Prognostic Delineation of Papillary Cholangiocarcinoma Based on the Invasive Proportion: A Single-Institution Study with 184 Patients

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Abstract

Background: Intraductal papillary neoplasm of the bile duct (IPNB) is a presumed precursor lesion in biliary carcinogenesis, clinicopathologically overlapping with papillary cholangiocarcinomas (PCC); however, as IPNB has no standardized definition, this relationship remains equivocal. Here, we aimed to develop a new PCC prognostic model, focusing on the invasive proportion.

Methods: Among 644 patients with surgically resected cholangiocarcinoma (1998-2011), 184 (28%) had intraductal exophytic papillary lesions. These were divided into four subsets based on the invasive component: non-invasive (PCC-1, n = 14), ≤10% (PCC-2, n = 32), 11-50% (PCC-3, n = 60), and >50% (PCC-4, n = 78). The remaining 460 were identified as non-papillary cholangiocarcinomas (NPCC).

Results: Invasion beyond the ductal wall and regional lymph node metastases were more frequent in NPCC than PCC ($P < 0.001$ for both). Five-year survival was better for PCC (55%) than NPCC (35%; $P < 0.001$), indicating the papillary component to be a significant independent prognosticator. PCC-4 and NPCC had similar clinicopathological features and overlapping survival curves: 33% and 35% at 5 years ($P = 0.835$), both lower than those of PCC-1, PCC-2, and PCC-3 (respectively, 92%, 74%, and 64% at 5 years; $P < 0.005$ in all combinations). Multivariate analysis in PCC showed >50% invasive component, nodal metastasis, and positive surgical margin as independent predictors.

Conclusion: PCC survival decreased with progression of the invasive component. PCC with >50% invasive component was clinicopathologically similar to NPCC. Although IPNB might be nosologically applied only for PCC cases with ≤50% invasive component, the present prognostic delineation indicated that all PCC subgroups belonged to a singular disease group.

Introduction

Papillary cholangiocarcinomas (PCC) are associated with better outcomes compared to nodular-sclerosing tumors [1-5]. PCC were recently divided into different classes of lesions, including mucin-producing bile duct tumors (MPBT) [6-9] and intraductal papillary neoplasm of the bile duct (IPNB) [10, 11], which have a favorable prognosis and may represent an alternative carcinogenesis pathway in the biliary tract [7-9, 11-13]. Radiographic studies of MPBT show apparent mucin production and a cystic or ductectatic tumor growth pattern [6, 8], and microscopic analyses show papillary adenocarcinoma to be the predominant histologic type. MPBT has communications with the bile duct, and superficial spread of the cancer is often observed [6-9]. On the other hand, IPNB is characterized as an intraductal papillary lesion, involving proliferation of atypical biliary epithelium along with delicate fibrovascular cores; IPNB is proposed to be the biliary counterpart of intraductal papillary mucinous neoplasms of the pancreas (IPMN-P) [10, 11, 14]. About one-third of IPNBs secrete mucin in the duct lumen [11, 14-17]; these mucin-producing IPNBs overlap with MPBT.

IPNB lacks a standardized definition, and thus remains an equivocal disease entity in PCC, clinically including a range from cholangiocarcinomas with intraductal epithelial lesion to invasive cancer [10, 11]. The 2010 WHO classification [14] describes “IPNB with low to high-grade intraepithelial neoplasia” and “IPNB with an associated invasive carcinoma”; however, it gives no suggestions for distinguishing invasive IPNB from nodular-sclerosing cholangiocarcinoma with focal papillary component [11, 14]. This distinction has also not been addressed by several recent reports on large series of IPNB [12, 13, 17-19] (Table 1). Furthermore, IPNB patients from Eastern and Western countries appear to have different clinical backgrounds (Table1). Recent large case series of this peculiar disease in exclusively Asian countries showed higher incidences of hepatolithiasis and liver flukes compared with Western series [12, 13, 17-20]. Additionally, all or most IPNB cases in Western series were diagnosed as malignant tumors or carcinomas *in situ* [13, 20], while the large Asian series included a substantial

number of benign or borderline lesions [12, 17-19].

To our knowledge, the present study includes the largest number of PCC cases of any report in the English literature to date. Here, we analyzed the clinical and histopathological features of these PCC cases in a group of consecutive patients surgically treated at a single institution. The aim of this study was to develop a new PCC prognostic model, focusing on the invasive proportion, and to further our understanding of IPNB.

Methods

Patient Selection

From 1998 to 2011, a total of 644 patients with intra- or extrahepatic cholangiocarcinoma underwent surgical resection at the First Department of Surgery, Nagoya University Hospital. From these patient records, we retrospectively surveyed the patients with PCC and reviewed the histologic slides of all patients with histopathologically confirmed cholangiocarcinoma. The institutional review board of Nagoya University approved the study protocol. PCC were defined as tumors characterized, at least focally, by exophytic proliferation of neoplastic papillary epithelium within the bile duct lumen, with or without an invasive component [5]; they included nodular-sclerosing cholangiocarcinomas with focal papillary component as well as IPNB.

Based on these definitions, 184 patients with intraductal papillary lesions were retrieved from the entire cohort. After reviewing slides, the pathologist drew a map of the entire lesion of PCC. Two authors (Y.S. and S.O.) semiquantitatively measured the percentage tumor volume occupied by the invasive component out of the entire PCC lesion. PCC were divided into non-invasive (PCC-1; n = 14; Figure 1 A, B) and invasive carcinomas (n = 170); the latter were further divided into three subgroups having invasive components of ≤10% (PCC-2; n = 32; Figure 1 C, D), 11-50% (PCC-3; n = 60; Figure 1 E, F), or >50% (PCC-4; n = 78; Figure 1 G, H). The remaining 460 of the 644

resected cases were identified as non-papillary cholangiocarcinoma (NPCC), and their characteristics and survival were compared with those of the PCC cases.

We found no benign biliary neoplasms (i.e., adenomas and papillomatoses); thus, all papillary tumors treated in our institution were malignant neoplasms. Furthermore, biliary cystadenomas and cystadenocarcinomas, and any papillary lesions of ampulla of Vater or gallbladder were excluded from this study. No patients in this study exhibited an ovarian-like stroma, which is associated with hepatic mucinous cystic neoplasms [14, 20].

Pathological Assessment

All cystic lesions in this study were confirmed to have anatomical communication with the bile duct; if bile duct communication could not be found before the operation, it was confirmed after the operation by specimen cholangiography. Next, the extrahepatic bile duct was opened longitudinally, from the distal resection margin up to the proximal margin. The specimens were investigated, and we recorded the main location of the tumor epicenter, tumor size, morphology, hepatolithiasis, and macroscopically visible mucin secretion. The main location around the hepatic hilum was defined according to our previous study on the definition of perihilar cholangiocarcinoma [21]. The border between the perihilar and distal extrahepatic bile duct was set at the level of the cystic duct [22].

