



Mini-symposium

Cholangiocarcinoma: Update and future perspectives[☆]

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ARTICLE INFO

Article history:

Received 18 November 2009

Accepted 28 December 2009

Available online 22 January 2010

Keywords:

Apoptosis

Cholangiocarcinoma

Cholangiocytes

Epidemiology

Proliferation

Risk factors

ABSTRACT

Cholangiocarcinoma is commonly considered a rare cancer. However, if we consider the hepato-biliary system a single entity, cancers of the gallbladder, intra-hepatic and extra-hepatic biliary tree altogether represent approximately 30% of the total with incidence rates close to that of hepatocellular carcinoma, which is the third most common cause of cancer-related death worldwide. In addition, cholangiocarcinoma is characterized by a very poor prognosis and virtually no response to chemotherapeutics; radical surgery, the only effective treatment, is not frequently applicable because late diagnosis. Biomarkers for screening programs and for follow-up of categories at risk are under investigation, however, currently none of the proposed markers has reached clinical application. For all these considerations, cancers of the biliary tree system should merit much more scientific attention also because a progressive increase in incidence and mortality for these cancers has been reported worldwide. This manuscript deals with the most recent advances in the epidemiology, biology and clinical presentation of cholangiocarcinoma.

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1. Introduction

Cholangiocarcinoma (CCA) is a malignant cancer arising from the neoplastic transformation of cholangiocytes, the epithelial cells lining the intra-hepatic and extra-hepatic bile ducts [1,2]. Globally, CCA is the second most common primary hepatic malignancy [2,3]. Several recent epidemiological studies have shown that incidence and mortality rates are increasing worldwide [3–6]. From the anatomical point of view, CCA is classified as intra-hepatic (IH-CCA) or extra-hepatic (EH-CCA), the latter being further divided into proximal (peri-hilar), middle, and distal, depending on the location of the cancer within the extra-hepatic biliary system [2]. The peri-hilar cancers (Klatskin tumour) are commonly classified as indicated by the Bismuth–Corlette classification [7]. This form of CCA characteristically presents with signs of ductal and vascular obliteration. Biliary tract sepsis, liver failure and/or cachexia and malnutrition are the most important causes of death associated with these tumours. IH-CCA usually appears as a mass-forming lesion within the liver, which is mostly confused with metastatic tumour. These tumours usually progress insidiously, are difficult to diagnose, and have poor prognosis [8,9]. Unfortunately, treatment

options are discouraging. In fact, radical surgery, the only effective treatment, is applicable in a minority of patients due to the late clinical presentation and diagnosis of this tumour [9–12]. Thus, to improve survival, the early detection of biliary tract cancer seems to be essential; serum and bile tumour markers, non-invasive and endoscopic-based imaging modalities, and histology and cytology have been attempted with varying success. The purpose of this review is to summarize the current literature concerning the epidemiology, risk factors, pathogenesis, diagnosis and treatment of biliary tract cancers.

2. Epidemiology

Primary liver cancer is the sixth most common cancer and third most common cause of cancer death worldwide. CCA is the second most common primary liver tumour after hepatocellular carcinoma [9,12,13]. Hepato-biliary malignancies account for 13% of the 7.6 million annual cancer-related deaths worldwide and for 3% of the 560,000 annual cancer-related deaths in the United States [14–16]. CCA accounts for 10–20% of the deaths for hepato-biliary malignancies. Given the poor prognosis of CCA, mortality and incidence rates are virtually similar. CCA incidence rates vary markedly worldwide, presumably reflecting differences in local risk factors and genetics [17]. Chile and Japan have the highest mortality rates in the world, followed by East Asia and India, while the lowest rates have been reported in Australia [16,17]. In the United States, the incidence of CCA has been reported to be 0.95/100,000 for IH-CCA and 0.82/100,000 for EH-CCA [13,14]. CCA prevalence in different racial and ethnic groups is heterogeneously distributed, with the high-

[☆] D. Alvaro was supported by MIUR grants: PRIN #2007, prot. 2007HPT7BA.003. E. Gaudio was supported by MIUR grants: PRIN#2007, prot. 2007HPT7BA.001 and Federate Atheneum funds from University "Sapienza" of Rome.

