INCREASINGLY RECOGNIZED DISEASES OF BILE DUCTS, GALLBLADDER, AND AMPULLA: EMPHASIS ON MULTIFOCAL AND DIFFUSE DISEASES

Precursor neoplastic lesions:

Biliary carcinomas may develop through two key precursor lesions: Biliary intraepithelial neoplasia (BiIIN) and intraluminal papillary neoplasms. These lesions are probably analogous to pancreatic intraepithelial neoplasia (PanIN) and intraductal papillary mucinous neoplasm of the pancreas (IPMN), respectively. The vast majority of invasive carcinomas of the pancreaticobiliary tract arise from flat (non-tumoral) forms of intraepithelial neoplasia. The cut-off size for the definition of tumoral intraepithelial neoplasia in the pancreaticobiliary tract as defined by WHO (2010) is 1 cm.

Intraluminal papillary neoplasms

Intraluminal papillary neoplasms of the biliary tract are referred to as “intracystic” in the gallbladder and “intraductal” in the extrahepatic bile ducts. Dysplasia in these neoplasms ranges from low grade dysplasia to high grade dysplasia/carcinoma in situ. They are divided into pancreaticobiliary, intestinal, gastric, and oncocytic forms depending on the cellular phenotype. These types have the following immunohistochemical characterization: pancreaticobiliary and oncocytic: MUC1-positive, intestinal: MUC2- and CDX2-positive, gastric: MUC5- and MUC6-positive. However, immunohistochemical markers are not required as cellular lineages can usually be identified with morphology alone.

Intraductal papillary neoplasm of the bile duct (IPN)

IPNs are uncommon, representing approximately 10% of all resectable cases of bile duct neoplasms. Previously, localized low-grade lesions were sometimes designated as “papillary adenomas”, high grade lesions as “non-invasive papillary carcinoma”, diffuse (low or high grade) lesions as “papillomatosis”. The pancreaticobiliary phenotype is most common, followed by intestinal, gastric, and oncocytic phenotypes. Unlike pancreatic IPMN’s, most (two-thirds) biliary IPNs are non-mucinous. Accordingly, some authors have subclassified biliary IPNs into mucinous and non-mucinous types. IPNs can be cystic and confused with mucinous cystic neoplasm. Connection of the cyst with the bile duct lumen and absence of ovarian-type stroma are helpful diagnostic features of IPNs.

In comparison to IPMNs of the pancreas, the incidence of invasive carcinoma in biliary IPN is much higher; 50-70% of IPNs are associated with invasive carcinoma in resected cases. The invasive component is often tubular or mucinous (colloid). However, undifferentiated, small cell, or large cell neuroendocrine type carcinomas can occur. MUC1 is commonly seen in the invasive component and can be used to evaluate the malignant potential of the tumor. Non-invasive and minimally invasive papillary neoplasms have an excellent long-term prognosis. Invasive papillary carcinomas also appear to
have a better prognosis than non-papillary cholangiocarcinomas. In a recent study from MSKCC, the overall median survival of IPNs was 62 months. Factors associated with a worse median survival included presence and depth of tumor invasion, positive resection margin, and expression of MUC1 and CEA. In this study, the median survival was similar among different locations or subtypes. However, another study from Korea with a larger number of cases demonstrated a significantly higher rate of invasive tubular carcinoma in pancreatobiliary IPNs compared with other subtypes. The pancreatobiliary form was also associated with a poorer prognosis.

Similar to pancreas, intraductal tubular neoplasm of the common bile duct has also been described. In this lesion, the intraductal component shows predominantly tubular morphology, without any papillary architecture.

Synchronous involvement of the pancreatic duct and bile duct by intraductal papillary mucinous tumors rarely occurs, indicating that intraductal papillary neoplasms of the pancreas and biliary tree may be a single disease entity.

