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The DOI for this manuscript is doi: 10.5858/arpa.2016-0262-SA

The final published version of this manuscript will replace the Early Online Release version at the above DOI once it is available.

The Evolving Role of Pathology in New Developments, Classification, Terminology, and Diagnosis of Pancreatobiliary Neoplasms

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• Pancreatobiliary tract lesions are increasingly being discovered because of more sensitive imaging modalities. Magnetic resonance imaging has identified incidental pancreatic cysts in 13.5% of patients of progressively increasing age. Pancreatobiliary tissue is more accessible through endoscopic ultrasound and magnetic resonance imaging–guided biopsy procedures, and is now an integral part of pathologists' routine practice. Accordingly, several new tumor categories have been recently recognized, including intraductal tubulopapillary neoplasm, a new addition to tumoral intraepithelial neoplasms. Other entities have been reclassified, including the recent transition to 2-tiered grading of preinvasive neoplasms, as well as new perspectives on the distinctive biologic behavior of oncocytic intraductal papillary mucinous neoplasms (IPMNs) compared with other IPMN subtypes. This has led to proposals for revised staging of virtually every segment of the pancreatobiliary tree, with theranostic markers becoming an integral part of workup. Ki-67 is now an integral part of the classification of neuroendocrine tumors, with new definitions of "high-grade neuroendocrine carcinoma." Although bile duct brushings have opened new avenues for diagnosis, their sensitivity remains low and often requires concomitant fluorescent in situ hybridization to better define ambiguous cases. Various molecular pathways have been elucidated for pancreatic cysts, including *KRAS* for ductal neoplasia, *GNAS* for intestinal IPMNs, *RNF3* for mucinous cysts, and *VHL* for serous cystic neoplasms, all key players in diagnostic workup. Integration of these updates into our understanding of pancreatobiliary disease requires active engagement of pathologists for appropriate specimen triage, judicious interpretation of results, and incorporation into reporting and staging. They also provide exciting opportunities for targeted therapy.

(*Arch Pathol Lab Med.* doi: 10.5858/arpa.2016-0262-SA)

Accepted for publication July 20, 2016.

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The authors have no relevant financial interest in the products or companies described in this article.

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We herein describe new concepts in the diagnosis of the most common pancreatic neoplasms, with emphasis on recent advances that potentially impact their classification, diagnosis, and prognosis.

RECENT DEVELOPMENTS IN CLASSIFICATION AND TERMINOLOGY OF PANCREATOBILIARY NEOPLASIA: NEW CONCEPTS, ENTITIES, AND RECENT MODIFICATIONS IN TERMINOLOGY

Tumoral Intraepithelial Neoplasia As an Important Category and Distinct Pathway of Carcinogenesis

It has now become clear that a dichotomy exists in pancreatobiliary ductal carcinogenesis, with the conventional intraepithelial neoplasia (PanINs; BilINs) representing incidental, microscopic forms of dysplasia on one hand, and mass-forming preinvasive (tumoral intraepithelial) neoplasms—most of them cystic and papillary—as the other arm of the carcinogenetic pathway.[1] These mass-forming lesions are now well characterized in every segment of the ampulla and pancreatobiliary tract, and include intraductal papillary mucinous neoplasm (IPMN), intraductal tubulopapillary neoplasm (ITPN), mucinous cystic neoplasm (MCN), intra-ampullary papillary-tubular neoplasm, and intracholecystic tubulopapillary neoplasm of the gallbladder, all with established malignant risk, representing the "adenoma-carcinoma sequence" (Figure 1, A and B).[2–4] The different subtypes of tumoral intraepithelial neoplasms are defined not only by their location and morphology, but also by their unique molecular makeup.