For all 644 patients, surgical specimens of cholangiocarcinoma were cut at intervals of 0.5 to 1.0 cm, making 10 to 40 blocks for each patient. We macroscopically confirmed the spread of the tumors, and cut around them as much as possible. The pathological findings were described using the TNM Classification of Malignant Tumors by the International Union Against Cancer (7th edition, 2009) [22], applying classifications of intrahepatic, perihilar extrahepatic, and distal extrahepatic bile ducts where appropriate according to the location. According to the WHO classification [14], we

identified the histological types and the epithelial subtypes. Based on the component of predominant histological type in the intraductal and invasive component, each tumor was classified as papillary, mucinous, tubular adenocarcinoma, and so on. The epithelial subtypes were classified as gastric, intestinal, pancreatobiliary, and oncocytic, as applied in earlier studies of IPNB [11, 13, 16, 19, 23]. Invasive carcinomas were classified as mucinous if they consisted of >80% large pools of extracellular mucin containing relatively scant neoplastic cells in strips, clusters, and individual cells. Mucin secretion was judged by pathologic examination and by the cut surface of the specimens. Superficial extension was defined as non-invasive carcinoma spread beyond the mass [24-26]. PCC invasive patterns were divided into two types: pushing and infiltrating growth margins. Positive margins with non-invasive cancer were treated as negative margins in the present study, since residual carcinoma *in situ* does not impact survival [26, 27].

All histologic slides were reviewed twice by two authors (Y.S. and S.O.) who were blinded to clinical information. In occasional instances of discrepancy, cases were discussed at a multi-headed microscope to achieve consensus.

Immunohistochemistry

All cases of PCC were subjected to immunohistochemical staining. Formalin-fixed paraffin sections were analyzed using immunoperoxidase staining via an avidin-biotin peroxidase complex method. The following monoclonal antibodies were used: MUC1, MUC2, MUC5AC, MUC6, HGM (Novocastra Laboratories, Newcastle, UK), Ki-67, CK7, CK20, p53 (DAKO, Glostrup, Denmark), and CDX2 (Bio Genex, San Romon, CA). The antibodies were used after antigen retrieval following microwave oven heating treatment—with the exception HGM, which required proteolytic digestion.

The expressions of MUC1, MUC2, MUC5AC, MUC6, HGM, CDX2, CK7, and CK20 were considered positive when more than 10% of the tumor cells stained positive. For Ki-67 and p53, the

nuclear labeling index was determined by counting the number of heavily labeled nuclei per 1000 cells.

Follow-up and Statistical Analysis

Patient survival was determined from the time of surgery to the time of death or the most recent follow-up. The median follow-up periods were 53.0 months in PCC and 68.4 months in NPCC. Two of 184 (1%) PCC patients and 14 of 460 (3%) NPCC patients died of postoperative complications. Patients who died of causes other than postoperative complications were treated as censored cases.

Measurement values for continuous variables are expressed as the mean ± standard deviation or median (range). Characteristics of cases were compared using the Mann-Whitney U test for continuous variables and the χ^2 test for categorical variables. The Kruskal-Wallis test and Fisher's exact test were also applied where appropriate. Postoperative survival was calculated using the Kaplan-Meier method. Differences in survival curves were compared using the log-rank test. Univariate and multivariate survival analyses were performed using the Cox proportional hazards regression model. In the multivariate model, forward variable selection was used to find independent prognostic factors. The results were considered statistically significant if the *P* value was <0.05. All calculations were performed using the IBM SPSS Statistics 19 software package (IBM Japan Inc., Tokyo, Japan).

Results

Comparison Between PCC and NPCC

Distributions regarding age, sex, and surgical procedure were similar between patients with PCC and NPCC (Table 2). The mean ages for these two groups were 66.3 ± 10.0 and 64.8 ± 9.9,

respectively ($P = 0.050$). There were 116 men and 68 women with PCC, and 302 men and 164 women with NPCC ($P = 0.677$). In both groups, the most frequent procedure was left-sided hepatectomy, followed by right-sided hepatectomy, and pancreatoduodenectomy without hepatectomy ($P = 0.175$).

Microscopic features revealed significant between-group differences in most variables (Table 2). All PCC had a papillary component, with tubular adenocarcinoma observed predominantly in 131 of 184 (71%) patients. Predominant histological types in the remaining patients were papillary (n = 40, 22%) and mucinous adenocarcinoma (n = 13, 7%). Meanwhile, tubular adenocarcinoma was found in 450 of 460 (98%) NPCCs ($P < 0.001$). Regarding invasion depth, 33 of 184 PCCs (18%) were confined to the ductal wall, while the majority (455 of 460, 99%) of NPCC invaded beyond the ductal wall ($P < 0.001$). Regional lymph node metastases were observed more frequently in NPCC (224 of 460, 48%) than PCC (57 of 184, 31%) ($P < 0.001$), as were lymphovascular and neural invasions ($P < 0.001$ for all variables).

The survival of patients with PCC was superior to that of patients with NPCC (64.9% vs. 44.0% at 3 years, 54.7% vs. 35.3% at 5 years; $P < 0.001$) (Figure 2). The presence of an intraductal papillary component was an independent prognostic factor in both univariate ($P < 0.001$) and multivariate analysis (hazard ratio, 0.70; $P = 0.012$), as were venous invasion and nodal metastasis (hazard ratio, 1.53 and 2.34, respectively; $P < 0.001$ for both) (Table 3).

Comparison According to the Invasive Component in PCC

Table 4 shows the comparison of clinicopathological features between the PCC subgroups. Age and sex distributions were not significantly different between the four groups ($P = 0.597$ and 0.091, respectively). PCC-1 was most frequently observed in intrahepatic bile duct (43%), followed by perihilar extrahepatic (36%) with subclassifications into gastric and intestinal types (36% each).

In the other three groups, the perihilar extrahepatic bile duct was most commonly affected (PCC-2, 75%; PCC-3, 55%; PCC-4, 60%), and both intestinal (44%, 57%, 28%, respectively) and pancreatobiliary types (44%, 30%, 58%, respectively) were more frequent (location, $P = 0.003$; epithelial subtype, $P = 0.001$). No significant between-group differences were observed in the average tumor size, mucin secretion, and superficial extension ($P = 0.404$, 0.200, and 0.511, respectively). Notably, only six patients had intrahepatic stones, and no liver flukes were observed in the cohort.

Microscopic analyses revealed that PCC were more advanced when they had a greater invasive component. Regarding predominant histological type, papillary adenocarcinoma was more often detected in PCC-1 (71%) and PCC-2 (56%), while tubular adenocarcinoma was observed mainly in PCC-3 (78%) and PCC-4 (88%) ($P < 0.001$). The most frequent invasive pattern was pushing growth margin in PCC-2 (91%), and infiltrating growth margin in PCC-3 (68%) and PCC-4 (81%) ($P < 0.001$). Significant between-group differences were also noted in the following variables: depth of invasion, pathological T classification, lymphovascular/neural invasion, and regional lymph node metastasis ($P < 0.001$ each). Regarding these variables, PCC-4 was the most advanced, with gradual improvement seen between PCC-3, PCC-2, and PCC-1. Periaortic lymph node metastasis was only observed in PCC-4 (5 of 78, 6%), while the other groups had no distant metastasis. The clinicopathological features of PCC-4 were quite similar to those of NPCC.

Immunohistochemistry of PCC

Table 5 shows the immunohistochemical characteristics of PCC. MUC1 expression increased in parallel with increasing proportion of invasive component, resulting in significant between-group differences ($P < 0.001$). We also found statistically significant differences in expressions of p53 ($P = 0.037$) and MUC5AC ($P = 0.014$), with the highest expressions in PCC-3

(20%) and PCC-1 (86%), respectively. MUC2 was expressed similarly among the four subgroups ($P = 0.929$), and the other stains showed no significant between-group differences.

