# 86108.docx

WORD COUNT 5040
TIME SUBMITTED 14-AUG-2023 06:00PM
PAPER ID 101976741

**Name of Journal:** *World Journal of Gastroenterology*
**Manuscript NO:** 86108
**Manuscript Type:** MINIREVIEWS

# Intraductal papillary neoplasm of the bile duct (IPNB): The new frontier of biliary pathology

## IPNB: A recently-identified biliary disease

**Abstract**

*Background* Intraductal papillary neoplasms of the bile duct (IPNB) represent a rare variant of biliary tumors characterized by a papillary growth within the bile duct lumen. Since their first description in 2001, several classifications have been proposed, mainly based on histo-pathological, radiological and clinical features, though no specific guidelines addressing their management have been currently developed.

*Main body* Bile duct neoplasms generally develop through a multistep process, involving different precursor pathways, ranging from the initial lesion, detectable only microscopically, i.e. biliary intraepithelial neoplasia (BilIN) to the distinctive grades of IPNB till the final stage represented by the invasive Cholangiocarcinoma (CCA). Complex and advanced investigations, mainly relying on magnetic resonance imaging (MRI) and cholangioscopy, are required to reach a correct diagnosis and to define an adequate bile duct mapping, aiming at addressing the proper treatment. The recently introduced sub-classification in type 1 and type 2 appropriately highlights the histopathologic and clinical aspects of IPNB, their natural evolution with a particular focus on prognosis and survival. Aggressive surgical resection, including hepatectomy, pancreaticoduodenectomy or both, represents the treatment of choice, granting optimal results in terms of survival, though several endoscopic approaches have been described.

*Conclusion* IPNB are newly-recognized pre-invasive neoplasms of the bile duct with high malignant potential. The novel sub-classification in type 1 and 2 properly

defines the histologic and clinical aspects, the prognosis and survival. Diagnosis is mainly based on MRI and cholangioscopy. Surgical resection represents the mainstay of treatment, though endoscopic resection is currently applied to non-surgically fit patients. New frontiers in genetic research have identified the processes underlying the cancerogenesis of IPNB, aiming at identifying targeted therapies.

**Key Words:** Intraductal neoplasm of the bile duct; Bile duct neoplasms; Cholangiocarcinoma; Intraductal papilloma; Classification; Treatment

Mocchegiani F, Vincenzi P, Conte G, Nicolini D, Rossi R, Benedetti Cacciaguerra A, Vivarelli M. Intraductal papillary neoplasm of the bile duct (IPNB): The new frontier of biliary pathology. *World J Gastroenterol* 2023; In press

**Core Tip:** IPNB are rare premalignant lesions, described prevalently in series from East Asia. Due to lack of specific guidelines addressing their management, a recent sub-classification in type 1 and 2 aims at clarifying several aspects, particularly histo-pathological, clinical and prognostic. MRI and cholangioscopy occupy a central role in the diagnosis and in tailoring the therapeutic approach. Surgery represents the most appropriate treatment, yielding optimal results in terms of survival, though endoscopic techniques have been employed, particularly in non-surgically fit patients. Lastly, recent genetic research is focused on identifying targeted therapies acting in the stepwise progression of neoplastic biliary epithelium.

## INTRODUCTION

Intraductal papillary neoplasms of the bile duct (IPNB), first described by Chen *et al* in 2001[1], represent a variant of biliary tumors with a distinctive papillary growth inside the common bile duct lumen and typically may develop along the biliary tree; IPNB is identified macroscopically with bile duct dilatation and intraductal masses at radiologic imaging[2-4].

Together with biliary intraepithelial neoplasia (BilIN)[5], IPNB was first recognized as a premalignant lesion towards invasive cholangiocarcinoma (CCA) in the 2010 World Health Organization (WHO) classification of tumors of the digestive system[6].

The term IPNB was introduced for the similarities with intraductal papillary mucinous neoplasms of the pancreas (IPMN-P), leading to consider the former as the biliary counterpart of the latter[3, 7, 8], even if the simultaneous occurrence of both types is extremely rare[9-19].

Though complex and generally requiring an extensive multidisciplinary investigation, the accurate diagnosis of IPNB is mandatory to guide the therapeutic approach, with aggressive surgical resection representing the treatment of choice, particularly in early stages, while endoscopic management has been described as a potential alternative in non-surgically fit patients[4, 20-24].

The purpose of this article is to summarize the current and latest concepts regarding IPNB, discuss the various morphologic features of IPNB and its mimickers, and describe clinical approaches in the diagnosis and treatment useful for their multidisciplinary management.