**Precursors lesions of the gallbladder:**

Gallbladder adenomas typically occur in adult females and are single in more than 90% of patients. Fifth-eight percent have associated gallstones. The adenomas are classified as pyloric, intestinal, foveolar, and biliary. The most frequently occurring adenoma is pyloric gland adenoma (PGA). PGAs show closely packed tubular glands, which are lined by monolayered columnar epithelium with abundant mucin-containing cytoplasm and basal nuclei. Cystically dilated glands are common. Squamoid morules are seen in 35% of PGAs. Pyloric gland metaplasia occurs in adjacent gallbladder mucosa in 50% of cases. Some pyloric gland adenomas may show eosinophilic cytoplasm and non-mucinous histology. Tubular adenoma is the most common type of intestinal adenoma. Papillary (villous) adenomas are the second most common type. Adenomas play a minor role in carcinogenesis. Only 1% of gallbladder PGAs are associated with invasive adenocarcinoma, although high-grade dysplasia and low-grade dysplasia are identified in 27% and15% of PGAs, respectively. High-grade dysplasia is recognized in 46.4% of intestinal-type adenomas, and the incidence of invasive adenocarcinoma is 3.5%.

The distinguishing features between papillary adenoma and intracystic papillary neoplasm are not clear, with no well-defined size or number criteria. A single lesion may likely be called papillary adenoma, while three or more lesions may be designated as multiple adenomas or papillary neoplasm. Recently, Adsay et al proposed a unified terminology of "intra-cholecystic papillary-tubular neoplasm" (ICPN) for all exophytic adenomatous/papillary preinvasive neoplasms that are more than or equal to 1.0 cm. It includes all lesions classified as adenoma by WHO 2010 classification (tubular, papillary, pyloric gland, foveolar gland, biliary, intestinal) and "intracystic papillary neoplasms". 6.4% of gallbladder adenocarcinomas arise from ICPNs. ICPNs occur predominantly in females with a mean age of 61 years. Gallstones are identified in 20% of cases.
Half of the neoplasms are detected incidentally. Other GI malignancies are seen in 20% of cases. High-grade dysplasia is seen in more than 75% of ICPNs. ICPNs often show mixed cellular lineages. Biliary phenotype is most common. Two types of gastric-type ICPNs are described: Foveolar and pyloric types. The foveolar type ICPNs show uniform MUC5 AC expression and is commonly accompanied by invasive carcinoma (in 60% cases). The pyloric-type ICPNs are characterized by diffuse MUC6 expression and are divided into mucinous and non-mucinous types. The mucinous form is similar to pyloric gland adenoma with a low risk of invasive carcinoma. The non-mucinous types are often large, complex, pedunculated, multinodular, intraluminal tumors that detach readily from the mucosal surface and may show morule formation; some of them may have nuclear overlapping and chromatin clearing resembling papillary thyroid carcinoma. Non-mucinous-type pyloric gland adenomas have an 18% risk of invasive carcinoma. Intestinal-type ICPNs are CDX2-positive in 75% of cases and MUC2-positive in 50% cases. ICPNs are typically positive for CK7 even in the cases that show intestinal differentiation. 

Invasive carcinoma is seen in more than half of ICPNs at the time of diagnosis, most of which are pancreatobiliary type. Invasion is more often seen in ICPNs with papillary configuration, with areas of high-grade dysplasia and with predominance of non-pyloric cell lines. The prognosis for invasive carcinoma arising in ICPNs is better than for other invasive carcinomas of the gallbladder, although deaths occur in non-invasive cases despite negative resection margins, usually long after the diagnosis of ICPN, suggesting a field effect phenomenon. Thorough sampling and careful evaluation is very important in these neoplasms to rule out the presence of invasive carcinoma, which may be grossly inapparent.