Intraductal Oncocytic Papillary Neoplasm Is a Distinct Tumor Type

Intraductal oncocytic papillary neoplasm (IOPN, or "IPMN-O") was initially described as a separate category, then was placed under the heading of IPMNs in the World Health Organization (WHO)–2010; however, it is now proving to be a distinct entity with a different molecular pathway, clinical presentation, and biologic behavior than other IPMN subtypes.[5–7] These tumors often present as large, complex masses that are rarely invasive and seldom fatal.[6,8] We recently showed that IOPN is also diagnosable on cytology (Figure 1, C and D).[8]

ITPN: The New Kid on the Block

Intraductal tubulopapillary neoplasm is a relatively new but rare indolent neoplasm of pancreatic and bile ducts

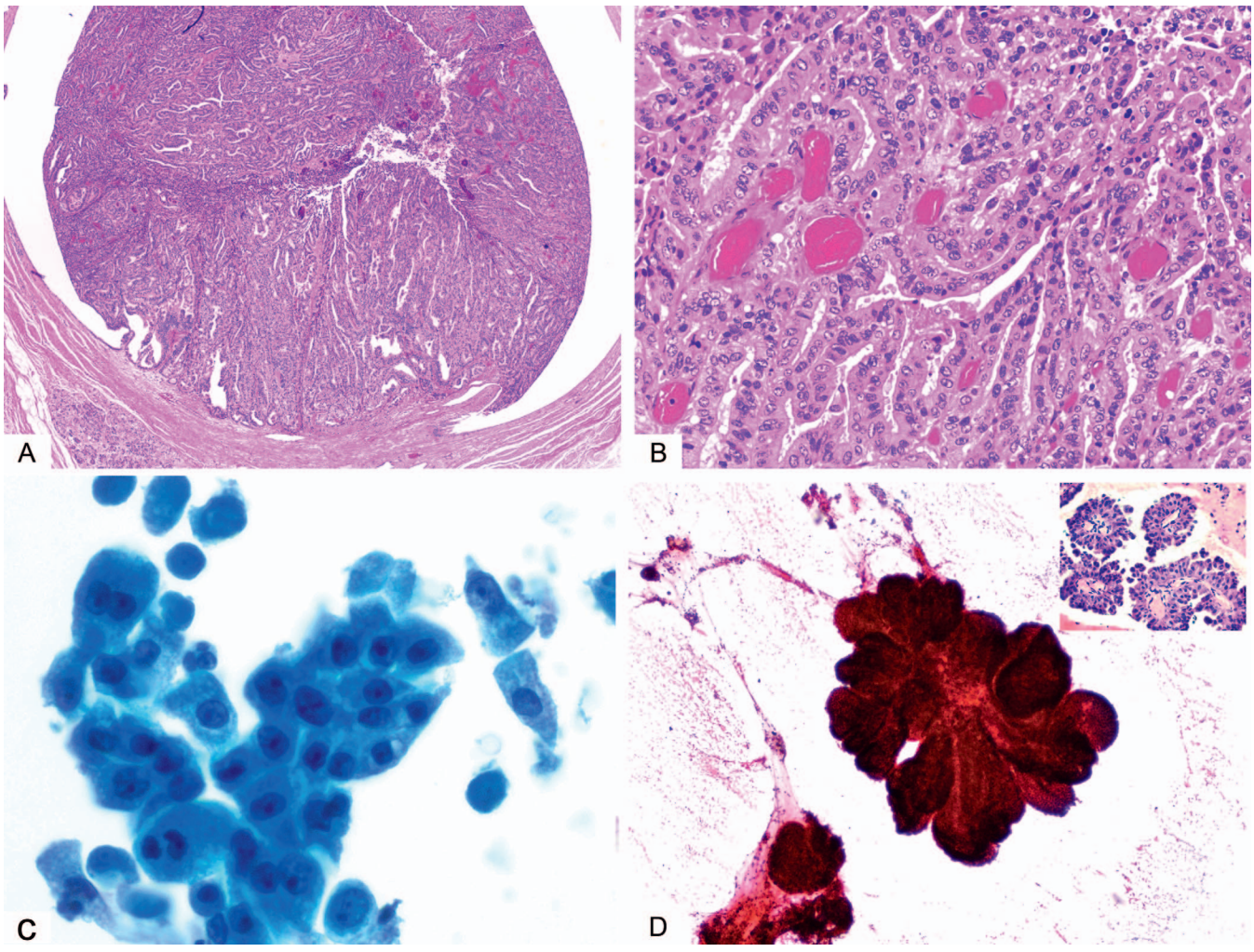


Figure 1. *Intraductal tubulopapillary neoplasm. A, Histologic section shows an intraductal solid tumoral mass composed of (B) back-to-back glands lined by epithelial cells with large vesicular nuclei, prominent nucleoli, and no cytoplasmic mucin. C and D, Oncocytic-type intraductal papillary mucinous neoplasm. C, ThinPrep shows a sheet of large oncocytic cells with abundant granular cytoplasm, large nuclei, and prominent nucleoli. D, Corresponding smear of the same case shows tumor cells forming large, branching papillae. The corresponding cell block (inset) shows papillae with hyalinized fibrovascular cores lined by large oncocytic cells (hematoxylin-eosin, original magnifications ×100 [A] and ×200 [B and inset]; Papanicolaou, original magnifications ×200 [C] and ×100 [D]).*

composed of back-to-back, nonmucinous tubules, occasionally with minimal papillae formation (Figure 1, A and B).[4] Tubules are lined by mitotically active, cuboidal epithelium showing high-grade dysplasia that closely mimics acinar carcinomas. Invasive carcinoma is seen in up to 40% of cases.[9] A third of biliary ITPNs show somatic mutations in the phosphatidylinositol 3-kinase (*PIK3CA*) gene,[10] which is associated with the phosphatidylinositol 3-kinase pathway and thus is a potential diagnostic/therapeutic target. *PIK3CA* mutations are associated with immunohistochemical overexpression of phosphorylated AKT in ITPNs. *p16/CDKN2A* mutations are also seen in ITPNs (slightly more than half), but *KRAS* and β-catenin–related mutations are not.