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Table 1
Risk factors for cholangiocarcinoma.

Risk factors	
Primary sclerosing cholangitis (PSC)	Refs. [24–28]
Infestation with liver flukes <i>Opisthorchis viverrini</i> <i>Clonorchis sinensis</i> <i>Schistosomiasis japonica</i>	Refs. [29–32]
Toxic compounds	Refs. [33–35]
Congenital disorders Caroli's disease Choledocal cyst Abnormal pancreatobiliary junction	Refs. [36–38] Refs. [41–43]
Hepatoolithiasis Viral hepatitis (HCV, HBV)	Ref. [39] Refs. [47–52]
Lifestyle and diabetes Alcohol Obesity Smoking	Refs. [53–54]

est age-adjusted prevalence in Hispanics (1.22/100,000) and the lowest in African Americans (0.17–0.5/100,000) [14–18].

Over the last 30 years, the estimated age-adjusted incidence rates of IH-CCA in the USA have increased by 165% [12] (from 0.32/100,000 in 1975–1979 to 0.85/100,000 in 1995–1999). The average age at presentation is the seventh decade of life with a male to female ratio of 1.5. The mortality rate and incidence of IH-CCA have parallel trends as age-adjusted mortality rate increased from 0.07 per 100,000 in 1973 to 0.69 per 100,000 in 1997 [12,19]. On the other hand, the age-adjusted incidence of EH-CCA has decreased by 14% compared to data from two decades earlier. Currently, it is 1.2 per 100,000 in men and 0.8 per 100,000 in women. Similarly to IH-CCA, 65% of EH-CCA present in the seventh decade of life [12]. The mortality rate of EH-CCA declined from 0.6 per 100,000 in 1979 to 0.3 per 100,000 in 1998 [12]. With the exception of Denmark [20–21], this scenario has been reported worldwide [14–18]. The significant increase in age-adjusted incidence of IH-CCA was confirmed even after correction for a prior misclassification of hilar CCA as IH-CCA [12,17]. In Europe, the increase in IH-CCA mortality was higher in Western Europe than in Central or Northern Europe [19,22,23]. Very recently, data on the mortality and incidence trend for CCA have been reported also in Italy where a 40-fold increase in mortality for IH-CCA has been documented from 1980 to 2003 [6]. For EH-CCA, in contrast, mortality rates were stable or slightly decreasing in the last 10 years. Thus, as described in most countries, also in Italy the increased mortality for CCA mainly involves the intra-hepatic form suggesting different etiology and risk factors for IH- and EH-CCA [6].

3. Risk factors

A number of different risk factors have been definitively identified, but only a minority of patients presenting with CCA have known risk factors [9–12] (Table 1). In Western countries, patients with primary sclerosing cholangitis (PSC) carry an increased risk of CCA [24]. The prevalence of CCA in PSC patients are 30–42%; different cohort and multicentric studies have identified that in 50% of CCA the diagnosis occurs together with the identification of PSC are detected during the first year of follow-up, with an annual incidence rate of 0.6–1.5% [24]. In 30–42% of PSC cases, CCA is often found incidentally at autopsy or in explanted livers of PSC patients submitted to transplantation.

A European multicenter study [25], including 394 PSC patients from five European countries with a median follow-up of 18 years, has demonstrated that the majority of CCA cases (50%) were diag-

nosed within the first year after diagnosis of PSC and in 27% of the cases at intended liver transplantation. There was no correlation between incidence of CCA and the duration of PSC. In a second study conducted at the Mayo Clinic [26], 161 patients with PSC, who did not have CCA at study entry, were followed for a median of 11.5 years and 6.8% of patients developed CCA. The rate of CCA development was approximately 0.6% per year. Compared with the incidence rates of CCA in the general population, the relative risk of CCA in PSC was significantly increased [26]. Therefore, patients who present with their first diagnosis of PSC should be carefully screened and regularly followed-up for CCA development mainly during the first 2 years after PSC diagnosis [27,28]. Unfortunately, no follow-up strategy for patients at risk has been yet validated. Recently, an algorithm based on CA 19-9 serum levels (cut-off value of 20 U/mL) combined with abdominal US at 12-month intervals was proposed as a useful strategy for the screening/surveillance of CCA in PSC [28]. In PSC patients [24–27], older age at PSC diagnosis, history of colorectal dysplasia or carcinoma, smoking, and current or former alcohol use (>80 g/day), have all been suggested as additional risk factors for CCA development.