IAPNs have also been recently described. IAPN comprise about 30% of all ampullary tumors. The male:female ratio is 2.4, and the mean age is 64 years. Similar to analogous tumors of the gallbladder and bile duct, these tumors also have a mixture of papillary and tubular growth, demonstrate different cellular lineages, and may exhibit a spectrum of dysplasia. The non-invasive IAPNs have an excellent prognosis. IAPNs with invasion exhibit a malignant behavior but have a significantly better prognosis than typical invasive ampullary carcinomas unaccompanied by IAPNs.

**Biliary intraepithelial neoplasia (BiILN)**

BiILN, also known as biliary dysplasia, is characterized by atypical epithelial cells with increased nuclear-to-cytoplasmic ratio, multilayering of nuclei, partial loss of nuclear polarity, nuclear hyperchromasia, and micropapillary projections into the bile duct or gallbladder lumen. BiILN has been noted in 40% to 60% of patients with invasive carcinoma, 30% of patients with sclerosing cholangitis, incidentally in 1% to 3.5% of cholecystectomy specimens, in patients with choledochal cyst,
hepatolithiasis, FAP, and pancreatobiliary reflux. Recently intrahepatic \BILINs have been reported in patients with viral hepatitis. BiILIN is usually not recognized on macroscopic examination, although the mucosa may be granular. Microscopically, BiILINs may be flat or form abortive papillae. It usually occurs in a background of pyloric and intestinal metaplasia. BiILIN is multicentric in many cases. BiILINs are categorized by WHO into BiILIN-1, BiILIN-2, and BiILIN-3, where BiILIN-1 corresponds to low-grade dysplasia, BiILIN-2 to intermediate grade dysplasia, and BiILIN-3 to high-grade dysplasia. Carcinoma in situ is considered as part of the spectrum of high-grade dysplasia. See Table 1 for diagnostic criteria for BiILIN and reactive epithelial changes.

Table 1. Modified Diagnostic Criteria for Reactive Change of the Biliary Epithelium and Biliary Intraepithelial Neoplasia

(Zen et al, Modern Pathology, 2007)

Hyperplasia or regenerative change
These lesions are most commonly flat. Low-papillary or micropapillary architecture, when observed, is often associated with hepatolithiasis or choledochal cyst. Tall papillary structures are rare. Cellularity is only slightly increased compared to normal biliary epithelium. Nuclei are round or oval, and slightly enlarged with smooth nuclear membrane. Chromatin is fine and homogeneously distributed. Intraepithelial neutrophil infiltration can be seen. Mitoses can be observed in some lesions.\textsuperscript{a}

\textit{BiILIN-1 (biliary intraepithelial neoplasia-1)}
These lesions show flat or micropapillary architecture. Nuclei are basally located. Some lesions show focal nuclear pseudostratification; however, the nuclei remain within the lower two thirds of the epithelium. Cytologically, mild nuclear abnormalities, such as subtle irregularities of nuclear membranes, high nuclear/cytoplasmic ratios and nuclear elongation are seen. Nuclear size and shape are relatively uniform. The presence of large nuclei suggests a diagnosis of BiILIN-2 or BiILIN-3.

\textit{BiILIN-2 (biliary intraepithelial neoplasia-2)}
These lesions show flat, pseudopapillary or micropapillary architecture. Loss of cellular polarity is easily found, but it is not a diffuse feature. Nuclear pseudostratification reaching the luminal surface is common. Cytologically, dysplastic nuclear changes, which include enlargement, hyperchromasia and irregular nuclear membrane, are evident. Some variations in nuclear size and shape are seen. Peribiliary glands are sometimes involved (glandular involvement). Mitoses are rare.

\textit{BiILIN-3 (biliary intraepithelial neoplasia-3)}
These lesions usually show pseudopapillary or micropapillary architecture, and are only rarely flat. They cytologically resemble carcinoma, but invasion through the basement membrane is absent. Cellular polarity is diffusely and severely distorted with nuclei reaching and piling on the luminal surface. 'Budding off' of
small clusters of epithelial cells into the lumen and cribriforming can be seen. Cytologically malignant features with severe nuclear membrane irregularities, hyperchromasia or abnormally large nuclei are typically noted. Mitoses can be observed. Peribiliary gland involvement is sometimes found.