We searched the international English literature for the purpose of reviewing this topic.

## DEFINITION, EPIDEMIOLOGY AND CLINICAL FEATURES

### *Definition*

Invasive bile duct neoplasms, i.e. CCA[25], always develop through a multistep process, including the two above-mentioned precursors: BilIN and IPNB[5].

While BilIN is a flat or low-papillary growth of dysplastic biliary epithelium, detected only microscopically, IPNB consists of an intraductal papillary growth of neoplastic biliary epithelium, that can be identified macroscopically and therefore visible on imaging[3, 5].

BilIN has been generally classified into three grading systems delineated as mild, moderate and severe dysplasia, or as low-grade, high-grade dysplasia and carcinoma *in situ*[26]. Recently, several cytological features, namely detailed cellular and nuclear

changes, glandular involvement, mitosis, nuclear location and intraepithelial neutrophils, have been incorporated into the categorization of BilIN, allowing to identify three histological grades: BilIN-1, BilIN-2, and BilIN-3, the latter one including the so-called carcinoma *in situ*[26].

IPNB is typically represented by a papillary or villous tumor growing inside the bile duct lumen and composed of papillary stalks with fine vascular cores that can develop anywhere along the biliary tree, involving both the intra- and extrahepatic bile ducts[2-4].

Although already described in 2001[1], the term IPNB was introduced by the revised WHO Classification of Tumors of the Digestive System in 2010[6], incorporating different entities, for example the previously-called mucin-producing CCA, papillary cholangiocarcinoma (PCC), mucin-hypersecreting bile duct tumor, IPMN of the bile duct (IPMN-B), biliary papilloma or papillomatosis, papillary adenocarcinoma of the bile duct, and intraductal growth type CCA[5, 6].

***Epidemiology, risk factors and clinical features***

IPNB is a quite uncommon disease with a prevalence of 5-15% among bile duct tumors and mainly reported in series from East Asia[22, 27-36], in relation to specific and common risk factors identified such as hepatolithiasis and liver parasitic infections (*Clonorchis Sinensis* and *Opistorchis Viverrini*)[2, 4].

Other series described the association of IPNB with hepatocellular carcinoma[37], primary sclerosing cholangitis [38] and mixed adeno-neuroendocrine carcinoma[39].

Conversely, IPNB has been described only in isolated reports from Europe with a propensity to a more invasive course and an extra-hepatic location, in comparison to Eastern Centers[5, 40-42].

Similarly, previous reports describing the association of IPNB with IPMN-P were extremely sporadic as summarized in Table 1[9-19].

IPNB tends to be more frequent in male patients over 65 years old, presenting clinically with right upper abdominal discomfort/pain, jaundice, and cholangitis, though 5 to 29% of patients might be asymptomatic[2, 43].

Elevation of total and direct bilirubin, alkaline phosphatase (ALP), gamma-glutamyl transferase (γ-GTP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are common laboratory findings, while increase in tumor markers, i.e. CEA and CA19-9, has been documented in approximately 25% and 40% of patients, respectively[43].

Though the mucin produced by IPNB is usually retained inside the neoplastic cells, in up to 1/3 of cases, elevated quantities of mucin are secreted into the bile duct lumen leading to intermittent obstruction of the bile flow with consequent upstream and downstream duct dilatation[5]. This particular type of IPNB has been named by some Authors intraductal papillary mucinous neoplasms of the bile duct[5].

## HISTOLOGIC AND MACROSCOPIC FEATURES

### *Histologic aspects*

No precise criteria for grading the biliary epithelium dysplasia within the IPNB have been yet established. Some Authors divided the IPNB into four types, based on the worsening degree of dysplasia, ranging from type 1, i.e. low grade, to type 4, i.e. stromal invasion of adenocarcinoma[2, 5], whereas others classified the IPNB exclusively into non-invasive and invasive lesions, including adenoma, borderline tumor, and carcinoma *in situ* in the first group and tubular or mucinous adenocarcinoma in the other group[36, 43].

Invasive carcinoma arising from IPNB have been described in a range between 30% and 75%, according to the different surgical series, with a rate of lymph-node metastasis of 9-15% at the time of surgical resection [2, 27-29, 44] and overall a better prognosis when compared to normal CCA[2, 4, 21, 22, 28, 42, 45, 46].