Infestation with liver flukes (*Opisthorchis viverrini*, *Clonorchis sinensis* and *Schistosomiasis japonica*) has been associated with an increased risk of CCA [29–32]. In areas where *Opisthorchis viverrini* is endemic, the adjusted prevalence for CCA by age and gender is as high as 14% [31]. It is hypothesized that these parasites colonize the biliary system causing chronic inflammation and predisposing to malignant transformation [31].

Several toxic compounds have been suspected of causing CCA [33–35]. Thorotrast (thorium dioxide) needs a special mention because it was used as a radiological contrast in the years 1920–1950 and was found to increase the risk of CCA up to 300-fold in comparison with the general population [34,35]. Because of its long biological half-life (400 years), the latency period of thorotrast-induced CCA ranges between 16 and 45 years [34], with the highest incidence between 20 and 30 years after exposure.

Caroli's disease, congenital choledocal cyst [36–38], intra-hepatic lithiasis [39,40] and abnormal pancreatobiliary junction (PBM) [41] are additional risk factors for CCA. In particular, an abnormal pancreatic-bile duct junction, with a common channel length of 8–15 mm, could influence the degree of pancreatic fluid regurgitation, resulting in an increased incidence of biliary tract malignancy [42]. A retrospective nationwide survey (1990–1999) of PBM in Japan revealed that 10.6% of PBM patients with bile duct dilatation were complicated by biliary tract cancer, and 33.6% of these were bile duct cancer, while 64.9% were gallbladder cancer. A prophylactic excision of the gallbladder and extra-hepatic bile duct has been proposed in these patients [42]. From a pathogenetic point of view, it has been considered that lysolecithin, formed as consequence of the mixing between pancreatic juice and bile, acts as detergent on the biliary epithelium favouring chronic inflammation [43]. This mechanism has been also considered for patients submitted to bilio-enteric surgical drainage for benign diseases, which represent another well-recognized category at risk. In contrast to this latter category, patients submitted to endoscopic sphincterotomy during endoscopic retrograde cholangio-pancreatography (ERCP) do not express increasing risk for CCA and this has been definitively demonstrated in three different studies performed in large series of patients with long follow-ups [44–46].

Among patients with CCA in the U.S.A., the prevalence of hepatitis C viral infection (HCV) was found to be four times higher than in the general population (0.8% vs. 0.2%) [47]. These results have been confirmed in Italy [48], in Taiwan [49] and in Japan [50] where HCV and viral hepatitis B (HBV) infection were detected in 23% and 11.5% of CCA compared to 6% and 5.5% of controls, respectively, with cumulative rates much greater than the general population [50]. Similar results were recently confirmed in

a case-control study performed in China, where at multivariate analysis, significant risk factors associated with CCA development were hepatolithiasis (adjusted OR: 5.7; 95% CI: 1.9–16.8) and HBV infection (adjusted OR: 8.8; 95% CI: 5.9–13.1) [51]. A large epidemiological study from the United States confirmed that HCV infection is a risk factor for IH-CCA (hazard ratio 2.55; 95% CI: 1.3–4.9) but not for EH-CCA (hazard ratio 1.5; 95% CI: 0.6–1.85) [52].

Multiple case-control analyses have reported an association between CCA and alcohol use [53]. More recently, obesity, diabetes and smoking have been taken into consideration, especially for IH-CCA but further confirmations are needed [53,54].

