** a que dicho proyecto sea realizado por el siguiente personal investigador:

- Jesus Bañales Asurmendi *Instituto Biodonostia*

Lo que firmo en Vitoria, a 19 de diciembre de 2016

19 DIC 2016

Dª. Idoia Camaron Echeandia
Secretaria del CEIC de la Comunidad Autónoma del País Vasco (CEIC-E)

Nota: Una vez comenzado el estudio, se recuerda la obligación de enviar un **informe de seguimiento anual** y el **informe final** que incluya los resultados del estudio (si el estudio dura menos de un año, con el informe final será suficiente). Más información en la página web del CEIC-E:
https://apps.euskadi.eus/r85-pkfarm03/es/contenidos/informacion/ceic_proyectos_investigacion/es_ceic/proyectos_investigacion.html

CEIm
Euskadiko Medikamentuen gaineko Ikerkuntza Batzorde Etikoa
Comité de Ética de la Investigación con medicamentos de Euskadi
E

EUSKO JAURLARITZA
GOBIERNO VASCO
OSASUN SAILA
DEPARTAMENTO DE SALUD

INFORME DEL COMITÉ DE ÉTICA DE LA INVESTIGACIÓN CON MEDICAMENTOS DE EUSKADI (CEIm-E)

Iciar Alfonso Farnós
Vicepresidenta del CEIm de Euskadi (CEIm-E)

CERTIFICA

Que este Comité de acuerdo a la ley 14/2007 de Investigación Biomédica, principios éticos de la declaración de Helsinki y resto de principios éticos y legislación aplicables, en su reunión del día 21/04/2021, Acta 07/2021, ha evaluado la propuesta del promotor para que se realice la modificación: **"Modificación del protocolo"** en el estudio:

Título: CHOLANGIOCARCINOMA (CCA) REGISTRY

Código Interno: PI2016137

Versión Protocolo evaluada: Version: 2.0 (dated 19-April-2021)

Y que este Comité ha decidido emitir INFORME FAVORABLE A LA REALIZACIÓN DE DICHA ENMIENDA.

Lo que firmo en Vitoria, a 26 de abril de 2021

Iciar Alfonso Farnós
Vicepresidenta del CEIm de Euskadi (CEIm-E)

Nota: Una vez comenzado el estudio, se recuerda la obligación de enviar un **informe de seguimiento anual** e **informe final** que incluya los resultados del estudio (si el estudio dura menos de un año, con el informe final será suficiente). Más información en la página web del CEIm-E:
http://www.euskadi.eus/comite-etico-investigacion-clinica/

Abbreviations

^{18}FDG	18-Fluorodeoxyglucose
5-FU	5-Fluorouracil
^{90}Y	Yttrium-90 microspheres
95% CI	95% confidence interval
χ^2	Pearson's Chi-square test

A

AEG	*"Asociación Española de Gastroenterología"*
AJCC	American Joint Committee on Cancer
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	One-Way analysis of variance
ASC	Active Symptom Control
AST	Aspartate aminotransferase

B

BilIN	Biliary intraepithelial neoplasia
BMI	Body mass index
BRAF	Serine/threonine-protein kinase B-raf
BSC	Best supportive care
BTC	Biliary tract cancer

C

CA19-9	Carbohydrate antigen 19-9
CAR	Chimeric antigen receptor
CC	Choledocal cysts
CCA	Cholangiocarcinoma
CEA	Carcinoembryonic antigen
CT	Computed tomography

D

dCCA	Distal CCA
DNA	Deoxyribonucleic acid
DSOC	Digital single operator cholangioscope

E

eBD	Extrahepatic bile duct
EBV	Epstein-Barr virus
eCCA	Extrahepatic CCA
ECOG-PS	Eastern Cooperative Oncology Group Performance Status
e-CRF	Electronic Case Report Form
ENSCCA	European Network for the Study of Cholangiocarcinoma
ERBB2	Receptor tyrosine-protein kinase erb-2
ERCP	Endoscopic retrograde cholangiography
ESMO	European Society for Medical Oncology
EU	European Union
EUS	Endoscopic ultrasound

F

FGFR	Fibroblast growth factor receptor
FISH	Fluorescence in situ hybridization
FLR	Future liver remnant
FNA	Fine-needle aspiration
FOLFIRINOX	5-FU, oxaliplatin, and irinotecan
FOLFOX	5-FU and oxaliplatin

G

Gem	Gemcitabine
GemCis	Gemcitabine and cisplatin
GemOx	Gemcitabine and oxaliplatin
GGT	Gamma-glutamyl transpeptidase
GST	Glutathione S-transferase

H

HAI	Hepatic arterial infusion
HBV	Hepatitis B viruses
HCC	Hepatocellular carcinoma
HCV	Hepatitis C viruses
hOGG1	Human oxoguanine glycosylase 1
HR	Hazard ratio

I

iBD	Intrahepatic bile duct
IBD	Inflammatory bowel disease
iCCA	Intrahepatic CCA
ICD	International Classification of Diseases
IDH1	Isocitrate dehydrogenase-1
IG	Intraductal-growing
ILCA	International Liver Cancer Association
IPNB	Intraductal papillary neoplasm of the bile ducts
IQR	Interquartile range
ITPN	tubulopapillary neoplasms of the bile ducts
IU	International Unit

L

LAD	Locally advanced disease
LD	Local disease

M

M	Distant organ metastases
MAPK	Mitogen-activated protein kinase
MD	Metastatic disease
MDT	Multidisciplinary team
MF	Mass-forming
mL	Milliliter
mOS	Median overall survival
mPFS	Median progression-free survival
MRCP	Magnetic resonance cholangiopancreatography
MRI	Magnetic resonance imaging
MRP2/ABCC2	Multidrug resistance-associated protein 2
MTHFR	5,10-Methylenetetrahydrofolate reductase
MUTYH/MYH	MutY homolog