Refined Criteria for Tumoral Versus Nontumoral Intraepithelial Neoplasms

Tumoral intraepithelial neoplasms are defined as mass-forming preinvasive neoplasms characterized by clinically, radiologically, and grossly detectable adenomatous lesions. For pancreatic IPMNs and ITPNs, the size criterion of 1 cm has been proposed to distinguish them from PanINs, which are typically less than 0.5 cm.[11] Those 0.5- to 1-cm borderline lesions with gastriclike epithelium are designated "incipient."[12] Additionally, in the pancreas, the flat, mucinous gastric-type epithelial cysts that are greater than 1 cm and without ovarian-type stroma or papillary units, but with the cytology of IPMNs, are now deemed "simple mucinous cysts" in order to distinguish them from IPMNs. These revised diagnostic criteria will hopefully improve our understanding and management of these borderline, "difficult-to-classify" pancreatic lesions.

From a 3-Tiered to a 2-Tiered Classification System for Preinvasive Neoplasia

New consensus recommendations were published (December 2015 and January 2016) for the reporting of pancreatic precursor lesions and invasive carcinoma, including PanIN, IPMN, and MCN.[11,13] To improve concordance and match clinical/molecular observations, a 2-tiered system (low-grade versus high-grade) was proposed for all precursor lesions, with PanIN-2 and "intermediate-grade"

examples of neoplastic mucinous cysts (IPMN, MCN) now being categorized as low grade, whereas the high-grade dysplasia category is reserved for carcinoma in situ–type lesions.[11,13] Although low-grade lesions are believed to be clinically insignificant, high-grade ones have frequent association with invasive cancer and warrant close clinical attention, especially if encountered in isolation (without invasive carcinoma), including at surgical margins.