4. Molecular and cellular pathogenesis

CCA results from malignant transformation of cholangiocytes, although recent attention has been given to the development of CCA from stem/progenitor cells located in the peribiliary glands and/or canal of Hering/bile ductules [55]. However, evidence for a role of resident stem cells exists, so far only for primitive hepatic cancers characterized by mixed hepatocellular carcinoma/CCA phenotypes [56,57], that are currently classified as hepato-cholangiocarcinoma (CHC) [58]. A recent study has demonstrated the presence of progenitor cell markers in CHC like K7, K19, prominin-1, receptor for stem cell factor c-kit, octamer-4 transcription factor, and leukemia inhibitory factor, which were up-regulated while albumin gene expression was down-regulated [58]. Functional analyses of polycomb-group protein Bmi1, a molecule that is responsible for the self-renewal capability of many types of stem cells, was examined in IH-CCA [59]. The level of mRNA Bmi1 was significantly higher in 13 (81.3%) of 16 IH-CCA compared with the corresponding non-neoplastic tissues. Moreover, the Bmi1 knockdown in cell lines resulted in decreased colony formation, decreased cell proliferation, and increased cell senescence. Therefore, overexpression of polycomb-group protein Bmi1 is essential for colony formation and cell proliferation, probably by the repression of cellular senescence in IH-CCA [59].

A common and important contributor to the malignant transformation of cholangiocytes is chronic inflammation, often coupled with the injury of bile duct epithelium and obstruction of bile flow, which increase cholangiocyte turnover [60,61]. Persistent inflammation is thought to promote carcinogenesis by causing damage in DNA mismatch repair genes/proteins, proto-oncogenes and tumour suppressor genes [62], and by creating a local environment enriched with cytokines and other growth factors capable to accelerate cell cycle and to favour accumulation of somatic mutations [63,64].

Current evidence supports a primary role played by nitric oxide (NO) induced by pro-inflammatory cytokines (TNF- α , IL-6, etc.) [65–68]. Nitric oxide can directly, or through the formation of peroxynitrite species, lead to the deamination of guanine, formation of DNA adduct thereby promoting DNA mutations. The resultant DNA damage leads to an increased mutation rate and alteration of genes critical to cell proliferation control, thus triggering oncogenic mutations [67,68]. Dysregulation of the proto-oncogene k-ras [69], nuclear accumulation of p53 [70–72] and up-regulation of the related protein mdm-2 and WAF-1 have been observed in CCA [73–75]. Other inactivated suppressor genes include p16^{INK4a} [76,77], DPC4/Smad4 and APC [78,79]. The majority of these genetic changes were described in IH-CCA. NO, together with different cytokines, can also inhibit cholangiocyte apoptosis by nitrosylation of caspase 9, and also, may induce proliferation thus favouring accumulation of somatic mutations.

Cytokines, such as interleukin-6 (IL-6), appear to play an important role in evasion from apoptosis, a mechanism that normally eliminates dysfunctional cells accumulating DNA damage [80,81].

CCA cells have been shown to secrete IL-6 that in an autocrine fashion activates the pro-survival p38 mitogen activated protein kinase [82,83]. In addition, IL-6 up-regulated the expression of myeloid cell leukemia-1 (Mcl-1), through STAT3 and AKT related signalling pathways [84,85]. Mcl-1 is an anti-apoptotic protein of the Bcl-2 family of apoptotic proteins.

Cyclooxygenase 2 (COX-2), activated by inflammatory cytokines and NO play an important role in CCA carcinogenesis through inhibition of apoptosis and growth stimulation [86–89]. Additional induction of COX-2 is mediated by bile acids [90] and oxysterols. Other COX-2-inducing molecules include the tyrosine kinase ErbB-2, which is overexpressed in CCA and involved in CCA carcinogenesis and progression [91,92]. ErbB-2 is an epidermal growth factor receptor (EGFR) homologue able to homodimerize or heterodimerize with other members of the EGF superfamily, resulting in activation of the Raf/MAPK-pathway [92,93]. Constitutive overexpression of ErbB2 and/or ErbB1 in malignant cholangiocytes has been documented in more than 50% of IH-CCA [94–96]. Relevance of ErbB2 or ErbB1 related pathways in CCA has raised interest in the possibility that agents which selectively target these receptors, could potentially be effective in CCA therapy [97]. However, current experience with such ErbB targeted therapies produced only modest responses in patients with biliary tract cancers. Another recently proposed mechanism linking chronic inflammation with CCA development is related with activation-induced cytidine deaminase (AID), a member of the DNA/RNA editing enzyme family, implicated in human carcinogenesis via its mutagenic activity [98]. AID was found to be increased in biopsies from patients with PSC or CCA; whereas, only trace amounts of AID were detected in the normal liver.

