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TOPIC HIGHLIGHT

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Pathological aspects of so called "hilar cholangiocarcinoma"

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Abstract

Cholangiocarcinoma (CC) arising from the large intrahepatic bile ducts and extrahepatic hilar bile ducts share clinicopathological features and have been called hilar and perihilar CC as a group. However, "hilar and perihilar CC" are also used to refer exclusively to the intrahepatic hilar type CC or, more commonly, the extrahepatic hilar CC. Grossly, a major distinction can be made between papillary and non-papillary tumors. Histologically, most hilar CCs are well to moderately differentiated conventional type (biliary) carcinomas. Immunohistochemically, CK7, CK20, CEA and MUC1 are normally expressed, being MUC2 positive in less than 50% of cases. Two main premalignant lesions are known: biliary intraepithelial neoplasia (BilIN) and intraductal papillary neoplasm of the biliary tract (IPNB). IPNB includes the lesions previously named biliary papillomatosis and papillary carcinoma. A series of 29 resected hilar CC from our archives is reviewed. Most (82.8%) were conventional type adenocarcinomas, mostly well to moderately differentiated, although with a broad morphological spectrum; three cases exhibited a poorly differentiated cell component resembling signet ring cells. IPNB was observed in 5 (17.2%), four of them with an associated invasive carcinoma. A clear cell type carcinoma, an adenosquamous carcinoma and two gastric foveolar type carcinomas were observed.

Key words: Cholangiocarcinoma; Bile duct carcinoma; Hilar cholangiocarcinoma; Perihilar cholangiocarcinoma; Klatskin tumor; Extrahepatic bile duct carcinoma; Hepatic hilum

Core tip: The controversy regarding the definition of hilar and perihilar cholangiocarcinoma (CC) is addressed. The authors review the main pathological features (gross and microscopic findings, immunophenotype) of hilar CC, including rare histological variants as well as precursor lesions (biliary intraepithelial neoplasia and intraductal papillary neoplasm of the biliary tract). Considerations regarding staging and other histological prognostic factors are also included. The authors also provide a series of 29 cases of resected hilar CC.

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NOMENCLATURE AND TOPOGRAPHY

Considerations on the concept and classification of hilar cholangiocarcinoma

As stated in the World Health Organization (WHO) classification of tumors of the bile duct, the use and meaning of the terms hilar (and perihilar) may differ among pathologists, surgeons and radiologists[1]. The right and left hepatic ducts, their confluence and their first to third

branches are collectively called hilar and perihilar bile ducts and they are intra and extrahepatically located. The boundary between the intra and extrahepatic biliary tree has been somewhat confused in the literature. Hilar bile ducts proximal to the junction of the second-order bile ducts are intrahepatic because the peritoneum is attached there and they are called large intrahepatic bile ducts, whereas the main hilar bifurcation (*i.e.,* the right and left hepatic ducts) can be considered extrahepatic[2-5].

Depending on its anatomical location, cholangiocarcinoma (CC) has normally been classified in intrahepatic or extrahepatic types, with extrahepatic CC further categorized in proximal (upper third), middle (middle third) and distal (lower third) subtypes[6]. Intrahepatic cholangiocarcinomas (ICC) have classically been separated into two groups: CC arising in small intrahepatic bile ducts (peripheral type CC) and CC originating from the major intrahepatic bile ducts, including the hilum (hilar type CC). CC originating from the large intrahepatic bile ducts (hilar type CC) exhibit significant clinicopathological differences from tumors of small bile duct origin (peripheral type CC)[7] and several decades ago it was suggested that it was more practical to treat such hilar carcinomas together with extrahepatic bile duct carcinomas (EBDCs) because of the similarity in symptomatology[8]. In addition, bile duct carcinoma arising near or at the confluence of the right and left hepatic ducts has also been classically known as hilar CC or Klatskin tumor[9,10]. The term hilar CC is very commonly used in a narrow sense to refer to this extrahepatic hilar bile duct carcinoma (*i.e.,* Klatskin tumor or proximal type extrahepatic CC), but hilar CC has also been used in a broad sense to refer to CCs involving the hepatic hilum regardless of their intra or extrahepatic location (*i.e.,* including intra and extrahepatic hilar CC)[11,12]. Because hilar CC is the commonest CC, the incidence of intra and extrahepatic CC in the literature has largely depended on how the hilar CC has been considered[13].

More recently, with respect to not only anatomical distribution but also preferred surgical treatment, CC has been classified as intrahepatic, perihilar and distal types. In this classification, perihilar CC has been defined as that tumor involving or requiring resection of the hepatic duct bifurcation even if it has a significant intrahepatic component[14,15]. Some authors consider that perihilar CC is a single entity that includes all the tumors involving the hepatic hilum irrespective of whether they are extrahepatic (*i.e.,* extrahepatic hilar CC) or intrahepatic (*i.e.,* intrahepatic hilar CC) as these tumors have comparable biological behavior with similar clinical management. These authors have suggested that perihilar tumors should preferably be staged by a staging system specific for extrahepatic bile duct cancer[11]. However, other authors argue that perihilar CCs are potentially divisible in ICC involving the hepatic hilum and extrahepatic hilar bile duct carcinomas because they have found different prognoses after hepatobiliary resection[16]. Perihilar CC is considered by the American Joint Committee Cancer/Union for International Cancer Control (AJCC/UICC) TNM system as an EBDC but the term perihilar CC is also sometimes used to refer to intrahepatic large bile duct carcinomas[1,17]. Therefore, as with hilar CC, the term perihilar CC has been used in both a broad and narrow sense.