N

n	Number
N	Regional lymph node infiltration

NAFL	simple steatosis
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
NCI	National Cancer Institute
NKG2D	Natural killer cell receptor G2D
NTRK	Neurotrophic receptor tyrosine kinase

O

OR	Odds ratio

P

PAHO	Pan-American Health Organization
PBC	Primary biliary cholangitis
pCCA	Perihilar CCA
PD-L1	Programmed death ligand 1
PDT	Photodynamic therapy
PET	Positron emission tomography
PI	Periductal-infiltrating
PSC	Primary sclerosing cholangitis
PTC	Percutaneous transhepatic cholangiography
PVE	Portal vein embolization

R

R0	Negative resection margin
R1	Microscopic residual disease
R2	Gross residual disease
REDCap™	Research Electronic Data Capture

S

SBRT	Stereotactic body radiotherapy
SEER	Surveillance, Epidemiology and End Results

T

T	Primary tumor
TACE	Transarterial chemoembolization
TARE	Transarterial radioembolization
TSER	Thymidylate synthase enhancer region

U

UC	Ulcerative colitis
UICC	Union for International Cancer Control
US	Ultrasonography
USA	United States of America

W

WHO	World Health Organization

List of publications

* shared co-first authorship

† shared co-seniorship

1. **Izquierdo-Sanchez L**, Lamarca A, La Casta A, Buettner S, Utpatel K, Klümpen HJ, Adeva J, Vogel A, Lleo A, Fabris L, Ponz-Sarvise M, Brustia R, Cardinale V, Braconi C, Vidili G, Jamieson NB, Macias RI, Jonas JP, Marzioni M, Hołówko W, Folseraas T, Kupčinskas J, Sparchez Z, Krawczyk M, Krupa Ł, Scripcariu V, Grazi GL, Landa-Magdalena A, Ijzermans JN, Evert K, Erdmann JI, López-López F, Saborowski A, Scheiter A, Santos-Laso A, Carpino G, Andersen JB, Marin JJ, Alvaro D, Bujanda L, Forner A, Valle JW, Koerkamp BG, Banales JM. Cholangiocarcinoma landscape in Europe: diagnostic, prognostic and therapeutic insights from the ENSCCA Registry. **J Hepatol**. 2021 Dec 21:S0168-8278(21)02252-2. doi: 10.1016/j.jhep.2021.12.010. PMID: 35167909.

2. Olaizola P*, Rodrigues PM*, Caballero-Camino F, **Izquierdo-Sanchez L**, Aspichueta P, Bujanda L, LaRusso N, Drenth JPH, Perugorria MJ†, Banales JM†. Genetics, pathobiology and therapeutic opportunities of polycystic liver disease. **Nat Rev Gastroenterol Hepatol**. 2022 (accepted)

3. Nooijen LE, Banales JM, De Boer MT, Braconi C, Folseraas T, Forner A, Holowko W, Hoogwater FJH, Klümpen HJ, Koerkamp BG, Lamarca A, La Casta A, López-López F, **Izquierdo-Sanchez L**, Scheiter A, Utpatel K, Swijnenburg RJ, Kazemier G, Erdmann JI. Impact of positive lymph nodes and resection margin status on the overall survival of patients with resected perihilar cholangiocarcinoma: the ENSCCA registry. **Cancers**. 2022 (accepted)

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5. Lee-Law PY*, Olaizola P*, Caballero-Camino FJ, **Izquierdo-Sanchez L**, Rodrigues PM, Perugorria MJ, Azkargorta M, Elortza F, Martinez-Chantar ML, Aspichueta P, Marzioni M, Bujanda L, Drenth JPH†, Banales JM†. Inhibition of NAE-dependent protein hyper-NEDDylation in cystic cholangiocytes halts cystogenesis in experimental models of polycystic liver disease. **United European Gastroenterol J**. 2021 Sep;9(7):848-859. doi: 10.1002/ueg2.12126. PMID: 34310849.

6. Lobe C, Vallette M, Arbelaiz A, Gonzalez-Sanchez E, **Izquierdo L**, Pellat A, Guedj N, Louis C, Paradis V, Banales JM, Coulouarn C, Housset C, Vaquero J, Fouassier L. Zinc Finger E-Box Binding Homeobox 1 Promotes Cholangiocarcinoma Progression Through Tumor Dedifferentiation and Tumor-Stroma Paracrine Signaling. **Hepatology**. 2021 Dec;74(6):3194-3212. doi: 10.1002/hep.32069. PMID: 34297412.

7. **Izquierdo-Sanchez L**, Banales JM, Rodrigues PM. A look back at cholangiocarcinoma in Finland. **United European Gastroenterol J**. 2021 Dec;9(10):1103-1104. doi: 10.1002/ueg2.12157. PMID: 34581503.

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10. Lee-Law PY*, Olaizola P*, Caballero-Camino FJ, **Izquierdo-Sanchez L**, Rodrigues PM, Santos-Laso A, Azkargorta M, Elortza F, Martinez-Chantar ML, Perugorria MJ, Aspichueta P, Marzioni M, LaRusso NF, Bujanda L, Drenth JPH†, Banales JM†. Targeting UBC9-mediated protein hyper-SUMOylation in cystic cholangiocytes halts polycystic liver disease in experimental models. **J Hepatol**. 2021 Feb;74(2):394-406. doi: 10.1016/j.jhep.2020.09.010. PMID: 32950589.

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