We further evaluated the expression patterns of MUC1 and MUC2 in each predominant histological type (Table 6). MUC2 was expressed by a majority of mucinous adenocarcinomas (9 of 13, 69%), and by very few papillary and tubular adenocarcinomas [5 of 40 (13%) and 18 of 131 (14%), respectively; $P < 0.001$]. No significant differences in MUC1 were observed among the three histological types. Comparing them in each invasive component, MUC1 expression increased in parallel with increasing proportion of the invasive component in all histological types. MUC2 was expressed more frequently in mucinous carcinoma of PCC-2 (100%), PCC-3 (100%), and PCC-4 (43%), and was detected in few cases of papillary and tubular adenocarcinoma (0-16%) in PCC-2, PCC-3, and PCC-4, resulting in significant differences in the former two ($P = 0.008$ and 0.004, respectively). The patterns of MUC1+/MUC2− and MUC1+/MUC2+ were associated with papillary/tubular- and mucinous-type invasion, respectively.

Survival of PCC

Survival rates of PCC-1 (91.7% at 5 years), PCC-2 (73.5%), and PCC-3 (64.2%) were significantly better than those of PCC-4 (32.7%; $P = 0.002$, <0.001, and <0.001, respectively) and NPCC (35.3%; $P = 0.004$, =0.001, and <0.001, respectively). PCC-4 and NPCC showed overlapping survival curves ($P = 0.835$; Figure 3). There were no significant differences in disease-specific survival among the former three categories (PCC-1 vs. PCC-2, $P = 0.376$; PCC-2 vs. PCC-3, $P = 0.340$; PCC-1 vs. PCC-3, $P = 0.117$).

Of the 15 five-year survivors in the groups with better survival (i.e., PCC-1, PCC-2, and PCC-3), two died of late recurrence. One had fibro-muscular layer invasion (T1N0M0, PCC-3) and died of local recurrence in the resected margin after 1859 post-operative days, even though all ductal

margins were negative. The other had invasion of the round ligament (T3N0M0, PCC-3) and died of liver metastasis and peritoneum dissemination after 2873 post-operative days, despite having undergone curative resection. In the PCC-4 group, five of the eight five-year survivors died of late recurrence.

Seventeen clinicopathological variables were analyzed as potential prognostic factors in the 184 PCC patients (Table 7). Univariate analysis showed that >50% invasive component, infiltrating growth pattern, invasion beyond ductal wall, pT3/pT4, lymphatic invasion, venous invasion, perineural invasion, nodal metastasis, and positive surgical margin were potential predictors of survival. Multivariate analysis identified >50% invasive component, nodal metastasis, and positive surgical margin as independent prognostic factors.

Discussion

The current results show that presence of an intraductal papillary component was an important determinant of survival in cholangiocarcinoma. Univariate and multivariate analyses in 644 patients revealed papillary component as one of the strongest survival predictors, in addition to nodal metastasis and venous invasion. PCC was documented as being more slow-growing, compared with the more aggressive NPCC, as reported previously [3-5]. However, it is unclear whether this is due to their inherent biology or the primarily intraductal growth pattern that may predispose to an early diagnosis from biliary obstruction before invasion into surrounding tissues occurs [13].

PCC patients with ≤50% invasive component (PCC-1, PCC-2, and PCC-3) that were characterized by predominantly intraductal papillary growth had better survival and less invasiveness. Tumors with >50% invasive component (PCC-4) were morphologically and prognostically closer to nodular-sclerosing cholangiocarcinoma (NPCC) than IPNB. Comparing PCC survival according to percentages of invasive component clearly revealed that the survival curve of the PCC group with the

highest invasive component was significantly worse than the others, and overlapped with that of NPCC. Interestingly, there was no significant difference in disease-specific survival among the three PCC groups with lower invasive components. These observations suggest that clinicopathological features of PCC are converted around this 50% threshold. Multivariate analysis supported this suggestion, identifying >50% invasive component as a prognostic factor in PCC. Our data supports the assertion of the WHO classification, which describes IPNB as a new type of precursor lesion showing the clinical development and progression of cholangiocarcinoma [14]. Our findings indicate that IPNB should be nosologically applied only for cases with ≤50% invasive component.

It has been unclear whether IPNB should be regarded as a neoplasm with biologically different pathogenesis compared to cholangiocarcinoma with focal papillary component or NPCC. We demonstrated that IPNB was well defined by the invasive area proportion (PCC-1, PCC-2, and PCC-3), and that this group was prognostically distinct from other more invasive carcinomas (PCC-4). However, we found no counterevidence for the assumption that IPNB and cholangiocarcinoma with focal papillary component constitute a continuous spectrum. In the present study, four cut-off values (non-invasive, ≤10%, 11-50%, and >50% invasive component) were proposed for the PCC classification system; these worked well for prognostic delineation, with the more advanced parameters in parallel with the higher category (Table 4). Other cut-off values were examined, and 50% was selected as the most appropriate for pragmatic diagnostic usage. Survival analysis among these four groups of PCC identified nine factors as potential predictors of survival in univariate analysis, three of which were identified as independent prognostic factors (>50% invasive component, nodal metastasis, and positive surgical margin) (Table 7). These factors largely overlapped with the previously reported prognostic factors of perihilar cholangiocarcinoma [21]. Overall, approaching PCC according to the proportion of the invasive component enabled us to understand the continuous biological behavior in PCC, although a few discrepancies existed.

Compared to other groups, PCC-1 more often affected the intrahepatic bile duct and was more frequently the gastric type (P = 0.003 and 0.001, respectively). However, morphologic appearance indicated that all PCC subgroups—i.e., both IPNB and cholangiocarcinoma with focal papillary component—belonged to a singular disease group.

The present large cohort of PCC including IPNB was associated with few hepatolithiasis and no liver flukes. This differs from other Eastern series, and was more similar to previous Western series. Only six (3%) of 184 patients with PCC had hepatolithiasis—a much lower incidence, compared to those of 21-31% reported in recent East Asian series (Table 1) [12, 17-19]. In general, hepatolithiasis has appeared less prevalently in Western populations (<1%) compared with in Far East Asian countries (9-21%) [28]; however, it was reported to be 2% in Japan [28]. None of our study patients had liver flukes, in contrast to the 12 and 18% incidences in China and Korea, respectively (Table 1) [18, 19]. The present series also included no benign or borderline lesions; this was also the case in Western cohorts, in which most cases of IPNB were malignant or carcinoma *in situ* [13, 20]. In contrast, previous large Asian series have included 13-25% and 48% benign or borderline lesions in Korea and China, respectively [12, 17-19]. It has been speculated that hepatolithiasis and liver flukes may contribute to the pathogenesis of these benign or borderline biliary tumors. Indeed, intrahepatic duct is reportedly more commonly affected by benign tumors, being more associated with hepatolithiasis or liver flukes than extrahepatic duct [18].