Another pertinent issue refers to the most appropriate use of the term CC. One option is to use CC for any bile duct carcinoma originating from the small intrahepatic bile ducts until the end of the common bile duct into the ampulla of Vater. In this option, the term CC is preceded by the anatomical location, such as intrahepatic, hilar, perihilar or extrahepatic CC. The other option, assimilated by the WHO system, is to restring the term CC for carcinomas arising in the intrahepatic bile ducts (ICC) and use the term bile duct carcinoma for the extrahepatically derived CC (*i.e.*, EBDC)[4]. According to the WHO classification, hilar CCs (*i.e.,* Klatskin tumors) are EBDC. However, the WHO system argues that, especially in locally advanced cases, their distinction from ICC of the major intrahepatic bile ducts is usually controversial and such cases could be included in the definition of perihiliar ICC[1,17]. Ultimately, it is up to the pathologist to establish the topographical origin of these hilar tumors in the surgical specimen, often with the essential collaboration of the surgeon. Since the histological classification of the ICC and EBDC is somewhat different, the location of the tumor also involves applying either of these classifications. In the case of EBDC, they are histologically subclassified the same as the carcinomas of the gallbladder by the WHO system[1].

In conclusion, several factors contribute to the designation of these tumors: topography (intrahepatic, extrahepatic, peripheral, hilar, perihilar, proximal, distal), histogenesis (small or large duct), preoperative staging by radiology (of interest for surgical approach-resectable, unresectable), definitive staging (of prognostic interest and for additional oncological treatment approach) with pathological staging of the resected tumor in the surgical specimen (intraepithelial, intraductal, invasive) and clinical aspects (obstructive or not). Ideally, all these parameters should be incorporated into the final diagnosis. With respect to the histological designation, we recommend naming this tumor biliary duct carcinoma (CC), specifying the exact location (for example: bile duct adenocarcinoma-CC-of the common hepatic duct and its confluence).

The different use of the term hilar CC complicates the review of its pathological features in the literature. In addition, many reports are confounded by the inclusion of patients with tumors arising from other locations of the biliary tract (for example, including mid and distal bile duct tumors).

MACROSCOPIC PATHOLOGY

Hilar CC has been grossly classified in polypoid or papillary, nodular, scirrhous constricting or nodular-infiltrating, and diffusely infiltrating types, each type showing different resectability and prognosis[18,19]. The polypoid or papillary type protrudes to the lumen in a bland and

friable cauliflower-like fashion. The nodular type is characterized by a firm, gray-white tumor bulging from the mucosa, with the border of the tumor to the adjacent tissue fairly well defined. Both papillary and nodular types have evident tumorous lesions. On the contrary, scirrhous constricting or nodular-infiltrating type exhibits only a slight protuberance of the mucosa and involves the thickness of the wall with distortion or annular fibrous constriction. In the diffusely infiltrating type, the tumor appears as an ill-defined stricture of the duct due to a hard fibrous thickening of the duct wall, with more linear extension than the scirrhous constricting type[19]. Nodular, and especially nodular-infiltrating and diffusely infiltrating types, usually infiltrate more intensively than polypoid (papillary) type and more frequently exhibit submucosal extension at the proximal border that may make their resection difficult. On the contrary, mucosal extension at the proximal border is observed more often in nodular and even more in papillary tumors. These macroscopic classifications present difficulties in practice because many tumors may have overlapping features. The papillary type has the best prognosis, is more often resectable and less invasive, and usually corresponds to well differentiated papillary tumors. Therefore, hilar CC can be classified macroscopically, making a major distinction between papillary and non-papillary (*i.e.*, nodular-sclerosing) types[9].

MICROSCOPIC PATHOLOGY

Conventional histology and immunohistochemistry

Most of hilar CC or Klatskin tumors histologically correspond to well to moderately differentiated biliary type adenocarcinomas[1,20]. These biliary type EBDCs are histologically very similar to those arising in intrahepatic large bile ducts (perihilar ICC)[1,3]. They are characterized by tubules or glands in a typical desmoplastic stroma with variable inflammatory response. There may also be solid nests and cords in less differentiated cases and same papillary groups are often seen on the surface. Tumor cells are columnar to cuboidal with moderate amount of clear to eosinophilic cytoplasm. Nuclei are generally small, although a major grade of nuclear atypia is also possible. They typically produce numerous peri and intraneural invasions. They also tend to produce lymphatic invasion, with venous invasion less frequent[11,21,22]. Perineural invasion is a specific route of invasion in bile duct carcinomas and it is an important prognostic factor[23]. Spread by direct invasion to periductal hilar tissues, portal vein branches, hepatic arterial branches and adjacent liver tissue are common findings in hilar CC[5,24]. Although hilar CC has traditionally been considered a slow-growing locally invasive tumor, different reports have found lymph node involvement in 30%-50% of patients on exploration and 20% had involvement of distant sites[11,22,25,26].

Biliary duct adenocarcinomas can be histologically graduated based on their degree of glandular or tubular differentiation. The previous edition of WHO classification established a quantitative grading system in which well, moderate and poorly differentiated biliary duct adenocarcinomas were composed of, respectively, 95%, 40%-94% and 5%-39% of glands[21,27]. However, the current WHO classification does not mention any quantitative criteria[1]. The College of American Pathologists (CAP) has proposed a quantitative grading system in which greater than 95%, 50%-95% and less than 50% of tumor composed of glands corresponds to, respectively, well, moderate and poorly differentiated bile duct adenocarcinomas[28]. We believe it is advisable to use a degree of differentiation based on cytological atypia in addition to architectural pattern (tubular, papillary, solid, single cells).

Immunohistochemically, cytokeratin (CK) 7 is nearly always positive in hilar CC, like the rest of the CC. The majority of perihilar ICC and EBDC also express CK20 (80% in case of hilar tumors), most commonly with a low or moderate labeling index. On the contrary, peripheral ICC is CK20 negative in just over half of the cases[29].

Several mucin-related glycoproteins and oncoproteins, such as carcinoembryonic antigen (CEA), human mucin (MUC) type 1 (MUC1) and 5AC (MUC5AC), B72.3 and CA 19-9 are normally expressed, although they may be focal[30,31]. Surface proteins such as CEA or MUC1 may be related to anti-adhesion molecular functions that promote the release of cells from the tumor and facilitate tissue invasion. CEA is generally limited to the apical membrane in biliary duct benign cells but it is commonly detected in the cytoplasm of biliary duct adenocarcinoma cells, more intensely in advanced tumors or poorly differentiated adenocarcinomas[32]. MUC1 is expressed in the majority of EBDC, with cytoplasmic expression more frequently observed in invasive lesions. MUC1 expression is related to poor differentiation, locoregional tumor progression, metastases to the liver and poor outcome[33,34]. In contrast, MUC2 (an intestinal-type secretory mucin) is expressed in less than half of the cases (usually with low or moderate labeling index), is highly expressed in well-differentiated adenocarcinomas, and is inversely related to tumor progression and poor outcome[34].