Anatomical location and geographical distribution of IPNB have been correlated with the risk of stromal invasion, reported higher in those tumors originating at the level of extra-hepatic bile ducts and in those affecting Caucasian patients, suggesting a more

indolent course of intra-hepatic IPNB and of those occurring in Asian countries[2, 3, 28, 44, 47].

Nakamuna *et al* recently reviewed the pathologic features of invasive carcinoma associated with IPNB, identifying three different patterns of increasing invasiveness: A, B and C, the first showing a similar favorable postoperative overall survival (OS) to non-invasive forms, in comparison to the latter two, that might be considered clinically advanced entities[48].

The neoplastic epithelia of the IPNB is categorized into four histologic phenotypes according to the hematoxylin and eosin staining: pancreatobiliary (PB), intestinal, gastric, and oncocytic[3, 4, 49] as shown in Figure 1.

The PB type consists of columnar or cuboidal cells with eosinophilic cytoplasm, round hyperchromatic nuclei and scarce mucinous appearance. This variant is usually immunohistochemically positive for MUC1, MUC5AC, CK7 and for S100P[3, 4, 49].

The intestinal type resembles a colo-rectal villous neoplasm, showing columnar cells with cigar-shaped nuclei, basophilic cytoplasm and variable amount of sopranuclear mucin. Immunohistochemically, the cells express MUC2 and MUC5AC but not MUC1[3, 4, 49].

The gastric type features the gastric foveolar epithelium, consisting of tall columnar cells with basally oriented nuclei and abundant cytoplasmic mucin, generally associated with pyloric glands. While the latter results typically positive for MUC6, the foveolar portions frequently express MUC5AC and CK7 but rarely MUC1 and MUC2[3, 4, 49].

The oncocytic type presents convoluted and branching papillae lined by one or different layers of cuboidal/columnar cells with hyperchromatic, round and large nuclei and abundant, intensely eosinophilic cytoplasm, consistently expressing MUC5AC and focally MUC1 and/or MUC2[3, 4, 49].

The PB and intestinal patterns are the most common and usually associated with invasive lesions while the remaining two histologic forms are quite uncommon and generally presenting with a indolent course[3, 4, 49] (Figure 2).

*Macroscopic and radiologic aspects*

Four morphological subtypes have been recognized based on the gross pathological picture: polypoid, superficial-spreading, cystic, and cast-like[5, 49].

The polypoid type describes an intraductal lesion, pedunculated or sessile, sometimes reaching great dimensions. Differential diagnosis involves mainly the typical CCA and bile duct stones when tumor pieces disintegrate inside the bile ducts[5, 49].

The superficial spreading type depicts a tumor barely visible on imaging that spreads along varying lengths of the bile ducts. Radiologically, it appears as an isolated bile duct dilatation without an obvious obstructing lesion, secondary to copious mucin production by the tumor. Any biliary obstruction leading to bile duct dilatation can mimic this form, though a real stricture cannot generally be identified in this variant of IPNB[5, 49].

The cystic type develops as a focal cystic dilatation of a bile duct, that maintains the communication with the lumen of the adjacent bile duct, thus configuring actually the pattern of a pseudocyst. This feature allows to differentiate the cystic type IPNB from the mucinous cystic neoplasms (MCN), i.e. cystadenomas and cystadenocarcinomas, where, in absence of a luminal communication, mucin secretion is confined within the neoplastic cyst. In addition, the presence of ovarian-like stroma is necessary to diagnose MCN[5, 49].

Lastly, mural nodules or mucin aggregates are common findings in this form of IPNB[5].

In the cast like type, the tumor occupies the lumen of the bile duct, expanding along the longitudinal axis. Though radiologically it appears as a cast-like lesion, histologically it presents generally as a polyp, with only a small attachment to the biliary epithelium[5, 49].

This macroscopic classification presents some common elements with the classification of mucin-producing CCA proposed by Sakamoto *et al*[50] in 1997, which included the duct-ectatic, cystic, and intermediate types.

In the first group, papillary tumors developed within diffusely dilated intrahepatic ducts, whereas in the second, large cystic lesions with papillary projections were found inside the liver, communicating with intrahepatic bile ducts and in the latter pattern, large cystic lesions were intermingled with solid tumors invading the liver parenchyma[50].

Histologically, all the CCA were papillary adenocarcinoma, demonstrating a superficial spread along the bile duct mucosa in almost half of cases[50].

## CLASSIFICATIONS

Recently, Kim *et al*[45] resumed the duct-ectatic and cystic subgroups of intrahepatic IPNB (I-IPNB) that together accounted for the major portion of tumors in their surgical series (68.5%), while the remaining were represented by extrahepatic (26.6%) and diffuse (4.1%) forms, the latter including lesions diffusely affecting both the intra and extrahepatic bile ducts.