In this study, the predominant histological type in PCC was related to the proportion of the invasive component, influencing MUC2 expression (Tables 4 and 6), with increased tubular and mucinous adenocarcinomas relating to increasing invasive component. The majority of mucinous adenocarcinomas expressed MUC2 (69%), which was less frequently detected in papillary and tubular adenocarcinoma (13% and 14%, respectively; P < 0.001). Analyzing each PCC subgroup revealed that the mucinous type frequently showed MUC2 positivity regardless of the degree of

invasiveness, resulting in statistically significant differences in PCC-2 and PCC-3 (P = 0.008 and 0.004, respectively) compared with papillary/tubular carcinomas. On the other hand, MUC1 expression did not significantly differ among the three predominant histological types, and it correlated with increasing proportion of the invasive component. The presently identified MUC1 and MUC2 expression patterns differed somewhat from those previously documented[11, 29]. Previous studies proposed two progression pathways of IPNB to tubular and mucinous adenocarcinomas, featuring the phenotypic selection of MUC1+/MUC2+ and MUC1−/MUC2+ types, respectively, suggesting an expression pattern analogous to that in IPMN-P [11, 29, 30]. The presently reported high MUC1 positivity (77%) in mucinous adenocarcinoma differed from the previously reported values of 11 or 20% [11, 29]. Additionally, the present 14% MUC2 positivity in tubular adenocarcinoma was lower than that previously reported (80%) [29]. Taken together, most PCC in this cohort showed a MUC1+/MUC2− carcinogenetic pathway progressing to papillary/tubular adenocarcinoma, whereas a few PCC progressed to mucinous adenocarcinoma characterized by a MUC1+/MUC2+ pathway. These pathways also applied to IPNB, i.e., PCCs with ≤50% invasive component. Thus, our immunohistochemical data did not support the similarities between IPNB and IPMN-P. To further investigate whether IPNB may be a biliary counterpart of IPMN-P based on their similarities in pathological characteristics and immunohistochemical patterns [9-11], future studies will have to examine the molecular mechanisms of this peculiar neoplasm to elucidate the mechanisms of carcinogenesis.

In conclusion, the presence of an intraductal papillary component was an important determinant of better survival in cholangiocarcinoma. PCC exhibited a more aggressive histologic character and worse survival with progression of the invasive component. PCC with >50% invasive component was morphologically and prognostically similar to NPCC. Although IPNB might be nosologically applied only for PCC cases with ≤50% invasive component, the present prognostic

delineation indicated that all PCC subgroups belonged to a singular disease group.

Legend

Figure 1: Papillary cholangiocarcinoma (PCC) was divided into four groups based on the invasive proportion. (A, B) Non-invasive carcinoma (PCC-1). Intraductal components were composed of thin arborizing papillary structures covered biliary epithelium with severe dysplastic changes corresponding to carcinoma in situ. (C, D) PCC with ≤10% invasive component (PCC-2). Tumor cells minimally infiltrated into the bile duct wall (arrows). (E, F) PCC with 11-50% invasive component (PCC-3). The tumor included invasive component less than 50% of the lesion with infiltrative margin (arrow heads). The invasive component was characterized as moderately differentiated adenocarcinoma. (G, H) PCC with >50% invasive component (PCC-4). The main bulk of the tumor was formed of invasive moderately differentiated adenocarcinoma. Focal papillary component was also seen (arrow heads).

Figure 2: Survival for patients with papillary cholangiocarcinoma (PCC) and those with non-papillary cholangiocarcinoma (NPCC). *A log rank test.

Figure 3: Survival for patients with subgroups of papillary cholangiocarcinoma (PCC) and those with non-papillary cholangiocarcinoma (NPCC). *A log rank test.

PCC-1: Non-invasive carcinoma, PCC-2: PCC with ≤10% invasive component, PCC-3: PCC with 11-50% invasive component, PCC-4: PCC with >50% invasive component.