Variably dispersed endocrine cells, immunoreactive for neuroendocrine markers such as synaptophysin and chromogranin, may be observed in just under a third of the EBDC, especially in well to moderate differentiated tumors[21,35].

p53 is a tumor-suppressor gene very commonly mutated in different human tumors. DPC4 is another tumor-suppressor gene, known to be inactivated in around 55% of pancreatic adenocarcinomas and less often in other tumors. Immunohistochemically, p53 overexpression and loss of DPC4 expression have been observed in, respectively, 25% and 15% of hilar CC. On the contrary, carcinoma of the distal bile duct exhibited a higher frequency of p53 overexpression and DPC4 inactivation, in proportions more similar to those observed in pancreatic adenocarcinoma[36,37]. The reported rates of K-ras mutations in EBDCs has also shown lower frequencies in proximal compared to distal EBDC[38-40]. These findings suggest that the molecular mechanisms in the tumorigenesis along the biliary tract might be different, reflecting

different etiologies, whereas distal bile duct and pancreas would share similar molecular alterations. However, the reported rates of p53 and K-ras alterations have differed widely in different studies[41]. The immunohistochemical reported results of HER-2/neu (c-erbB-2) overexpression in EBDC has also varied considerably. More recent studies have observed c-erbB-2 overexpression in 4% to up to one third of EBDC[42-45].

Non conventional histology: histological variants

Hilar CC rarely corresponds to other histological variants. Adenosquamous carcinoma exhibits variable amount of malignant squamous cells with keratin pearls and/or intercellular bridges and a glandular component identical to conventional adenocarcinoma. Either of the two components can be predominant. The squamous areas are positive for high molecular weight cytokeratins, CK5/6, p63 and S100A2, these markers being negative in the adenocarcinoma component[31,46]. The survival time for patients with adenosquamous carcinoma seems to be significantly worse when compared with adenocarcinoma of the bile ducts. An inverse relationship between the proportion of the squamous component and patient survival has been observed[46,47]. Currently, the WHO classification makes no comment on the need for a minimum amount of squamous cell component required for the diagnosis of adenosquamous carcinoma of the extrahepatic bile ducts[1]. Squamous cell carcinoma of the hilar bile duct is very rare. The presence of any amount of glandular component excludes this diagnosis. The majority of reported cases were already advanced at the time of diagnosis so it has been assumed that its prognosis is poor[48,49]. Malignant transformation of squamous metaplasia in biliary epithelium has been suggested for the origin of squamous cell carcinoma and adenosquamous carcinoma, whereas histopathological alteration from adenocarcinoma to squamous cell carcinoma has been suggested as an alternative etiology for adenosquamous carcinomas[48].

Mucinous (colloid) adenocarcinoma is characterized by prominent extracellular or stromal mucin deposition. In the literature, mucinous (colloid) adenocarcinoma of the extrahepatic bile ducts is normally described as the invasive component related to some papillary non-invasive neoplasms[50,51]. Intestinal type adenocarcinoma is composed of tubular glands closely resembling those of colonic adenocarcinomas. It has been pointed out that some conventional bile duct carcinomas (*i.e.,* pancreatobiliary type) can exhibit a somewhat pseudostratified appearance with tall columnar cells or some expression of intestinal makers, such as MUC2 and CDX2, which may have led to classifying these cases as intestinal type adenocarcinoma[30]. In any case, pure mucinous adenocarcinoma and intestinal adenocarcinoma are very rare in the biliary ducts[20,30].

Clear cell carcinoma of the extrahepatic bile ducts is composed of tumor cells with clear cytoplasm containing PAS-positive diastase-labile cytoplasmic granules. It can exhibit trabecular, nesting, glandular and sheet pattern in variable proportions and must be differentiated from metastatic renal cell carcinoma. The presence of areas of conventional biliary adenocarcinoma and the immunohistochemical study permit the correct diagnosis. For instance, primary clear cell carcinoma of the hilar ducts express CK7, whereas this is not the case for renal clear cell carcinoma[52,53].

Gastric foveolar type hilar CCs are well-differentiated tumors with tubular glands lined by slender tall columnar cells with mucin-containing cytoplasm and basal nuclei with small nucleoli, resembling gastric foveolar cells. Long and irregular tubular glands, nuclear pseudostratification and minor foci of less differentiated tumor cells can also be observed[1,54]. Very recently, three hilar CCs with pyloric gland phenotype have been described as a new morphological variant of EBDC. Although they were extremely differentiated, their infiltrative pattern and perineural invasion facilitated the diagnosis and their clinical behavior was similar to that of conventional biliary adenocarcinoma. They exhibit complex glands with a stellar pattern that seems to be a unique feature of this distinctive variant. Immunohistochemically, this variant coexpress MUC5AC (gastric foveolar mucin) and MUC6 and is negative for MUC2 and CDX2. This immunophenotype is similar to that observed in foveolar adenocarcinomas[20].

Other carcinomas that have very rarely been described in the extrahepatic bile ducts are signet ring cell carcinoma, carcinosarcoma and undifferentiated carcinoma[1,20,55].

Premalignant lesions

Currently, two premalignant lesions related to the development of CC are known. They are referred to in the WHO classification of biliary tumors with the names of biliary intraepithelial neoplasia (BilIN) and intraductal papillary neoplasm of the biliary tract (IPNB)[1]. They are usually found in the intrahepatic large bile ducts and extrahepatic ducts (including the hilar bile ducts) and are precursors of some ICC and EBDC. BilIN e IPNB are postulated to be in many respects the counterpart of respectively, pancreatic intraepithelial neoplasm (PanIN) and intraductal papillary mucinous neoplasm (IPMN) of the pancreas (IPMN-P) because they share common morphological features and biological behaviors[51].