In this "modified anatomical classification", as named by the Authors, no significant difference in terms of overall survival was recorded among the three groups[45].

Another radiological-pathological classification was introduced by Luvira *et al*[51] in 2017. According to the Authors, five different categories of IPNB were described in their series of 103 patients: I, classical intrahepatic IPNB with an intraductal tumor determining unilateral duct dilatation; II, extrahepatic IPNB (E-IPNB) with an intraductal tumor producing bilateral intrahepatic duct dilatation; III, cystic lesion with a papillary tumor inside, communicating with the lumen of the bile duct; IV, micropapillary tumor causing a disproportionate bile duct dilatation without a clear lesion radiologically evident; V, mass forming tumor with macroinvasion[51].

Their analysis concluded that the category of IPNB and the presence of lymph-node metastasis represent the only significant prognostic factors, with class II and V showing the worse OS[51].

### 4.1 Novel subclassification in type 1 and type 2 IPNB

In 2020, the Japan Biliary Association (JBA) and the Korean Association of Hepato-Biliary-Pancreatic Surgery (KAHBPS), after retrospectively reviewing the clinico-pathological data of 694 patients who had undergone surgery for IPNB over a 20 year-period, proposed new histo-pathological diagnostic criteria and identified two main types of IPNB: 1 and 2[28].

Indeed, histo-pathologically, while the type 1 showed a regular growth with papillary, villous or tubular homogenous structures, thin papillary fibrovascular stalks and large amount of mucin, the type 2 displayed a non-homogenous appearance and an irregular growth with complicated structures, such as cribriform, compact tubular, solid or large cystic components and rare mucin overproduction[28] (Figure 3).

In addition, associated invasive carcinoma was reported in almost all cases of type 2 Lesions (93.6%), while low-intermediate or high-grade dysplasia were observed in 9.7% and 40.2% of type 1 Lesions, respectively, with approximately 50% of these exhibiting stromal invasion[28].

The intestinal and gastric histological subtypes were generally associated with the type 1, while the incidence of PB subtype was significantly higher in the second group[28].

Clinically, the type 1, representing the major portion (75%) of tumors reviewed in this multi-center analysis, tended to be more frequent at the level of intra-hepatic bile ducts, conversely the other type tended to develop inside the extra-hepatic ducts leading to significantly higher levels of bilirubin, ALP, γ-GTP, ALT, AST and tumor markers (CEA and Ca19-9) with increased occurrence of liver dysfunction, jaundice and pain[28].

Radiologically, type 1 IPNB presented typically with the aspects of extensive cystic duct dilatation or intraductal cauliflower-like lesion whereas intraductal solid mass determining upstream duct dilatation was the classic pattern of the type 2[52].

Lastly, the above-mentioned study highlighted how the bile duct margin status after surgical resection did not impact on the OS and disease-free survival (DFS) in both groups, as well as no significant difference in the recurrence rate (RR) was observed between the type 1 and 2, though lymph-node metastasis rate was significantly higher in the latter[28].

Nevertheless, patients with type 2 IPNB presented a significantly lower OS and DFS at 1, 3 and 5 y, when compared to type 1 IPNB, leading the Authors to conclude that their new classification might be strongly predictive of the patient outcome[28].

Though the above-mentioned binary classification was included in the last WHO classification of the Digestive System Tumors[53], the differential diagnosis between these two types of IPNB has resulted challenging, particularly in the hands of not-experienced pathologists.

Accordingly, Onoe *et al*[36] developed a scoring system based on six pathological features aimed at helping in differentiating between type 1 and 2 IPNB; accordingly, lesions with a total score of 5-6 can be categorized as type 1, whereas those with a score of 0-1 and of 2-4 as type 2 or unclassifiable, respectively.

The Authors confirmed the findings of a prevalent intrahepatic location of type 1 IPNB in comparison to the extrahepatic site for type 2 and the survival rates being higher in the first group compared to the type 2 or unclassifiable lesions[36].

In addition, as other Authors suggested[2, 52], the type 1 IPNB might be considered the real biliary counterpart of IPMN-P, particularly the main-duct variant, while the type 2 might be identified as a PCC, indicating that papillary bile duct tumors occupy a single continuous spectrum, ranging from less advanced forms, i.e. type 1, to more advanced forms, i.e. type unclassifiable and type 2.