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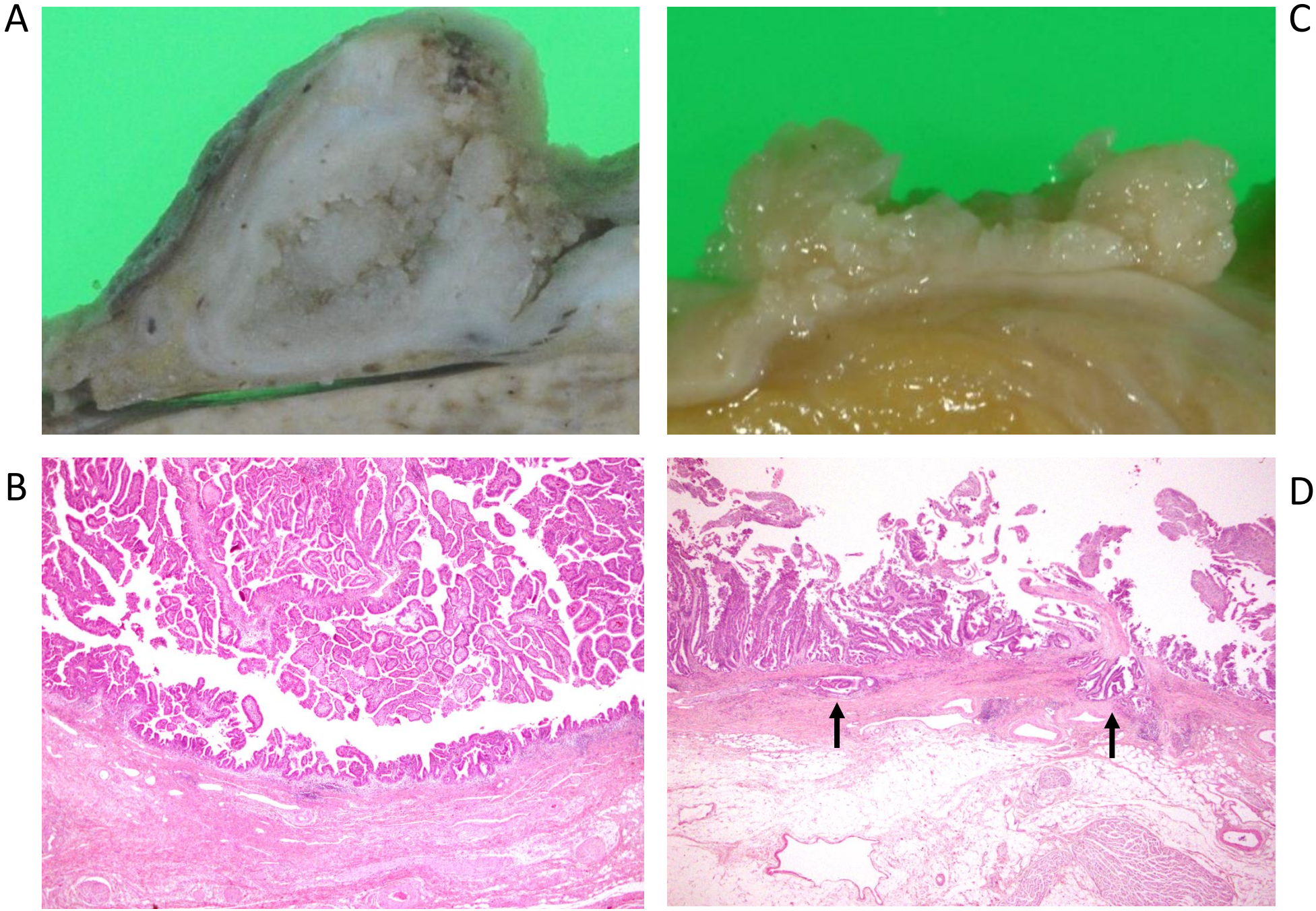
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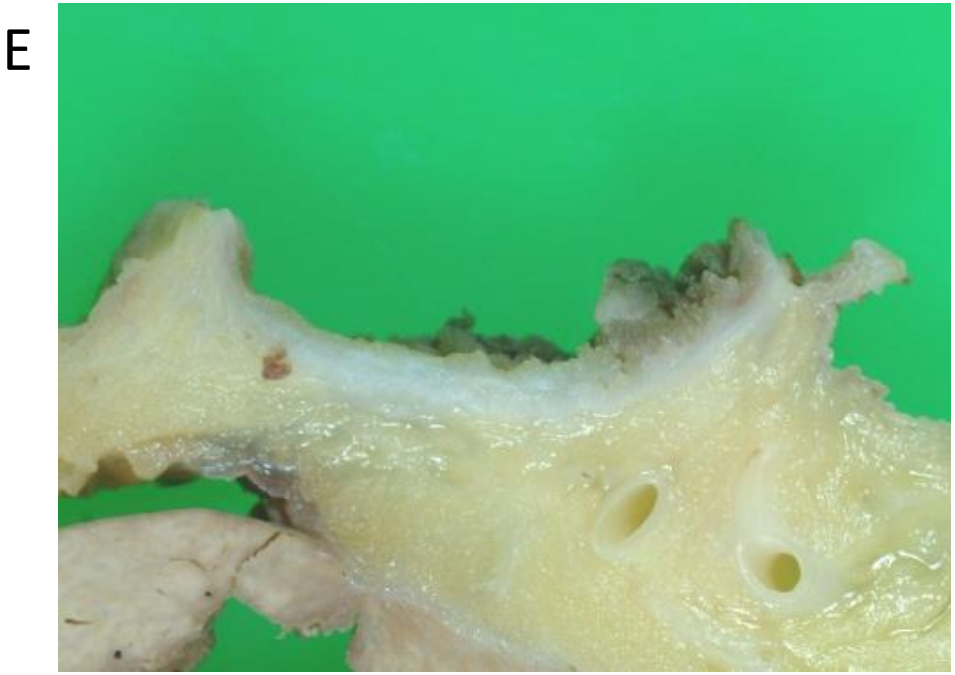
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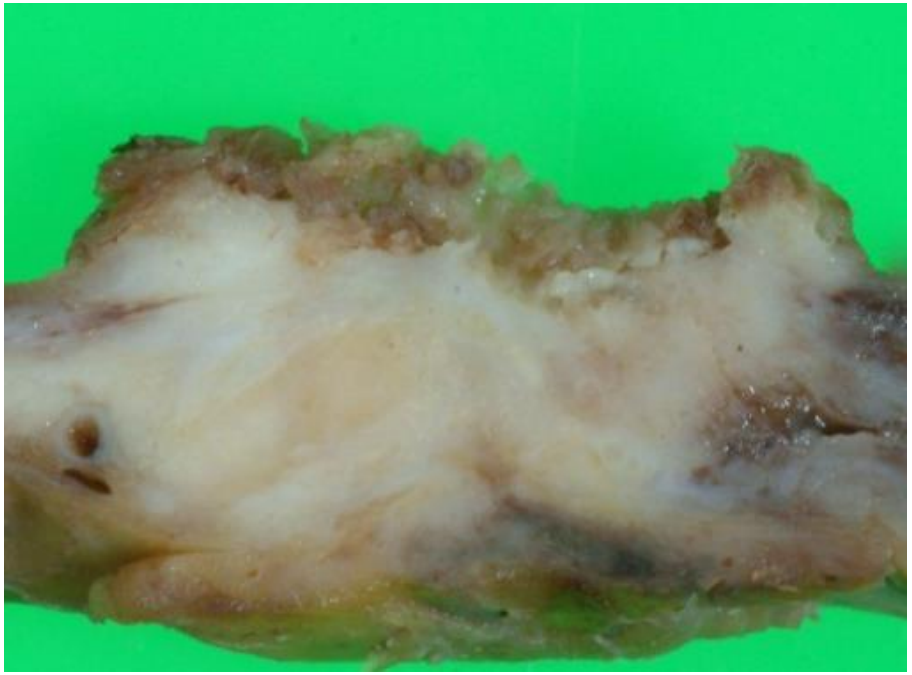