Previously known as atypical biliary epithelium, biliary dysplasia or carcinoma *in situ*, BilIN are flat or low-papillary lesions, therefore only recognizable microscopically. Currently, BilIN are classified into three histological grades based on the degree of atypia, suggesting a spectrum of lesions with increasing neoplastic potential. Diagnostic criteria of consensus, for which a moderate interobserver agreement among experienced pathologists has been observed, are available[56,57]. BilIN-1 lesions are most commonly flat, with cellularity only slightly increased and round or oval nuclei only slightly enlarged. BilIN-2 are flat, pseudo or micropapillary lesions with loss of cellular polarity, nuclear pseudostratification and enlargement of the nuclei with hyperchromasia and irregular nuclear membrane. BilIN-3 cytologically resemble carcinoma (includes the lesion previously called

carcinoma *in situ*), mostly being pseudo or micropapillary, sometimes with budding of small cluster of cells into the lumen, with large hyperchromatic nuclei with severe membrane irregularities[57]. When accompanied by invasive lesions, BilIN is known to progress to conventional CC (*i.e.,* tubular adenocarcinoma) with biliary type phenotype. This pathway is characterized by MUC2-/CK7+/CK20-, with an increased expression of MUC1 along with the disease progression. MUC2 and CK20, reflecting the intestinal type, is rarely observed in this lineage[58].

Intraepithelial carcinoma (BilIN-3, carcinoma *in situ*) have been described in 10% to 75% of invasive EBDC. When observed associated with invasive carcinoma, these intraepithelial malignant cells might belong to a primary carcinoma *in situ* or could be due to cancerization of the surface epithelium from the invasive carcinoma. Extensive intraepithelial spread (also called superficial spread) have been defined as the presence of intraepithelial carcinoma 20 mm or more in length from the margin of the main lesion to the proximal or distal side and has been observed in 13%-18% of EBDC[18,59]. The majority of cases with extensive intraepithelial spread are histologically well differentiated papillary tumors. In resected specimens, the presence of extensive intraepithelial spread has been observed to be associated with a better postoperative prognosis, although it might be related to late relapses of the tumor in the bile duct stump.

Biliary papilloma, biliary papillomatosis, papillary CC, CC of the intraductal growth type, mucin hypersecreting CC, mucin hypersecreting bile duct tumors, mucin ball-producing EBDC and others are different terms to designate a constellation of biliary papillary tumors that currently are considered to belong to a single tumor entity called IPNB[60-62]. The incidence of IPNB among all bile duct carcinomas range from 7% to 38%[63]. IPNB produce papillary projections macroscopically evident into the lumen of the bile ducts. Around a third of the IPNB secrete mucus grossly visible into the lumen (IPNB with excess mucin secretion or mucin secreting biliary tumor)[1,64]. Infrequently, IPNB appears as a cystic tumor (IPNB with prominent cystic changes or cystic variant of IPNB)[64,65].

Histologically, IPNB are composed of papillary fronds with delicate fibrovascular cores, with or without gland formations that may be lined with four different cell types: pancreatobiliary, intestinal, gastric and oncocytic type. The most frequent phenotype is the pancreatobiliary, characterized by columnar cells with moderate amphophilic cytoplasm and enlarged nuclei resembling biliary epithelium (MUC1+, MUC2-, MUC5AC+ being the most common immunophenotype). Next in frequency are the intestinal and gastric types, with different outcomes in terms of its prevalence. The intestinal type show columnar cells and goblet cells resembling intestinal epithelium (MUC1-, MUC2+, MUC5AC+), whereas the gastric type is composed of columnar epithelial cells with abundant cytoplasmic mucin that resemble the gastric foveolar epithelium (MUC1-, MUC2-, MUC5AC+). The oncocytic type (MUC-/+, MUC2+, MUC5AC+) is considered a variant of the pancreatobiliary type and is the rarest form. In some cases it may be difficult to identify the type only by its microscopic appearance as it is necessary to carry out its classification on the basis of immunohistochemistry[60,62,63,66]. Although pancreatobiliary type is the most common phenotype, the majority of IPNB with macroscopically visible mucin secretion (mucin producing biliary tumors) are intestinal type (also referred as columnar type). These mucin secreting papillary tumor, also known as biliary tract IPMN, are the IPNB who most resemble IPMN of the pancreas pathologically, especially the main pancreatic duct type[61,67,68]. Recently, a rare case of pseudomyxoma peritonei preceded by IPNB has been described[69].

IPNB exhibit a spectrum of architectural complexity and cytological atypia but, unlike BilIN, there are no well-defined criteria for their grading. Instead, consensus criteria for IPMNP have been applied to grade IPNB. Accordingly, the current WHO system classifies IPNB into low, intermediate and high grade[1,58].

Most IPNB (70%-80% of cases) are associated with a component of invasive adenocarcinoma[63]. Invasive papillary carcinoma has been the classical name for this invasive adenocarcinoma. However, currently this tumor is better named "intraductal papillary neoplasm with associated invasive adenocarcinoma". In fact, invasive papillary structures are very rarely observed, the invasive component associated with IPNB being a conventional type (tubular or pancreatobiliary type) adenocarcinoma in the majority of cases, although can also frequently be a colloid carcinoma and other invasive type components may occasionally be seen[58,67]. During carcinogenesis from IPNB to invasive carcinoma, most of IPNB are characterized by an intestinal immunophenotype (MUC1-/MUC2+) which is commonly conserved in associated colloid carcinomas, whereas the majority of tubular adenocarcinoma related to IPNB acquires MUC1 expression (MUC1+, MUC2+). MUC1 is also expressed in the majority of biliary duct adenocarcinomas not related to IPNB. The CK7+/CK20+ pattern is the commonest both in tubular and colloid carcinomas related to IPNB. CK20 is most frequently expressed in invasive adenocarcinoma associated with IPNB compared to CC not related to IPNB[58,62]. It has been suggested that invasive tumors arising from IPNB have a better prognosis than invasive carcinomas not related to IPNB. However, it is now believed that evolution depends largely on the stage of the invasion and histological type, the prognosis being better in colloid carcinomas. Minimally invasive papillary carcinomas (*i.e.*, with only superficial stromal infiltration) of the extrahepatic bile duct have been found to have a good prognosis, similar to noninvasive papillary carcinomas[50,70].