Furthermore, the geographic distribution has been reported divergent between the type 1 and 2, being the former predominant in Asia and the latter conversely in Europe and in the USA[52, 54].

## DIAGNOSIS: CROSS SECTIONAL IMAGING, ENDOSCOPY AND CHOLANGIOSCOPY

Routine diagnostic modalities employed in IPNB are represented by computed tomography (CT) and magnetic resonance imaging (MRI), commonly showing intraductal masses associated with bile duct dilatation[49].

At CT, IPNB generally present an enhancement pattern isointense or hyperintense during the late arterial phase with sometimes an intense rim enhancement at the base of lesions, while at MRI, IPNB tend to be hypointense on T1-weighted images and hyperintense on T2-weighted images, carrying the advantage of identifying small intraductal or multiple tumors[2, 4, 43] (Figure 4).

Lee *et al* recognized significant features at MRI useful in differentiating IPNB with an associated invasive carcinoma from non-invasive forms, represented by an intraductal visible mass, tumor size ≥ 2.5 cm, multiplicity of the tumor, adjacent organ invasion and bile duct wall thickening, the last one displaying the highest diagnostic accuracy[20].

However, the superficial spread and progression of IPBN along the bile duct mucosa might be underestimated at conventional imaging[21]. For this reason, intraductal ultrasonography (IDUS) and direct cholangioscopy play a central role in assessing these parameters, i.e. the depth and extent of invasion, and in performing a biopsy of the lesion[2, 21].

Nevertheless, the discrepancy between the preoperative biopsy and the definitive histology after surgical resection might be notable, particularly in E-IPNB, with a reported negative predictive value of approximately 40%, meaning that around 60% of patients with a preoperative diagnosis of non-malignancy actually had an invasive carcinoma at definitive pathology[47].

Since these two methods have been widely applied in the evaluation of IPNB, the current applications of endoscopic retrograde cholangiography (ERCP) are very limited, apart from determining the presence of mucobilia or a direct communication between a cystic lesion and the bile duct[4] as displayed in Figure 5.

Therefore, classifications of IPNB based on their cholangiographic patterns, such as the one proposed by Yeh *et al*[55], have no longer a significant clinical utilization.

**High-risk stigmata of malignancy**

Few studies have focused their attention on the analysis of possible features that might anticipate the risk of invasiveness and/or malignant degeneration in IPNB[29, 47]. In a recent large surgical series, the presence of a CEA > 5 IU/mL, a CA19-9 > 37 IU/L, a

mural nodule > 12 mm, intrahepatic calculi and lymph-nodes enlargement were identified as potential predictors of malignancy in I-IPNB, while a total bilirubin > 3 mg/dL, enhancement of mural nodules and CA19-9 > 37 IU/L as potential risk factors in E-IPNB[47].

However, at the multivariate analysis, the mural nodule > 12 mm (RR: 5.33, 95%CI: 1.05–26.89, $P$ = 0.043) and the enhancement of mural nodules (RR: 19.08, 95%CI: 1.08–335.5, $P$ = 0.044) were confirmed as the only significant predictors of malignancy in I- and E-IPNB, respectively[47].

Interestingly, these two features were identified as absolute surgical indications in IPMN-P, according to the 2018 European Guidelines and the 2017 International Consensus Guidelines[56, 57].

## TREATMENT

Based on the remarkable frequency of high-grade dysplasia or invasive carcinoma and of symptoms reported in IPNB and on the poor sensitivity of preoperative biopsy, particularly in invasive forms, treatment should be considered mandatory, with surgery representing the main therapeutic option[21].

### 7.1 Surgical resection

The type and extent of surgical resection depends on the location of IPNB, with bile duct resection or pancreatico-duodenectomy applied to E-IPNB and hepatic resection to I-IPNB. Regional lymphadenectomy should be always carried out since incidence of regional lymph-node metastasis is estimated in the range of 9-15%[2, 27-29, 44].

R0 resection was reportedly achieved in 90% of patients[4] and the presence of residual disease, including dysplasia, in the resection margin should indicate further resection, though specific guidelines are not available[21].

Indeed, according to Uemura *et al*[30] and Luvira *et al*[58], only the bile duct margin positive for carcinoma represented a significant poor prognostic factor, while Jung *et al*[59] reported the presence of any dysplasia, even if low, in the bile duct margin as a significant

element impacting the OS and DFS to the same extent of carcinoma in situ or invasive carcinoma.