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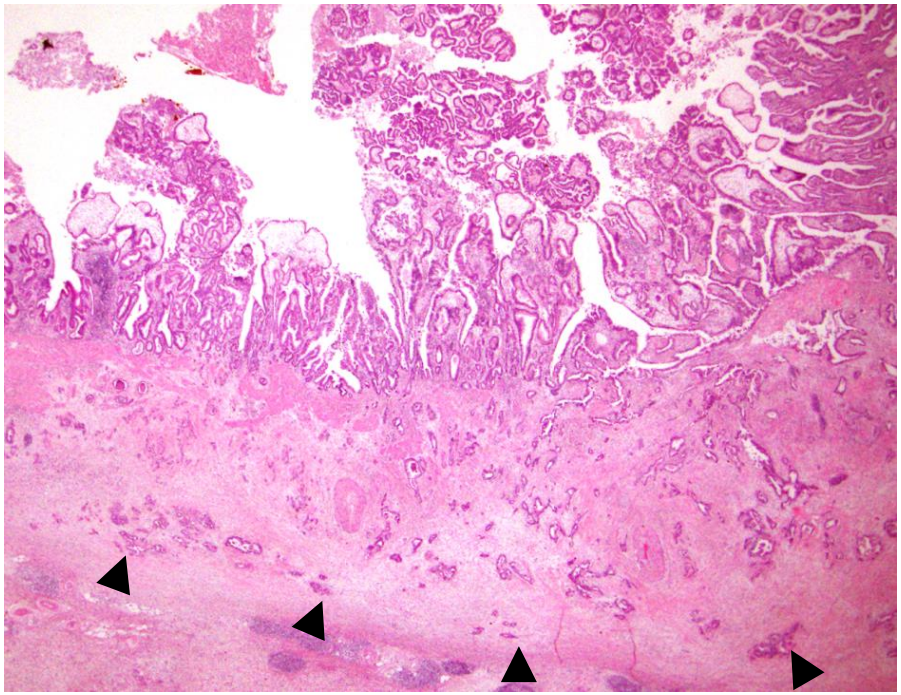
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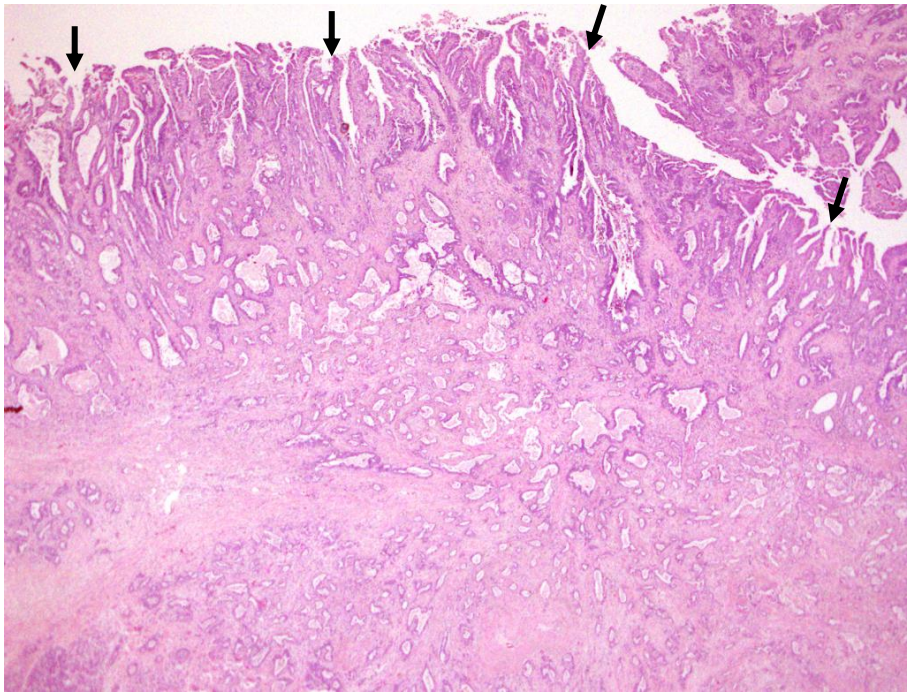
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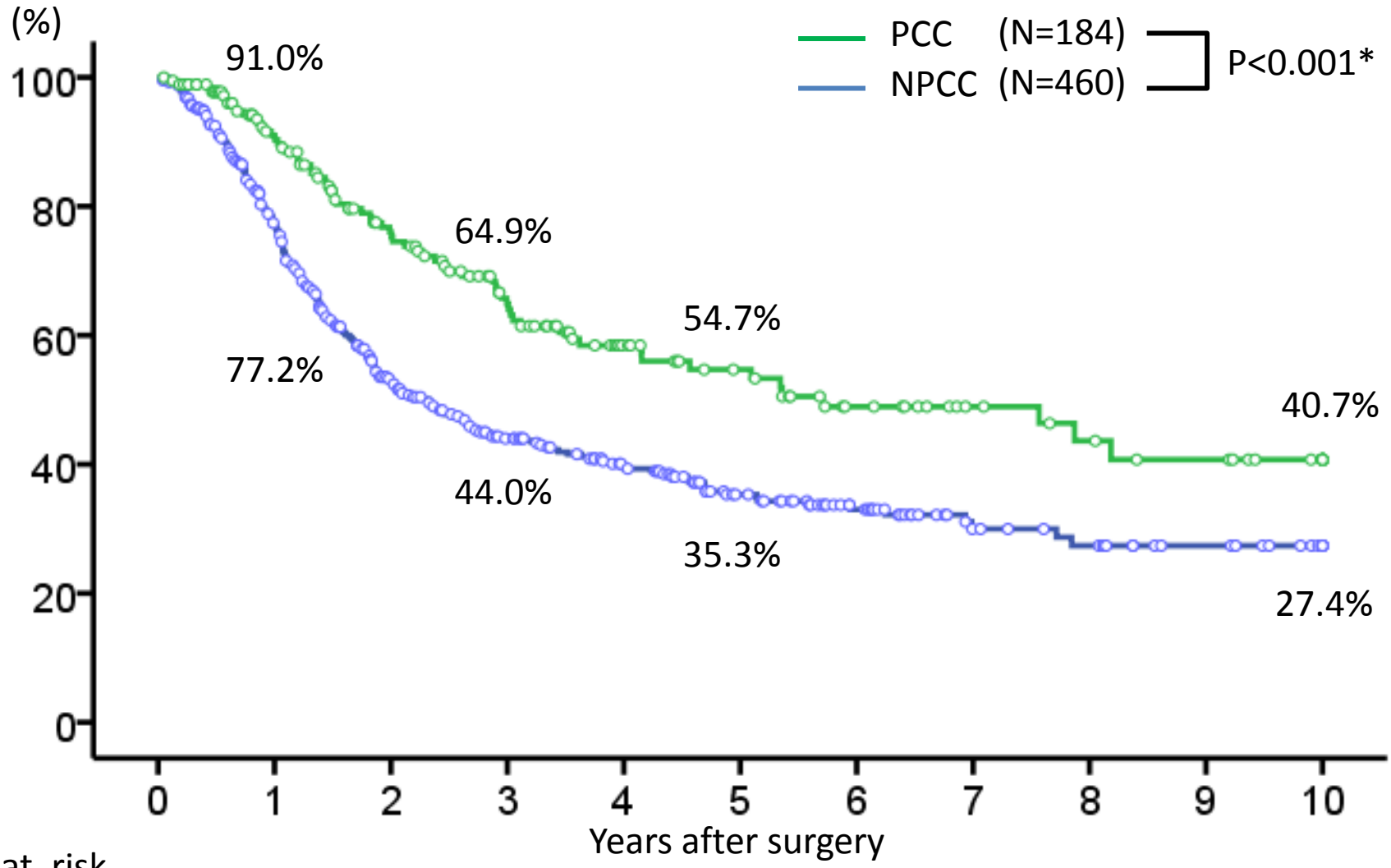
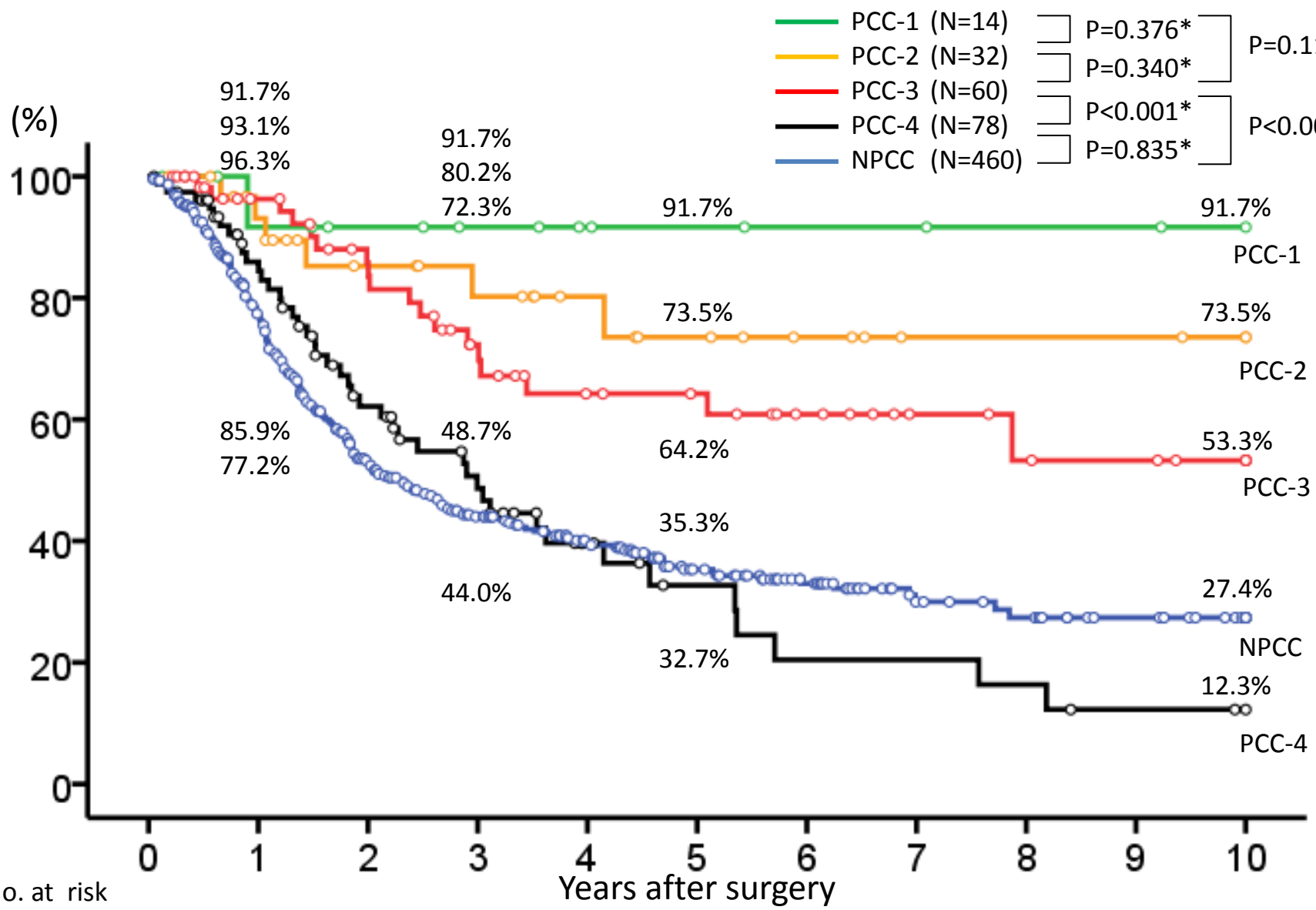