Recently, two cases have been described of so called biliary intraductal tubulopapillary neoplasm (ITPN) in the hilar bile ducts. It has been proposed that biliary ITPN is a distinct tumor entity with a suggested origin from preexisting peribiliary cysts[71].

Table 1 Observed survival of a series of 29 patients with resected hilar cholangiocarcinoma

Period (yr)	Observed survival (95%CI)
1	68.4 (51.3-85.5)
2	39.6 (21.5-57.7)
3	35.6 (17.7-53.5)
4	31.2 (13.5-48.9)
5	22.3 (6.2-38.4)

Table 2 Histological types of 29 cases of perihilar bile duct carcinomas *n* (%)

Histology	
Adenocarcinoma, biliary type	24 (82.8)
Without IPNB	20 (69)
Well or moderately differentiated	18
Poorly differentiated with signet ring cells	2
With IPNB	4
Well or moderately differentiated	3
Poorly differentiated with signet ring cells	1
IPNB high grade (papillary carcinoma)	5 (17.2)
Without invasive carcinoma	1 (3.4)
With invasive carcinoma, biliary type	4 (13.8)
Well-moderately differentiated	3
Poorly differentiated with signet ring cells	1
Adenocarcinoma, gastric foveolar type	2 (13.8)
Adenocarcinoma, clear cell type	1 (3.4)
Adenosquamous carcinoma	1 (3.4)
Total number of cases	29

IPNB: Intraductal papillary neoplasm of the biliary tract.

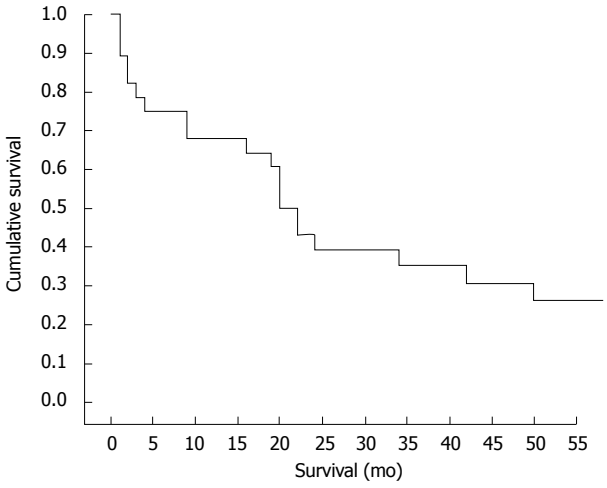

Figure 1 Survival curve of a series of 29 patients with resected hilar cholangiocarcinoma.

Table 3 Other histological features in 29 cases of perihilar bile duct carcinomas *n* (%)

Gross type	
Sclerosing	10 (34.5)
Nodular	8 (27.6)
Nodular-sclerosing	6 (20.7)
Papillary	2 (6.9)
Papillary-nodular	2 (6.9)
Papillary-sclerosing	1 (3.4)
BilIN	
BilIN-1 and/or 2	10 (34.5)
BilIN-1	9 (31)
BilIN-2	5 (17.2)
BilIN-3 (*in situ* carcinoma)	6 (20.7)
Lymphatic invasion (L1)	9 (31)
Venous invasion (V1)	11 (37.9)
Perineural invasion	23 (79.3)
T-staging (according to AJCC/UICC (7th ed)	
pTis	1 (3.4)
pT1	1 (3.4)
pT2a	10 (34.5)
pT2b	12 (41.4)
pT3	5 (17.2)
Lymph node status:	
Positive	7 (24.1)
Negative	16 (55.2)
No lymph nodes histologically studied	6 (20.7)
Margin status	
Negative (R0)	12 (41.4)
Positive (R1) (invasive carcinoma)	17 (58.6)
Bile duct margin	10 (34.5)
Radial margin	11 (37.9)

BilIN: Biliary intraepithelial neoplasia; AJCC/UICC: American Joint Committee Cancer/Union for International Cancer Control.

Staging and other histological prognostic factors

There are different staging schemes to evaluate hilar CC: the modified Bismutt-Corlette system[72], the Memorial Sloan-Kettering Cancer Center classification[73] and the more recently described new proposal by the International CC Group for the Staging of Perihilar CC[74]. Currently, the system most widely used by pathologists to stage these tumors after surgical resection is the pathological TNM included in AJCC/UICC TNM classification[5]. The AJCC/UICC establishes three different staging systems for intrahepatic, perihilar and distal bile duct carcinomas. Proximal or perihilar CC (Klatskin tumors) are defined anatomically by the AJCC/UICC as tumors located in the extrahepatic biliary tree proximal to the origin of the cystic duct, which may extend proximally into either the right or left hepatic ducts, or both. Recently, some problematic aspects that may arise when applying the pathological TNM for EBDC have been revised, looking for opportunities for improvement[75]. For instance, perihilar CC is considered pT1 if it is confined to the bile duct, with extension up to the muscle layer or fibrous tissue, and pT2a when it invades beyond the wall of the bile duct to the surrounding adipose tissue. However, the muscle layer is only well-defined in the very distal common bile duct and in many bile ducts there are no hallmarks to determine where the ducts end, especially in the setting of fibrosis which often accompanies these tumors. As an alternative, some authors have proposed the use of the depth of invasion as part of the T-staging of EBDC. They have found that the cutoff points of 5 and 12 mm separate patients with EBDC into three groups with different lengths of survival. For hilar CC, tumor depth $\geqslant$ 5 mm was predictive of poor survival in one study[76,77]. pT3 and pT4 definitions involve determining whether the portal vein and hepatic artery or their branches are affected, as