We have demonstrated that proliferating cholangiocytes serve as a neuroendocrine compartment during chronic liver diseases, and as such, secrete and respond to a number of hormones and neuropeptides contributing to the autocrine and paracrine pathways that modulate liver inflammation and fibrosis [99]. A number of recent studies have shown that the nervous system, neuropeptides and endocrine hormones play a key role in the modulation of CCA growth [100]. Recently, it was demonstrated that CCA cells express GABA-A, -B, -C receptors and respond to GABA stimulation with growth inhibition. GABA inhibitory effect on malignant cholangiocyte proliferation was evident *in vitro* and also *in vivo*, by reducing the growth of CCA tumours injected subcutaneously in nude mice. Moreover, GABA has been shown to be able to inhibit malignant cholangiocyte migration, a peculiar characteristic of the CCA cells [101].

Very recently, a relevant role in modulating CCA growth and proliferation has been attributed to dopamine, found to be increased in CCA cell lines. These data represent the first evidence that dopamine metabolism is dysregulated in CCA and that modulation of dopamine synthesis may represent an alternative target for the development of therapeutic strategy [102].

Estrogens have also been shown to play a role in the promoting of CCA cell growth. Our group has demonstrated that IH-CCA express the receptors for both estrogen receptor subtypes alpha and beta and for insulin-like growth factor (IGF-1), indicating that estrogens and IGF-1 coordinately regulate CCA growth and apoptosis [103,104]. In addition, it has been shown that the estrogen proliferative effect on CCA cells is also due to the stimulation of VEGF synthesis and secretion [103,104].

In this experimental background, recent advances in molecular pathogenesis have highlighted the importance of epigenetic alterations including promoter hypermethylation and histone deacetylation in addition to genetic changes in the process of cholangiocarcinogenesis [105,106]. Among hypermethylated genes, it was found that the CpG island hypermethylation is a suppressor of cytokine signaling 3 (SOCS3) gene promoter in CCA.

Table 2
Recently proposed serum and bile biomarkers for the diagnosis of CCA.

Biomarker	Sensitivity (%)	Specificity (%)
Serum		
CA 19-9	53–92	50–98
CEA	33–68	79–100
IL-6	73	92
Trypsinogen-2	AUC = 0.804	
MUC5AC	71.01	90
CYFRA 21-1	74.7	92.2
Bile		
CA 19-9	46–61	60–70
CEA	67–84	33–80
IGF1	100	100
Pancreatic elastase/α-amylase ratio	82	89
Mcm5	62	67

IL-6-mediated signal transducers and activators of transcription 3 (STAT3) activation are aberrantly sustained in CCA cells, resulting in resistance to apoptosis [107]. This aberrant overexpression of IL-6 has recently been shown to be a consequence of the epigenetic silencing of the suppressor of cytokine signaling 3 (SOCS-3). SOCS-3 promoter methylation was observed in a subset of CCA samples as well as in a number CCA cell lines. Enforced overexpression of SOCS-3 in these cell lines effectively reduced the IL-6-mediated signal transduction cascade [107].

Non-coding RNAs have recently been recognized as gene specific regulators, and therefore, are similar in function to transcription factors [108,109]. These RNAs can regulate every stage of gene expression, including transcription, mRNA stability and translation. A role for these RNAs in the neoplastic transformation of cells is emerging. Several research groups have provided evidence that microRNAs may act as key regulators of processes as diverse as early development, cell proliferation and cell death. In addition, recent studies suggest a possible link between microRNAs and various cancers, including CCA [108,109].

5. Helpful serum and bile biomarkers in CCA diagnosis

For many years, efforts have been performed to identify biomarkers with adequate diagnostic accuracy for CCA in serum or biological fluids [110,111], which could also be useful for population screening or for the surveillance of high-risk subjects including PSC patients [111–113]. Serum tumour markers are attractive because of the ease of obtaining samples and their relatively low cost. Therefore, they have been the objects of extensive investigation to aid CCA diagnosis, but unfortunately, none of these markers has reached adequate specificity for CCA [114–118] (Table 2).