Similarly, Youn *et al*[29] identified R1 resection, though not specifying if any dysplasia or carcinoma, as the most important factor for a poor outcome in terms of OS and RR, together with high serum levels of CA19.9 (>37 IU/mL).

Conversely, as emerged in the large surgical series of Kubota *et al*[28], the status of the surgical margin, positive *vs* negative, did not influence the OS, DFS and RR in the two different groups of type 1 and 2 IPNB, indicating that this parameter does not affect the prognosis.

**7.2 Role of liver transplantation**

Regarding other types of surgical treatment, liver transplantation might be considered as the only definitive and curative treatment in patients with extensive superficial spread along the bile duct mucosa, bilobar disease or with positive surgical margins, even after multiple resections, though only limited experiences have been described so far in literature[60-63].

**7.3 Other therapeutic approaches**

In non-surgically fit patients, some palliative treatments, such as percutaneous transhepatic biliary drainage, biliary stenting and endoscopic approaches including cholangioscopic electrocoagulation, submucosal resection, argon plasma coagulation (APC), radiofrequency ablation (RFA) and transpapillary intraluminal radio-therapy (TIRT), have been reported[23, 24, 64-68].

Among the endoscopic treatments described, the IPNB lesion can be resected through the methodic of electrocoagulation using a high frequency electric needle knife that directly targets and destroys the tumor tissue(68), through the classical method of submucosal resection by using polypectomy snares(23), through APC that dehydrates the tissue by blocking the blood flow into the lesion and dries the area around(64-66) and through RFA by inducing coagulative necrosis of the neoplastic tissue(24).

In particular, the advantage of the electrocoagulation technique relies on its concomitant utilization with cholangioscopy(68), allowing to reach intrahepatic lesions, differently from the other techniques that are employed by classical endoscopy. Alternatively, TIRT with high dose of iridium-192 can be delivered directly into the lesion by using a ultrathin flexible probe; response to treatment can be easily monitored through conventional imaging and sessions can be repeated according to treatment response(67).

## PROGNOSIS AND SURVIVAL

Few reports exist in literature investigating the natural evolution of non-treated IPNB. In the series of Han *et al*[47], among 196 pt with IPNB, only 16, of whom 9 with I-IPNB and 7 with E-IPNB, did not undergo surgery and were observed during a median period of approximately 3 years. Of those with intrahepatic lesions, 55.5% experienced a malignant transformation *vs* 28.5% of those belonging to other group, 33.3% were admitted due to cholangitis *vs* 57% with E-IPNB and the remaining did not develop either malignancy or any type of symptoms related to IPNB.

Though survival data varies widely among different series, the different studies generally agree on the concept of a significantly improved OS and DFS for surgically resected IPNB compared to conventional CCA, with an associated decreased rate of lymph-node and distant metastasis[2, 4, 22, 42, 45, 46].

Several factors have been reported to be significant predictors of outcome after surgical resection of IPNB, although most of them have not been well established or are still controversial[27, 28, 30, 45, 46, 58, 59].

For example, Rocha *et al*[7], already in 2012, reported the depth of invasion (≥ 5 mm, < 5 mm, or none) and the percentage of invasive carcinoma components (≥ 10%, < 10%, or none) as the main significant prognostic factors, with patient OS inversely proportional to the grade of invasion and to the proportion of malignancy detected in the IPNB.

Similarly, Onoe *et al*[69], investigating 184 pt with PCC, documented the presence of >50% invasive components, defined as PCC-4, as an independent predictor of survival that approached the 5 y OS of patients with non-papillary cholangiocarcinoma (NPCC).

The Authors concluded that, although IPNB might be nosologically applied only for PCC cases with <50% invasive component, their prognostic classification of PCC according to the proportion of invasive components indicated that all subgroups belonged to a singular disease group[69].

On the other hand, the classification of IPNB in type 1 and 2, according to their clinical and histo-pathologic features, has been generally recognized as a significant predictor of survival[27, 28, 30], independently from the grade of dysplasia and/or presence of invasive carcinoma, with a 1-, 3-, 5-, and 10-year OS for Type 1 IPNB of 96.1%, 85.2%, 75.2% and 58.5%, respectively, and for Type 2 IPNB of 94.6%, 69.1%, 50.9% and 26.8%, respectively (P<0.0001), while the 1-, 3-, 5-, and 10-year DFS for Type 1 were 88.3%, 73.8%, 64.1%, and 52.2%, respectively, and for Type 2 IPNB were 81.0%, 48.0%, 35.3%, and 25.8%, respectively (P<0.0001)[28].