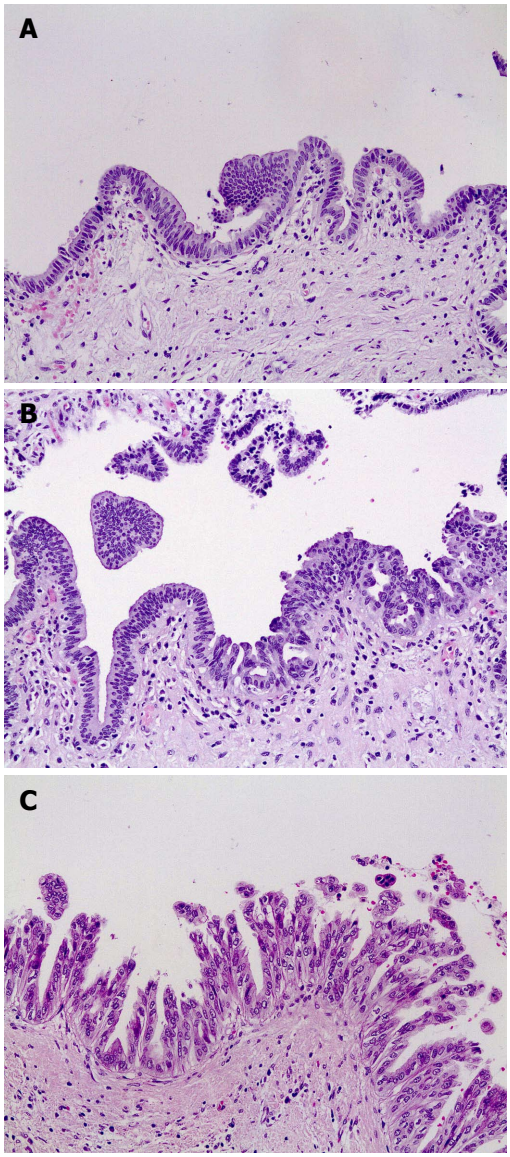

Figure 2 Biliary intraepithelial neoplasia. A: Biliary intraepithelial neoplasia (BilIN) 1 (HE stain, ×200); B: BilIN 2 (HE stain, ×100); C: BilIN 3 (HE stain, ×100).

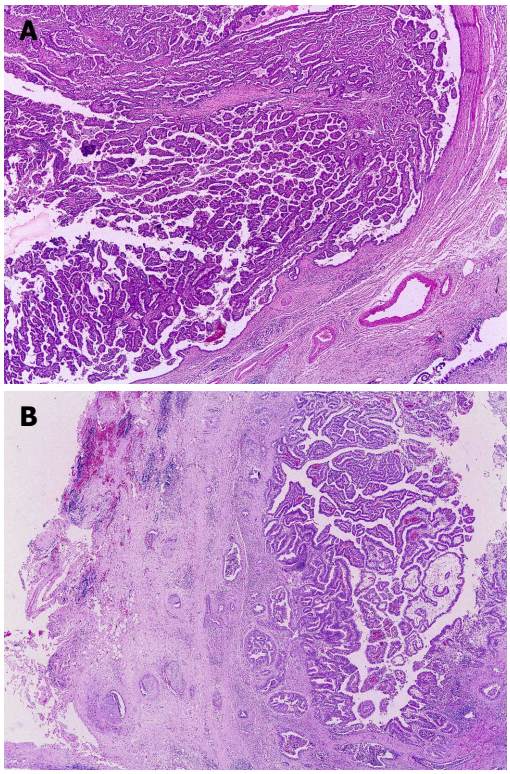

Figure 3 Intraductal papillary neoplasm of the biliary tract. A: Intraductal papillary neoplasm of the biliary tract (IPNB) without invasion, classically named biliary papillomatosis (HE stain, ×20); B: IPNB with associated invasive carcinoma, previously named invasive papillary carcinoma (HE stain, × 20).

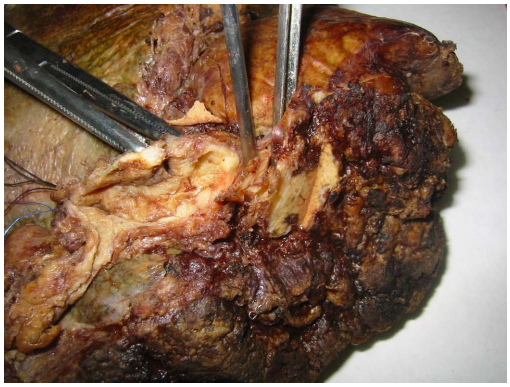

Figure 4 An example of a nodular-sclerosing cholangiocarcinoma in the common, right and left hepatic ducts.

well as the secondary biliary radicals. However, this assessment may be inaccessible to the pathologist unless some of these structures are specifically marked by the surgeon[75]. Positive lymph nodes represent one of the most relevant prognostic factors[11,14]. Regional lymph node metastases (nodes along the cystic duct, common bile duct, hepatic artery and portal vein) are considered pN1, whereas periaortal, pericaval, superior mesenteric artery and celiac artery lymph nodes are assigned pN2[5]. To avoid an incorrect staging, lymph nodes should be referred properly identified with regards to their origing to the pathologist.

The incidence of positive surgical resection margins in patients treated surgically with curative intent is very variable (9%-74%). The affected ductal resection margins by invasive carcinoma has a strong adverse effect on patient survival, whereas being affected by severe dysplasia or carcinoma *in situ* does not seem to have such a pernicious effect, although it could be responsible for some late recurrences in the stump. This suggests that carcinoma *in situ* could take several years to become invasive in the stump[14,15,25,78,79]. The dissection margin has been defined as the remaining surgical cleavage plane with the adjacent hilar structures. Although it seems that less emphasis has been placed on dissection margin, this radial margin should be taken into consideration. Positivity of the dissection margin in hilar CC has been observed to be considerably higher compared to the ratio of positivity of the ductal margins[22]. In addition, intraoperative examination by frozen sections is very useful for obtaining a ductal resection margin free of invasive carcinoma (the distinction between dysplasia, carcinoma *in situ* and