Carcinoembryonic antigen (CEA), which is mainly used for colorectal cancers, is of scarce utility, since it is increased only in approximately 30% of patients with CCA [115–117]. Carbohydrate antigen (CA 19-9) is the most widely used serum marker for CCA but it is also elevated in pancreatic cancer, gastric cancer, primary biliary cirrhosis and in smokers; in addition, it may be transiently increased during cholangitis or cholestasis. The sensitivity and specificity for detection of CCA in PSC are 79% and 98%, respectively, at a cut-off value of 129 U/mL. Other investigators have documented that only a higher cut-off (>180 U/mL) may achieve this degree of specificity [119]. According to Levy et al., a change from baseline of >63 U/L has a sensitivity of 90% and specificity of 98% for CCA detection [120]. In patients without PSC, CA 19-9 sensitivity is 53% at a cut-off of >100 U/L and its negative predictive value is 76–92% [121]. CA 19-9 can also be elevated in bacterial cholangitis and other gastrointestinal and gynecological neoplasias; patients lacking the blood type Lewis antigen (10% of individuals) do not produce this tumour marker [122–125]. In general, as extensively

discussed in recent reviews [115–118], a high value of sensitivity and specificity has been reported for CA 19-9 in patients with CCA depending on the study population and the cut-off values, and this is also true for CCA complicating PSC. In addition, elevated CA 19-9 usually allows CCA diagnosis in advanced stages when radical treatments are not allowed. Therefore, current efforts aim to identify novel serum markers which can substitute CA 19-9, or can improve, when measured together, the diagnostic accuracy of CA 19-9. The serum levels of interleukin 6 [80], at a 25.8 pg/mL cut-off, provide a diagnostic sensitivity of 73% and a specificity of 92%. Serum levels of IL-6 have been correlated with tumour burden in CCA patients, and interestingly, 1 month after treatment with photodynamic therapy, the mean IL-6 level decreased significantly. Although these findings are encouraging, serum IL-6 is also elevated in many patients with hepatocellular carcinoma, benign biliary disease and metastatic lesions [118]. Other biomarkers such as trypsinogen-2 [126], platelet-lymphocyte ratio (PLR), mucin-5AC [127,128], soluble fragment of cytokeratin 19 (CYFRA21-1) [129] have been recently shown to help in the diagnosis of CCA with, in some cases, a prognostic value. In particular, mucin 1 (MUC1) and MUC5AC are not expressed by hepatocellular carcinoma, suggesting a possible role in the differential diagnosis. Moreover, a very recent study focus on CCA immunohistochemical staining for mucin, has evidenced MUC1 expression in CCA closely related to dedifferentiation and infiltrative growth pattern, suggesting that the expression of MUC1 might be associated with the progression of CCA [118].

As far as bile is concerned, the pancreatic elastase/α-amylase ratio, mucin-4, minichromosome maintenance replication protein (Mcm5) and insulin-like growth factor 1 (IGF1) have been explored with biliary IGF1 concentration to discriminate CCA from benign biliary disorders and pancreatic cancer (Table 2). Specifically, we measured IGF1 and vascular endothelial growth factor (VEGF) in bile of patients undergoing ERCP for biliary obstruction and evaluated the performance of these markers in differentiating EH-CCA from pancreatic cancer or benign biliary abnormalities [112]. The biliary IGF1 concentration was 15–20-fold higher in EH-CCA than the other two groups. In contrast, biliary VEGF concentration was similar in the three groups. In substance, this study indicated a marker (bile IGF1), which could be used, with absolute diagnostic accuracy, in patients undergoing ERCP for biliary obstruction, to differentiate EH-CCA from either pancreatic cancer or benign biliary disorders [112].