For all these features, type 1 IPNB might be considered the real biliary counterpart of IPMN-P, while PCC might be included in the subgroup of type 2 IPNB, as stated above[2, 52].

Likewise, Matsumoto *et al* identified the I-IPNB as those with more regular histo-pathologic characteristics and favorable prognosis similar to the type 1 in comparison with E-IPNB that conversely might resemble the type 2[44].

Another significant poor prognostic factor recognized in several studies is represented by the presence of lymph node metastasis, carrying an increased risk of loco-regional recurrence[27, 28, 30, 45, 51].

Controversial results in terms of prognosis and survival have emerged from the analysis of other distinctive features of IPNB, such as the macroscopic appearance, the histologic subtype, the immunohistochemical phenotype and the level of CA19-9[27, 28, 30, 42, 45-47, 51, 58, 59].

## GENETIC CHANGES IN IPNB AND TARGETED THERAPIES: THE NEW FRONTIERS

Recent genetic analysis of IPNB have identified several mutations involving different oncogenes and oncosuppressor genes, such as KRAS, BRAF, GNAS, RNF43, TP53, APC, CTNN B1, ZNRF3, CDKNZA and SMAD4, whose altered expression is strictly related to the different immunohistochemical patterns observed[70-73].

Accordingly, at least three main signaling pathways have been identified in the cancerogenesis of IPNB: the RAS-MAPK, controlled by the KRAS and BRAF oncogenes; the WNT-β-catenin, regulated by the oncogene CTNNB1 and by two oncosuppressor genes, APC and ZNRF43; and the GPCR-CAMP, activated by mutations of the oncogene GNAS[70-73].

In particular, since mutations affecting the APC and CTNNB1 genes have been rarely detected in IPMN-P, the WNT-β-catenin pathway appears to have a unique role in the molecular alterations underlying the neoplastic transformation of the biliary epithelium, acting in the early phases of carcinogenesis, whereas dysregulation of other signaling pathways, peculiarly the RAS-MAPK, have been described in different neoplasms, such as the colo-rectal, the pancreatic and the lung cancer[70-73].

In addition, KRAS and GNAS mutations were frequently identified in type 1 IPNB, often of intestinal subtype, that, as already mentioned, share several clinico-pathological features with IPMN-P, while mutations in the APC oncosuppressor gene and in the CTNNB1 oncogene, i.e. the WNT-β-catenin pathway, were generally described in type 2, often associated with the pancreatobiliary subtype[70-73].

These genetic studies confirm that IPNB consist of at least two distinct entities, and that the type 1 and 2 sub-classification, recently introduced by the JBA and the KAHBPS, may reflect these genetic subcategorizations[70-73].

For all these reasons, recent research has focused its attention on these deregulated signal pathways, especially the WNT-β-catenin, identifying several targeted agents currently under-evaluation in clinical trials and pre-clinical studies applied to solid and hematologic neoplasms[74, 75].

Recently, preliminary valuable results have emerged from the NCT02675946 and NCT03507998 clinical trials evaluating the efficacy and safety of the WNT pathway

porcupine inhibitor CGX1321 in advanced gastro-intestinal neoplasms, including CCA, though, at the moment of writing, none of these targeted therapies have been adopted in the field of IPNB[76].

## CONCLUSION

IPNB are newly-recognized pre-invasive neoplasms of the bile duct with high malignant potential and a tendency to evolve to invasive CCA.

Though several classifications have been proposed over the past ten years, the recently introduced sub-classification in type 1 and type 2 appropriately highlights the histopathologic and clinical aspects of IPNB, their natural evolution with a particular focus on prognosis, OS, DFS and similarities/discrepancies with IPMN-P.

In relation to their complexity, advanced techniques employed in a multimodal approach are needed for a correct diagnosis and precise identification of their locations, extension, and pathological degree.

Surgery with a radical intent represents the most appropriate treatment, though different modalities, mainly consisting in endoscopic approaches, can be considered in non-surgically fit patients.

Genetic analysis of the specific mutations driving the stepwise progression of neoplastic biliary epithelium might represent an innovative research field in terms of targeted therapies, particularly those implicating the WNT-β-catenin pathway.

**Figure Legends**

Figure 1 Histologic subtypes of IPNB. (A) Pancreatobiliary (B) Intestinal (C) Gastric (D) Oncocytic Hematoxylin and eosin staining.

Figure 2 Histologic features of IPNB with irregular growth pattern and foci of invasive carcinoma (pancreatobiliary type). (A) Immunostaining positive for CK19 (B) Immunostaining negative for CDX2 (C) Immunostaining negative for CK20.