When present, discrimination from BilIN is important, because mitoses in BilIN suggest the diagnosis of higher grade lesions, mostly BilIN-3.

Dysplasia may be very difficult to differentiate from reactive epithelial changes. Inflamed biliary epithelium may have mild nuclear atypia, high nuclear-cytoplasmic ratio, hyperchromasia, and disorganization of nuclei. The presence of acute inflammation (especially intraepithelial neutrophils) favors a reactive process. See Table 2 for morphologic features that distinguish dysplasia from reactive atypia.

**Table 2. Morphologic Features to Distinguish Dysplasia from Reactive Atypia in Gallbladder**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Dysplasia</th>
<th>Reactive Atypia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute inflammation and/or ulceration</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Intraepithelial neutrophils</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Abrupt transition between normal and atypical epithelium</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Fine nuclear chromatin</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Prominent nucleoli</td>
<td>−/+</td>
<td>+</td>
</tr>
<tr>
<td>Surface maturation</td>
<td>−/+</td>
<td>+</td>
</tr>
<tr>
<td>Loss of polarity</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>History of instrumentation</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

Abbreviations: +, present; −, absent; +/-, can be present or absent.

Involvement of Rokitansky-Aschoff sinuses and peribiliary mucus glands by dysplasia can mimic invasive carcinomas. Table 3 highlights the morphologic features used to distinguish dysplasia extending into Rokitansky-Aschoff sinuses from invasive carcinoma in gallbladder.

**Table 3. Morphologic Features to Distinguish Dysplasia/In Situ Carcinoma Extending into Rokitansky-Aschoff Sinuses from Invasive Carcinoma in Gallbladder**
Rarely squamous cell dysplasia can be observed, and signet ring cell type in-situ carcinoma has also been reported.

The biologic significance of dysplasia is not well understood. If dysplasia is observed in a cholecystectomy specimen, thorough sampling of the remaining gallbladder is recommended to rule out the presence of invasive adenocarcinoma.

**Tumor-like Lesions**

**Adenomyoma and adenomyomatous hyperplasia**

Adenomyomas are commonly seen in the gallbladder, but can be seen in the stomach, small bowel, bile duct, and ampullary region. It is unclear whether these are neoplastic or malformative in nature. Histologically, the adenomyomas are characterized by lobules of small glands located around the larger duct. These glands are lined by single layered cuboidal or columnar epithelial cells with no atypia or mitotic figures. Some glands show cystic changes. Hyperplastic glandular lobules are surrounded by hyperplastic mesenchymal tissue consisting of muscle fibers, fibroblasts, myofibroblasts and varying numbers of mononuclear inflammatory cells.

Adenomyoma of the ampulla may cause biliary obstruction and is often misdiagnosed as adenoma or carcinoma. Endoscopic biopsies from adenomyomas of the ampulla often show epithelial cell atypia which is mistaken for dysplasia. Immunohistochemically, the adenomyomas are positive for cytokeratin 7 and negative for cytokeratin 20, whereas adenomas are often positive for both CK7 and CK20 positive. The biopsy diagnosis of adenomyoma is important to avoid unnecessary extensive surgery. However, regardless of a

<table>
<thead>
<tr>
<th></th>
<th>Dysplasia/In Situ Carcinoma</th>
<th>Invasive Carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desmoplasia</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Connection to surface epithelium</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Small to medium-sized glands in smooth muscle</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Dilated or long elongated gland structures</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Presence of luminal bile</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Mixture of benign and atypical glands</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Involvement of intermuscular connective tissue</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Involvement of the muscle itself</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Perineural or vascular invasion</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

Abbreviations: +, likely present; −, likely absent.
diagnosis of adenoma or adenomyoma, most ampullary lesions are treated by ampullectomy.