Proteomics of serum and bile are under investigation but definitive findings are currently unavailable [130,131]. Another strategy is to evaluate the diagnostic performance of serum CA 19-9 together with imaging techniques [132–134]. To this regard, it has been demonstrated that the combination of serum CA 19-9 with either computed tomography (CT), magnetic resonance imaging (MRI), magnetic resonance colangio-pancreatography (MRCP) or ERCP show the best sensitivity (~100%) but a low specificity (~40%) in diagnosing CCA occurring in PSC patients. In contrast, the combination of serum CA 19-9 and ultrasonography (US) had intermediate specificity (62%) with a good sensitivity (91%) for detecting CCA. On the basis of test properties, cost and availability, combination of serum CA 19-9 (cut-off value of 20 U/mL) and abdominal US at 12-month intervals was recently proposed as useful strategy for the screening/surveillance of CCA in PSC [28].

6. Staging

Understanding the patterns of spread of CCA is essential for staging and treatment planning. CCA can spread along biliary ducts, invade perineural and vascular tissues, infiltrate adjacent structures, invade lymph nodes or develop distant metastasis [135–137].

IH-CCA and EH-CCA are staged differently. A new staging system for IH-CCA was proposed by Nathan et al. [138] based on the number of tumours, vascular invasion, lymph node status and presence of metastatic disease. The presence of multiple tumours may be indicative of satellite neoplastic deposits or intra-hepatic metastatic disease from hematogenous or lymphatic spread; similarly to vascular and lymph node invasion, it is associated with poor survival. For EH-CCA, the goal of staging is to determine the local extent of the disease, as it predicts resectability and the extent of the resection. The American Joint Committee on Cancer (AJCC) staging system, also known as the TNM staging system for EH-CCA [139] is based on pathological data useful in identifying the patient prognosis but with little applicability for assessing the feasibility of surgical treatment [138]. Bismuth–Corlette classification for hilar CCA is useful for describing tumour location and its spread within the biliary tree, but it is not predictive of resectability [7]. The Memorial Sloan-Kettering Cancer Center (MSKCC) has proposed a staging system known as T-stage criteria [138]. The MSKCC staging system is based on the location and extent of ductal involvement, presence or absence of portal vein invasion, and presence or absence of hepatic lobar atrophy irrespective of metastases or lymph node status. The MSKCC staging system for hilar CCA correlates with resectability and survival, as 59% of T1 lesions are resectable with median survival of 20 months compared to 0% resectability for T3 lesions with a median survival of only 8 months [138].

7. Treatment

Tumour resection is the only potential cure for CCA. The median survival of patients with unresectable disease is 6–12 months [140]. All patients with resectable IH-CCA, hilar CCA, and the majority of patients with EH-CCA, require partial hepatectomy to increase the chances of negative resection margins. Preoperative evaluation includes an extensive assessment of patient fitness for major surgery, the absence of any metastatic disease and the possibility of resection margins free from cancer [141–143]. If any of these conditions are not satisfied, surgical therapy is not indicated and palliative modalities should be recommended [12,144]. Recently, portal vein embolization (PVE) is a valuable preoperative measure when anticipating extensive liver resections with subsequent small hepatic residual volume [145]. A compensatory hypertrophy of the remnant hepatic parenchyma is induced by selectively occluding the main portal vein branch to the lobe that will be resected. This can increase the volume of the anticipated liver remnant by 12–20%, thereby reducing the rate of postoperative liver dysfunction [146,147]. PVE is useful when the anticipated liver remnant volume is less than 20–25% of the total liver volume in patients with normal liver function, and when the anticipated liver remnant volume is 40% or less in patients with compromised liver function [147].

Transplantation is an emerging therapy for unresectable CCA without evidence of metastatic disease [148,149]. Early experiences with liver transplantation were discouraging, given the high rates of tumour recurrence and poor patient survival. Trials with aggressive transplantation methods and adjuvant chemotherapy did not yield significantly better outcomes. Recently, stringent patient selection and neoadjuvant chemoradiation have yielded promising results with 5-year survival rates as high as 76% [148,149]. At the Mayo Clinic, Rosen et al. [150,151] have developed a liver transplantation protocol for HCC that provides a disease-free 5-year survival of 82%. This protocol is aimed at treating unresectable CCA in PSC patients. To be eligible for this protocol, the diagnosis of CCA needs to be confirmed histologically, CCA has to be considered unresectable with no evidence of metastatic disease. Eligible patients receive neoadjuvant chemoradiation therapy

followed by staging laparotomy to rule out metastatic disease followed by living-related or cadaveric liver transplantation [151]. Currently, the use of liver transplantation for the treatment of CCA is reserved only for highly selected patients in specialized centres.