No. at risk

PCC	184	142		75		40				8
NPCC	460	319		134		69				5



No. at risk					
PCC-1	14	11	7	4	1
PCC-2	32	26	16	9	2
PCC-3	60	48	28	19	4
PCC-4	78	57	8	8	1
NPCC	460	319	134	69	5

Table 1. Definitions of IPNB in recent large series.

Year/ Author	Country	No. of cases	Hepato-lithiasis	Liver flukes	Benign or borderline lesion	Definition of IPNB
2010/ Choi SC [12]	Korea	55	15 (27%)	ND	11 (20%)	Preoperatively, tubular or cystic dilations of the bile duct with an intraductal growing mass or mucobilia by CT and cholangiography. Histologically tumors showed intraductal papillary growth, a microscopic or macroscopic existence of mucin, and positivity for any MUC2, MUC5AC, and CDX2 in the immunohistochemical staining.
2012/ Kim KM [17]	Korea	97	30 (31%)	ND	13 (13%)	Tumors that showed papillary proliferation of neoplastic biliary epithelial cells with delicate fibrovascular stalks within the bile ducts, and positivity for either MUC2, MUC5AC, or CDX2 according to immunohistochemical staining.
2012/ Jung G [19]	Korea	93	29 (31%)	17 (18%)	23 (25%)	Biliary epithelial neoplasms showing three following features: 1) marked bile duct dilation with intra-luminal filling defects in radiological images, 2) papillary or cast-like mass growing predominantly within the bile ducts revealed by gross examination, and 3) papillary or villous tumors having fibrovascular cores under the microscope.
2012/ Yang J [18]	China	52	11 (21%)	6 (12%)	25 (48%)	Certain types of papillary tumors occurring in the extrahepatic and intrahepatic large bile ducts which composed of papillary proliferation of biliary epithelium with delicate fibrovascular stalks with a histological spectrum ranging from benign disease to invasive malignancy.
2012/ Rocha FG [13]	USA	39	1 (3%)	0 (0%)	4 (10%)	All cases of resected bile duct tumors with gross and microscopic features of intraductal growth were retrieved from the database, and all papillary bile duct tumors were selected for histologic review. Patients with bile duct tumors containing intraductal growth were identified.

IPNB: intraductal papillary neoplasm of the bile duct, ND: not documented

Table 2. Comparison of clinical and pathologic features in cholangiocarcinoma.

	PCC (n=184)	NPCC (n=460)	*Univariate P*
Age (years)	66.3±10.0	64.8±9.9	*0.050*
Sex			*0.677*
Men	116 (63%)	298 (65%)	
Women	68 (37%)	162 (35%)	
Surgical procedure			*0.175*
Right-sided hepatectomy	64 (35%)	158 (34%)	
Left-sided hepatectomy	81 (44%)	225 (49%)	
Central bisectionectomy	2 (1%)	7 (2%)	
Partial hepatectomy	2 (1%)	7 (2%)	
Bile duct resection	7 (4%)	4 (1%)	
PD without hepatectomy	28 (15%)	59 (13%)	
Predominant histological type			*<0.001*
Papillary adenocarcinoma	40 (22%)	0	
Mucinous adenocarcinoma	13 (7%)	6 (1%)	
Tubular adenocarcinoma	131 (71%)	450 (98%)	
Adenosquamous carcinoma	0	4 (1%)	
Depth of invasion			*<0.001*
Within ductal wall	34 (18%)	5 (1%)	
Beyond ductal wall	150 (82%)	455 (99%)	
Lymphatic invasion (present)	101 (55%)	374 (81%)	*<0.001*
Venous invasion (present)	47 (26%)	215 (47%)	*<0.001*
Perineural invasion (present)	115 (63%)	413 (90%)	*<0.001*
Lymph node metastasis			
Regional (present)	57 (31%)	224 (48%)	*<0.001*
Periaortic (present)	5 (3%)	26 (6%)	*0.116*

PCC: cholangiocarcinoma with intraductal papillary component, NPCC: cholangiocarcinoma without intraductal papillary component, PD: pancreatoduodenectomy

Table 3. Univariate and multivariate analysis in 644 patients with cholangiocarcinoma.

Variable	No. of patients	Survival (%) 3-year	Survival (%) 5-year	P (Log-rank test)	Multivariate Hazard ratio (95% CI)	Multivariate P
Papillary component				*<0.001*		*0.012*
Absent (NPCC)	460	44.0	35.3		*1.00*	
Present (PCC)	184	64.9	54.7		*0.70 (0.53-0.93)*	
Predominant histological type				*0.004*		
pap/muc/asq	63	67.6	63.8			
tub	581	48.0	38.4			
Depth of invasion				*<0.001*		
Within ductal wall	39	79.0	79.0			
Beyond ductal wall	605	48.1	38.4			
Lymphatic invasion				*<0.001*		
Absent	169	69.0	58.3			
Present	475	42.7	34.1			
Venous invasion				*<0.001*		*<0.001*
Absent	382	59.1	50.4		*1.00*	
Present	262	33.9	23.1		*1.53 (1.21-1.94)*	
Perineural invasion				*<0.001*		
Absent	116	69.1	57.7			
Present	528	45.6	36.9			
Lymph node metastasis				*<0.001*		*<0.001*
Absent	363	66.1	57.2		*1.00*	
Present	281	29.2	18.8		*2.34 (1.85-2.97)*	

pap: Papillary adenocarcinoma, muc: Mucinous adenocarcinoma, tub: Tubular adenocarcinoma, asq: Adenosquamous carcinoma

Table 4. Comparison of clinical and pathologic features according to invasive component.