**Hyperplastic Luschka ducts**

The ducts of Luschka are located in the gallbladder fossa within the rim of adherent liver and gallbladder serosa. Awareness of these ducts is important in preventing an erroneous diagnosis of malignancy, since hyperplastic Luschka ducts can mimic adenocarcinoma. Histologically, they are composed of lobular aggregates of small ductules lined by bland, cuboidal to columnar biliary-type epithelium, with associated centrally located, larger ductules surrounded by concentric fibrosis. Sometimes Luschka duct proliferation is noted, with an irregular growth pattern and epithelial atypia, suggesting the possibility of invasive adenocarcinoma. Most cases of duct proliferation are associated with marked acute and chronic cholecystitis. See Table 4 for histologic features comparing Luschka ducts with invasive gallbladder adenocarcinoma.

**Table 4. Morphologic Features to Distinguish Luschka’s Ducts from Invasive Adenocarcinoma in the Gallbladder**

<table>
<thead>
<tr>
<th>Histologic Features</th>
<th>Luschka Ducts</th>
<th>Invasive Gallbladder Adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location in gallbladder wall</td>
<td>Adventitia only</td>
<td>Full thickness</td>
</tr>
<tr>
<td>Architecture</td>
<td>Lobular, linear</td>
<td>Haphazard</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>Concentric</td>
<td>Irregular</td>
</tr>
<tr>
<td>Cytology</td>
<td>Reactive atypia associated with inflammation</td>
<td>Nuclear variation (4:1) within a single gland</td>
</tr>
<tr>
<td>Mitoses</td>
<td>Minimal/none</td>
<td>Usually present Vascular and perineural invasion</td>
</tr>
<tr>
<td>Other findings</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Immunohistochemistry to differentiate reactive epithelium from neoplastic epithelium**

**S100p, IMP3 (insulin-like growth factor-II mRNA binding protein-3) and pVHL (von Hippel-Lindau gene product)**

This panel can be helpful in making the diagnosis of adenocarcinoma in a small biopsy specimen. Normal mucosa, inflamed mucosa, and low-grade dysplasia show no or weak expression for IMP3. In contrast, this marker is strongly expressed in malignant epithelium. Although the specificity of IMP3 is high, diffuse positivity is seen in only 50% of malignant cases. IMP3-positivity
correlates with overexpression of p53, high Ki-67, and significantly reduced overall survival. In a study by Levy et al, seventy percent of malignant biopsies were positive for S100p and IMP3 and negative for pVHL. The normal pattern of S100p-negative, IMP3-negative and pVHL-positive was not seen in any malignant biopsy. The overall sensitivity and specificity of S100p and IMP3 are 90% and 75% and 77% and 93%, respectively. The interpretation of the stains can be very difficult in some cases; pVHL negativity is not seen in 10% of malignant cases, and not all benign cases showed 100% staining. S100p staining is often seen in low-grade PanIN, and focal staining may be rarely seen in benign bile ducts. For all these reasons, it is best to use these immunostains as a panel. Positivity for S100p or IMP3 in the absence of epithelial atypia has been suggested by some to be a marker of early dysplasia, preceding recognizable histologic manifestations of dysplasia. More prospective studies are needed to evaluate the significance of focal staining for S100p and IMP3 in what appears to be benign/reactive epithelium.

CD10 (Tretiakova et al, AJSP 2012):
The absence of CD10 expression in strips of atypical biliary epithelial cells is described to be associated with malignant transformation and is proposed as a helpful marker to distinguish benign from malignant lesions on biopsy material. In this study, all cases of normal duct epithelium (n=64) showed strong continuous staining at the apical surface, and most (72/75) malignant lesions (invasive adenocarcinoma and high-grade dysplasia) (n=75) showed loss of CD10 immunoreactivity. Most benign (low grade and reactive) lesions were CD10 positive, but the staining pattern was discontinuous, with positive cells varying from 20% to 80%. Because of the interrupted staining pattern in reactive epithelium and the presence of focal staining in a few malignant cases, this stain may be difficult to apply in routine clinical practice.