For the majority of CCA patients who are not candidates for surgical resection or transplantation, recent proposals deal with the use of photodynamic therapy (PDT) based on the intravenous administration of photosensitizing agents that preferentially accumulate in malignant cells [152–154]. In these studies, in fact, PDT alone or plus stenting improved cholestasis and quality of life considerably, and had a favourable side-effect profile. In light of these findings, recent review articles recommended PDT for patients with non-resectable disease [154]. The role of PDT adjuvant treatment before or after surgical resection needs to be assessed. It is important to bear in mind that PDT requires careful patient management. Radiotherapy (external, intraluminal, brachitherapy) received recent attention and positive findings have been reported by different centres for peri-hilar-CCA [153,154].

As far as pharmacological therapy is concerned, CCA is characterized by a remarkable resistance to common chemotherapy. Several drugs have been tested alone in unresectable CCA and in restricted phase II studies (5-fluorouracil, methansulfon-m-anisidide, cisplatin, rifampicin, mitomycin C, paclitaxel, gemcitabine) with partial response rates of 0–9% and an average survival of 2–12 months. Certainly, 5-fluorouracil was the most commonly used drug but with the same disappointing results as the other drugs used in monotherapy [155]. Recently, the Italian Health Care System approved the use of gemcitabine. In 2005, a review [156] analysed over 13 single arm phase II studies suggesting the role of gemcitabine as an alternative to supportive care. However, randomized controlled trials are necessary to define any real and potential increase in survival determined by this drug which shows a low toxicity. Regarding combination therapies, one of the most used therapeutic strategies is ECF (epirubicin + cisplatin + 5-fluorouracil) but with disappointing results [157]. Several phase Ib and II studies evaluated the addition to gemcitabine [158] of cetuximab [159,160], oxaliplatin/cisplatin [161–163], erlotinib [164] or capecitabine [165]. Among these studies, the effect seems to be more consistent with the combination of capecitabine and addition of oxaliplatin/cisplatin. With regard to biological therapy, ongoing pilot studies are investigating the use of sorafenib, lapatinib or bevacizumab in the treatment of unresectable CCA. Very recently, it was demonstrated that sorafenib displayed significant tumour suppression resulting in a survival benefit for treated animals that mimic human disease. In this *in vivo* model, sorafenib also decreased tumour Tyr (705) STAT3 phosphorylation and increased tumour cell apoptosis [166].

Studies of cellular and molecular biology have clearly demonstrated, in a high percentage of CCA, the activation of signalling pathways such as PI3-kinase and MEK/ERK, multiple receptor pathways (epidermal growth factor EGF, IGF1, estrogen receptors, VEGF) and COX2/PgE2. On the basis of this evidence, there is a rationale for pilot studies testing biological drugs acting on these targets. With regard to adjuvant chemotherapy, paucity of data makes it impossible to use that therapy after surgical resection [167]. In particular, chemotherapy with 5-fluorouracil and mitomycin C failed to improve the survival of patients undergoing surgical resection, as recently observed in a phase III study [168].

Benefits of adjuvant radiotherapy after surgical resection have been recently reviewed [167]. Overall, there are retrospective data, which, altogether, show a benefit especially with regard to dose-scaling radiation [165]. However, the only prospective study [168] denies a real benefit of radiotherapy after surgical resection of peri-hilar CCA in terms of survival. Further prospective studies are needed to reach a definitive conclusion. Although data are scarce, there are some studies [167] demonstrating a slight but signifi-

cant improvement in survival for distal common bile duct cancers undergoing surgical resection and adjuvant radio-chemotherapy [169].

Conflict of interest statement

The authors have nothing to disclose in connection with the content of this article.

List of abbreviations

CCA, cholangiocarcinoma; IH-CCA, intra-hepatic cholangiocarcinoma; EH-CCA, extra-hepatic cholangiocarcinoma; PSC, primary sclerosing cholangitis.

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