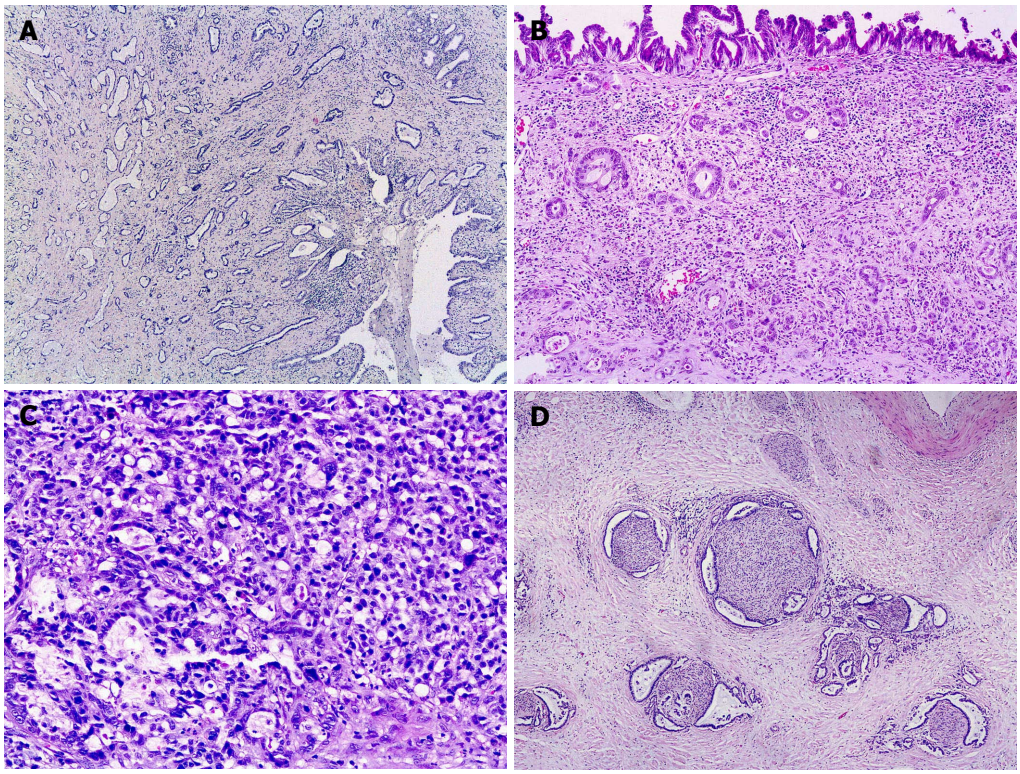

Figure 5 Examples of the broad morphological spectrum of hilar bile duct carcinoma. A: Well differentiated biliary type adenocarcinoma (HE stain, ×40); B: A case with well defined tumor glands interspersed with poorly differentiated small tumor groups an single tumor cells (besides, biliary intraepithelial neoplasia 3 can be observed on the duct surface) (HE stain, ×100); C: A case with poorly differentiated cell component with resembling signet ring cells (HE stain, ×200); D: Perineural invasion by well differentiated glands (HE stain, ×40).

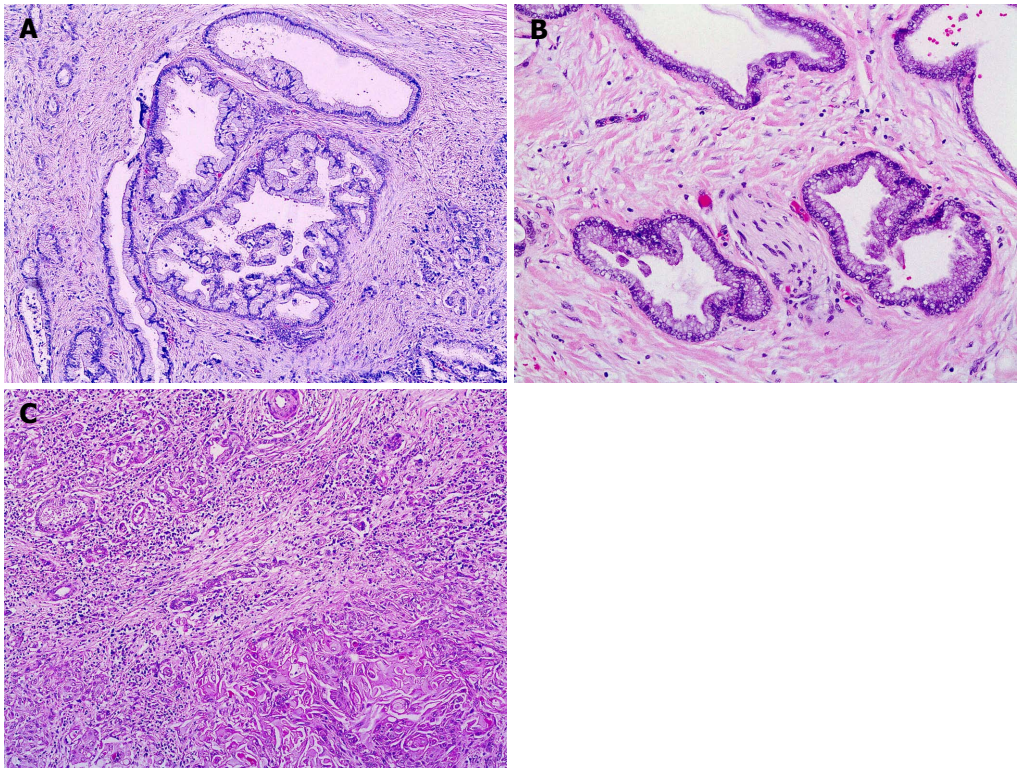

Figure 6 Histological variants. A: Gastric foveolar type carcinoma (HE stain, ×100); B: Very well differentiated glands of a gastric foveolar type carcinoma near a nervous fascicle (HE stain, ×200); C: Adenosquamous carcinoma showing the glandular and squamous component (HE stain, ×100).

reactive changes in frozen sections is less reliable) but is not really feasible for the dissection margin in most cases. Another issue concerns the minimal distance required between tumor and resection margin. Some authors have observed that a distance of tumor less than 5 mm from the transverse surgical margin is associated with the worst prognosis. In Japan, a distance of 5 mm has been proposed to define R0 resections, although the TNM AJCC/UICC system does not make any consideration in this regard[26,80,81]. We prefer to follow the notion of R0 according to its definition by the AJCC/UICC literally, so we only consider R0 if the tumor is not at the margin. In addition, we add the distance between the tumor and the margin in the report.