Pdx1 and HES1 (Igarashi et al, Histopathology 2012)
Pdx1 has been recently demonstrated to be expressed in 67% of invasive cholangiocarcinomas. In large bile ducts, expression of PDX1 increased while that of HES1 decreased during the progression of BiIN lesions to cholangiocarcinoma. These immunohistochemical markers may be targets for future studies as potential biomarkers for differentiating malignancy from reactive changes.

DNA ploidy analysis performed in conjunction with routine brush cytology achieves better sensitivity in detection of malignancy on ERCP specimens obtained for the assessment of pancreaticobiliary strictures.

Usefulness of IgG4 immunostaining of duodenal papilla/ampulla/bile duct biopsies in supporting a diagnosis of autoimmune pancreatitis

Pancreatic biopsy can be useful in the setting of autoimmune pancreatitis (AIP), since it can reliably rule out malignancy or even show histologic characteristics of
autoimmune pancreatitis. However, pancreatic biopsy is not always performed
due to technical challenges and the possibility of complications. The revised
version of HISORT (mnemonic for histology, imaging, serology, other organ
involvement and response to therapy) criteria for the diagnosis of AIP has now
included positive IgG4 immunostaining in the ampulla as a finding that is
supportive of AIP. Some authors have stressed the importance of bile duct
biopsy, as one can evaluate for features of IgG4 sclerosing disease such as
diffuse inflammatory infiltration and irregular fibrosis.
ERCP examination is often clinically necessary in suspected AIP cases in order
to rule out malignancy, obtain a direct pancreatogram, or relieve obstructive
jaundice. Biopsies of the ampulla, bile duct, or papilla can be easily acquired at
this time. We routinely perform IgG4 immunostains on ERCP-obtained biopsies
from suspected AIP patients, a practice driven by our gastroenterologists.
Although based on small numbers, multiple studies have shown that an
increased number of IgG4 plasma cells (more than 10 IgG4 positive cells in any
one high power field) in an ampulla/bile duct/papilla biopsy are supportive of the
diagnosis of autoimmune pancreatitis. While the sensitivity of IgG4
immunostaining is not high (50% to 80%), the specificity is reported to be 100%.
One group has suggested that the ratio of IgG4-positive to IgG-positive plasma
cells (IgG4/IgG ratio) is more useful. Using an IgG4-IgG ratio cutoff of 0.10 in
ampulla biopsies, the diagnostic sensitivity and specificity are reported to be 86%
and 95%, respectively. In our experience, finding > 10 IgG4-positive cells per
high power field in any ampulla/bile duct biopsy is not specific for AIP. However,
there have been cases where diffuse infiltrates of > 50 IgG4-positive cells in an
ampulla/bile duct biopsy have supported the clinical suspicion of AIP.
The low sensitivity of IgG4 immunostaining can be explained in part by variable
disease activity of AIP. It also depends on the number and size of the biopsy
samples.
One needs to keep in mind that the diagnosis of AIP is based on the combination
of clinical, laboratory, and imaging findings - not just immunohistochemistry alone
or any other single criterion. In AIP patients with a normal serum IgG4 level,
IgG4 immunostaining can be particularly helpful in obtaining a diagnosis as IHC
is positive in up to 43% of these patients. It should be noted that the information
above does not apply to idiopathic duct-centric type of AIP, as this entity is
usually negative for IgG4.

References:

3. Adsay V, Jang KT, Roa JC, Dursun N, Ohike N, Bagci P, et al. Intracholecystic papillary-tubular neoplasms (ICPN) of the gallbladder (neoplastic polyps, adenomas, and papillary neoplasms that are ≥1.0 cm): clinicopathologic and immunohistochemical analysis of 123 cases. Am J