Proportion of invasive component	PCC-1 Non-invasive (n=14)	PCC-2 ≤10% (n=32)	PCC-3 11-50% (n=60)	PCC-4 >50% (n=78)	*Univariate P*
Age (years)	68.5∓9.0	66.1∓11.1	67.1∓9.6	65.4∓10.1	*0.597*
Sex					*0.091*
Men	6 (57%)	23 (72%)	33 (55%)	54 (69%)	
Women	8 (43%)	9 (28%)	27 (45%)	24 (31%)	
Tumor location					*0.003*
Intrahepatic	6 (43%)	1 (3%)	6 (10%)	17 (22%)	
Perihilar	5 (36%)	24 (75%)	33 (55%)	47 (60%)	
Distal	3 (21%)	7 (22%)	21 (35%)	14 (18%)	
Hepatolithiasis (present)	2 (14%)	0	1 (2%)	3 (4%)	*0.113*
Mucin secretion (present)	8 (57%)	11 (34%)	27 (45%)	43 (55%)	*0.200*
Predominant histological type					*<0.001*
Papillary adenocarcinoma	10 (71%)	18 (56%)	10 (17%)	2 (3%)	
Mucinous adenocarcinoma	0	3 (9%)	3 (5%)	7 (9%)	
Tubular adenocarcinoma	4 (29%)	11 (34%)	47 (78%)	69 (88%)	
Epithelial subtype					*0.001*
Gastric type	5 (36%)	4 (13%)	7 (12%)	8 (10%)	
Intestinal type	5 (36%)	14 (44%)	34 (57%)	22 (28%)	
Pancreatobiliary type	2 (14%)	14 (44%)	18 (30%)	45 (58%)	
Oncocytic type	2 (14%)	0 (0%)	1 (2%)	3 (4%)	
Invasive pattern					*<0.001*
None	14 (100%)	0	0	0	
Pushing growth margin	0	29 (91%)	19 (32%)	15 (19%)	
Infiltrating growth margin	0	3 (9%)	41 (68%)	63 (81%)	
Depth of invasion					*<0.001*
Within ductal wall	14 (100%)	15 (47%)	4 (7%)	1 (1%)	
Beyond ductal wall	0	17 (53%)	56 (93%)	77 (99%)	
Lymphatic invasion (present)	0	6 (19%)	37 (62%)	58 (74%)	*<0.001*
Venous invasion (present)	0	0	14 (23%)	33 (42%)	*<0.001*
Perineural invasion (present)	0	7 (22%)	42 (70%)	66 (85%)	*<0.001*
Superficial extensiton (present)	9 (64%)	20 (63%)	44 (73%)	48 (62%)	*0.511*
Lymph node metastasis					
Regional (present)	1 (7%)	1 (3%)	17 (28%)	38 (49%)	*<0.001*
Periaortic (present)	0	0	0	5 (6%)	*0.151*

PCC: Cholangiocarcinoma with intraductal papillary component

PCC-1: Non-invasive carcinoma, PCC-2: PCC with ≤10% invasive component, PCC-3: PCC with 11-50% invasive component, PCC-4: PCC with >50% invasive component

Table 5. Comparison of immunohisitochemical stain according to invasive component.

Proportion of invasive component	PCC-1 Non-invasive (n=14)	PCC-2 ≤10% (n=32)	PCC-3 11-50% (n=60)	PCC-4 >50% (n=78)	*Univariate P*
Ki67 index (%)	8% (1-30%)	10% (1-70%)	10% (1-70%)	10% (1-80%)	*0.277*
MUC1	5 (36%)	12 (38%)	41 (68%)	64 (82%)	*<0.001*
MUC2	3 (21%)	6 (19%)	9 (15%)	14 (18%)	*0.929*
MUC5AC	12 (86%)	14 (44%)	39 (65%)	56 (72%)	*0.014*
MUC6	8 (57%)	8 (25%)	18 (30%)	24 (31%)	*0.174*
HGM	9 (64%)	12 (38%)	31 (52%)	45 (58%)	*0.210*
CDX2	2 (14%)	9 (28%)	7 (12%)	9 (12%)	*0.145*
CK7	13 (93%)	28 (88%)	55 (92%)	76 (97%)	*0.173*
CK20	4 (29%)	13 (41%)	29 (48%)	36 (46%)	*0.557*
p53 (%)	5% (1-80%)	8% (1-80%)	20% (1-95%)	10% (1-80%)	*0.037*

Expressed as number of positive samples (%) except for Ki67 index, p53 [median (range)]

Table 6. Immunohistochemical characteristics according to the predominant histological type.

Predominant hisitological type \ Inv. component (%)		PCC-1 Non-invasive (n=14)	PCC-2 ≤10% (n=32)	PCC-3 11-50% (n=60)	PCC-4 >50% (n=78)	All PCC (n=184)
pap						
	MUC1	4/10 (40%)	8/18 (44%)	7/10 (70%)	2/2 (100%)	21/40 (53%)
	MUC2	2/10 (20%)	2/18 (11%)	1/10 (10%)	0/2 (0%)	5/40 (13%)
muc						
	MUC1	0	1/3 (33%)	3/3 (100%)	6/7 (86%)	10/13 (77%)
	MUC2	0	3/3 (100%)	3/3 (100%)	3/7 (43%)	9/13 (69%)
tub						
	MUC1	1/4 (25%)	3/11 (27%)	31/47 (66%)	56/69 (81%)	91/131 (70%)
	MUC2	1/4 (25%)	1/11 (9%)	5/47 (11%)	11/69 (16%)	18/131 (14%)
Univariate	*P MUC1*	*>0.999*	*0.744*	*0.660*	*>0.999*	*0.098*
	P MUC2	*>0.999*	*0.008*	*0.004*	*0.129*	*<0.001*

pap: Papillary adenocarcinoma, muc: Mucinous adenocarcinoma, tub: Tubular adenocarcinoma

Expressed as N/Y (%): Number of cases that expressed the marker in ≥10% of the cells/number of cases examined

Table 7. Univariate and multivariate analysis in 184 patients with PCC.

Variable	No. of patients	Survival (%) 3-year	Survival (%) 5-year	P (Log-rank test)	Multivariate Hazard ratio (95% CI)	Multivariate P
Invasive component (%)				*<0.001*		*0.001*
≤50%	106	76.8	70.2		*1.00*	
>50%	78	48.7	32.7		*2.38 (1.40-4.04)*	
Mucin secretion				*0.171*		
Absent	95	71.2	62.5			
Present	89	57.9	46.6			
Predominant histological type				*0.297*		
pap/muc	53	67.1	67.1			
tub	131	64.2	50.0			
Invasive pattern				*0.004*		
None/Pushing growth margin	77	75.9	67.7			
Infiltrating growth margin	107	57.5	46.1			
Depth of invasion				*0.007*		
Within ductal wall	34	80.5	80.5			
Beyond ductal wall	150	61.3	48.7			
Lymphatic invasion				*0.001*		
Absent	83	80.5	70.0			
Present	101	51.4	41.2			
Venous invasion				*0.001*		
Absent	137	68.8	61.2			
Present	47	52.0	27.7			
Perineural invasion				*0.002*		
Absent	69	77.7	67.5			
Present	115	56.9	46.6			
Superficial extension				*0.386*		
Absent	63	57.9	48.4			
Present	121	68.2	58.0			
Lymph node metastasis				*<0.001*		*<0.001*
Absent	127	76.7	68.0		*1.00*	
Present	57	38.4	23.3		*2.54 (1.53-4.23)*	
Surgical margin				*<0.001*		*0.002*
Negative	162	70.5	60.9		*1.00*	
Positive	22	28.2	15.0		*2.58 (1.42-4.67)*	

pap: Papillary adenocarcinoma, muc: Mucinous adenocarcinoma, tub: Tubular adenocarcinoma