Other histological factors that have been adversely associated with prognosis in univariable and, in some cases,

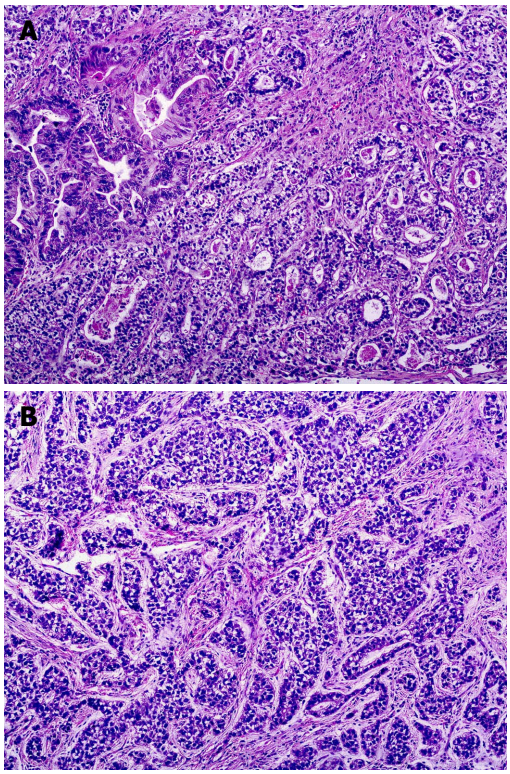

Figure 7 Histological variants: clear cell carcinoma (HE stain, ×100). A: An area with glandular pattern; B: An area with trabecular pattern.

multivariable analysis, are the high tumor grade, the presence of vascular, lymphatic or perineural invasion[11,23,28] and nodular or sclerosing gross features (*vs* papillary tumors)[9]. Some histological subtypes like adenosquamous and squamous carcinoma could have greater malignant potential than conventional biliary type adenocarcinoma[47,49]. According to the CAP protocol for perihilar CC, high-grade tumors, such as signet-ring cell carcinomas, small cell carcinomas and undifferentiated carcinomas, are associated with a poorer prognosis compared with conventional adenocarcinoma[28].

Contribution of a series of hilar CC from our institution

A total of 71 patients with extrahepatic non ampullary biliary duct carcinoma were obtained from the files of the Department of Pathology, Hospital "12 de Octubre" in Spain between January 1999 to December 2011. After excluding distal bile duct tumors and cases with only incisional biopsies or cytology samples, we reviewed the surgical specimens of 29 patients, 17 males and 12 females, aged 47 to 82 years (median age, 68 years; mean age, 67.5 years) with hilar CC. The observed survival was 39.6% and 22.3% at one and three years. The survival curve is shown in Table 1 and Figure 1. A summary of the histological features is given in Tables 2 and 3.

With respect to T-staging, in three cases the tumor extended to the cystic duct and/or gallbladder, although the main tumor mass was located in the hilar bile ducts. This situation is not covered by the current TNM system for perihilar CC.

The majority of the tumors (82.8%) were conventional type adenocarcinomas (biliary or pancreatobiliary), most of them well to moderately differentiated (21 cases, 72.4%). A component of *in situ* carcinoma BilIN-3 was present in 6 of the cases (20.7%) (Figure 2). Five cases exhibited IPNB (17.2%), four of them with an associated invasive carcinoma (Figure 3). We consider it more appropriate to call these latter cases adenocarcinoma with associated IPNB rather than invasive papillary carcinoma given that they did not infiltrate with a papillary pattern but with a conventional appearance. Four and one IPNB, respectively, showed a biliary and an intestinal differentiation. All the IPNB, including the one without an invasive component, exhibited a high cytological grade of dysplasia. With respect to their macroscopic appearance, two grossly papillary cases correspond to the IPNB without associated invasive carcinoma and one case of IPNB with associated superficial invasive carcinoma (*i.e.,* microinvasive carcinoma o pT1). Cases with papillary-nodular pattern (2) and papillary-sclerosing pattern (1) pertain to the remaining observed cases of invasive carcinoma associated to IPNB. Among non-papillary tumors, the separation between nodular and sclerosing types was a somewhat subjective in practice (Figure 4).

Histologically, conventional adenocarcinomas exhibited a broad morphological spectrum from one case to another and even within the same tumor, with cuboidal or tall cells, different sized glands, tubules, cords and single cells. Desmoplastic stroma was constantly present, at least in some tumor areas. Some cases were extremely well differentiated. In these cases, the diagnosis was aided by their infiltrative appearance in the surgical specimen; however, the diagnosis of these cases in small biopsies would be extremely difficult. We observed three cases with a poorly differentiated cell component resembling signet ring cells, one of then somewhat histiocytoid. In addition, we observed a biliary type adenocarcinoma with taller cells with greater nuclear stratification resembling intestinal adenocarcinoma, although with other glands showing biliary features clearly (Figure 5).

With respect to non-conventional histology, two well differentiated tumors belonged to the gastric foveolar type (although this variant, as well as the more recently described pyloric type would need studies of larger series for better characterization). In addition, a clear cell type carcinoma and one case of adenosquamous carcinoma was observed (Figures 6 and 7).

Most reviewed cases (23 cases, 79.3%) showed perineural invasion, which in most cases was extensive (Figure 5). Lymphatic and venous vessel invasion was observed in 9 (31.0%) and 11 (37.9%) cases, respectively. Lymphatic invasion especially showed a significant subjectivity in its assessment, in part due to tissue shrinkage around many neoplastic groups.

Seventeen specimens (58.6%) exhibited involvement of the surgical margins, with the bile duct margin positive in 10 (34.5%) and radial margin positive in 11 (37.9%) cases. Radial margin involvement frequently occurred in the periductal soft tissue and in some cases at the liver parenchyma.

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