Figure 3 Subclassification of IPNB according to the Japan Biliary Association and the Korean Association of Hepato-Biliary-Pancreatic Surgery (A) Type 1 consists of papillary, villous or tubular homogenous structures with thin papillary fibrovascular stalks (B) Type 2 consists of thick papillary glands with irregular branching, often intermingled with solid irregular components. Hematoxylin and eosin staining.

Figure 4 Magnetic resonance imaging of IPNB (A) Magnetic resonance colangio-pancreatography showing an intraductal lesion of the left hepatic duct with upstream bile duct dilatation (B) Diffuse dilatation of the intrahepatic left lobe bile ducts with low intensity tumors (T2 weighted image, coronal section) (C) Magnetic resonance colangio-pancreatography showing a cystic type IPNB (D) Cystic type IPNB (T2 weighted image, coronal section).

Figure 5 Endoscopic retrograde colangio-pancreatography of an intrahepatic IPNB showing a direct communication between the cystic lesion and the bile duct.

# 86108.docx

ORIGINALITY REPORT

8%
SIMILARITY INDEX

PRIMARY SOURCES

| | | |
|---|---|---|
| 1 | Keiichi Kubota, Jin‐Young Jang, Yasuni Nakanuma, Kee‐Taek Jang et al. "Clinicopathological characteristics of intraductal papillary neoplasm of the bile duct: a Japan‐Korea collaborative study", Journal of Hepato-Biliary-Pancreatic Sciences, 2020<br>Crossref | 73 words — 1% |
| 2 | Mondal, D., M.A. Silva, Z. Soonawalla, L.M. Wang, and H.K. Bungay. "Intraductal papillary neoplasm of the bile duct (IPN-B): also a disease of western Caucasian patients. A literature review and case series", Clinical Radiology, 2016.<br>Crossref | 57 words — 1% |
| 3 | www.mdpi.com<br>Internet | 51 words — 1% |
| 4 | rcastoragev2.blob.core.windows.net<br>Internet | 40 words — 1% |
| 5 | Marek Krawczyk, Bogna Ziarkiewicz-Wróblewska, Joanna Podgórska, Jakub Grzybowski et al. "Intraductal papillary neoplasm of the bile duct – A comprehensive review", Advances in Medical Sciences, 2021<br>Crossref | 39 words — 1% |
| 6 | www.wjgnet.com<br>Internet | 34 words — 1% |

| | | |
|---|---|---|
| 7 | www.ajronline.org<br>Internet | 28 words — 1% |
| 8 | Shoji Kubo, Shogo Tanaka, Masahiko Kinoshita, Hiroji Shinkawa, Takeaki Ishizawa, Yasunori Sato. "Development of intraductal papillary neoplasm of the bile duct in patients with occupational cholangiocarcinoma", Virchows Archiv, 2023<br>Crossref | 24 words — < 1% |
| 9 | Yasutaka Aoki, Masamichi Mizuma, Tatsuo Hata, Takeshi Aoki et al. "Intraductal papillary neoplasms of the bile duct consist of two distinct types specifically associated with clinicopathological features and molecular phenotypes", The Journal of Pathology, 2020<br>Crossref | 19 words — < 1% |
| 10 | academicjournals.org<br>Internet | 16 words — < 1% |
| 11 | nagoya.repo.nii.ac.jp<br>Internet | 15 words — < 1% |
| 12 | www.karger.com<br>Internet | 15 words — < 1% |
| 13 | Yoh Zen. "Biliary intraepithelial neoplasia: an international interobserver agreement study and proposal for diagnostic criteria", Modern Pathology, 06/2007<br>Crossref | 13 words — < 1% |
| 14 | "Gastrointestinal Malignancies", Springer Science and Business Media LLC, 2016<br>Crossref | 12 words — < 1% |
| 15 | Shunsuke Onoe, Tomoki Ebata, Yukihiro Yokoyama, Tsuyoshi Igami et al. "A | 12 words — < 1% |

clinicopathological reappraisal of intraductal papillary neoplasm of the bile duct (IPNB): a continuous spectrum with papillary cholangiocarcinoma in 181 curatively resected cases", HPB, 2021

Crossref

| | | | |
|---|---|---|---|
| EXCLUDE QUOTES | ON | EXCLUDE SOURCES | < 12 WORDS |
| EXCLUDE BIBLIOGRAPHY | ON | EXCLUDE MATCHES | < 12 WORDS |