For the bile ducts, the BilIN category still encompasses a 3-tiered system, although most authors continue to use the low-grade versus high-grade dysplasia terminology, which is more clinically relevant.[14] In fact, most of the principles recognized for pancreatic neoplasia (including the 2-tiered preinvasive neoplasia approach) are also valid for biliary tract.[13] The parallels between different segments of pancreatobiliary tract are very striking,[15] and the same is true for the gallbladder, where high-grade dysplasia warrants clinical intervention, unlike low-grade dysplasia, which is poorly reproducible and thought to be clinically inconsequential.

Refined Classification/Grading of Neuroendocrine Neoplasms

Current WHO-2010 guidelines require that pancreatic neuroendocrine neoplasms (NENs) be graded by immunohistochemical Ki-67 index calculation and measurement of mitotic activity. Mitotic activity seldom, if ever, results in a higher tumor grade than Ki-67 index, particularly if the Ki-67 count is performed accurately (see below). Thus, mitotic count is now becoming a moot point. Grade 1 is defined by WHO as tumors whose Ki-67 index is "<2%" and grade 2 is defined as ">3%," which left cases between 2% and 3% unaccounted for (a category to which 15% of NENs belong, in our experience).[16] However, the North American Neuroendocrine Tumor Society has addressed this issue by redefining grade 1 NENs as lower than 3% and grade 2 as 3% or higher Ki-67 index.[17]

The grade 3 category of WHO-2010, defined as Ki-67 above 20%, comprises 2 highly distinct groups of tumors[18,19]: (1) well-differentiated pancreatic neuroendocrine tumors (PanNETs) that happen to be more proliferative, and (2) true high-grade (poorly differentiated) neuroendocrine carcinomas of small cell and large cell types, as defined in the lungs. The former group, which typically has a Ki-67 below 40%, is also called "grade discordant" and has an intermediate-grade behavior, whereas the high-grade cases generally have Ki-67 indices between 40% and 50%, along with high-grade morphology, brisk mitoses, and necrosis. There are, however, "ambiguous cases" that are difficult to classify into one of these categories and are usually encountered at metastatic sites, such as the liver.[20]

Ki-67 grading methodologies include eyeball estimation, real-time microscopic counting of cells, manual counting of a static color photomicrograph of Ki-67–stained tumor cells, and automated counting systems.[16,21] Some advocate automated systems because of their alleged higher accuracy, although their cost and maintenance can be prohibitive.[16] Automated counting is also prone to errors unless software modification or operator education is performed to carefully exclude staining of contaminating endothelial cells, lymphocytes, or hemosiderin pigment. Manual calculation from a printed image of a static color photomicrograph represents a significantly cheaper alternative that offers higher reproducibility than eyeballing and real-time microscope counting (Figure 2).[16]

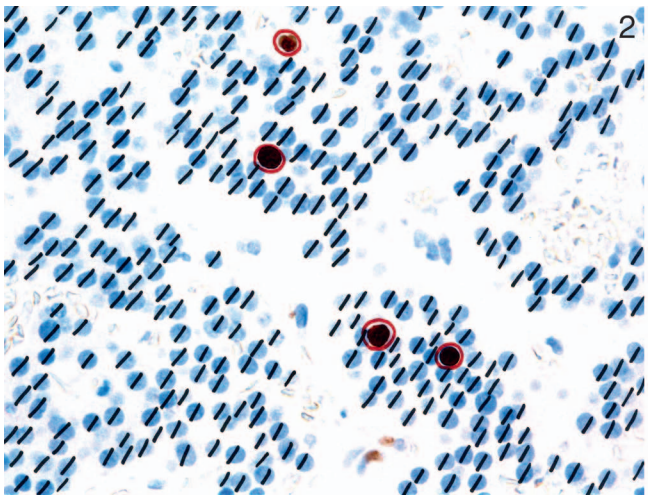
Ki-67 index calculation can be performed on cytologic samples, which is the more common initial diagnostic procedure and which gives results comparable to those of corresponding resections.[16,22,23] False low/high indices may occur in specimens with low cellularity, abundant hemosiderin-laden macrophages, and Ki-67–positive lymphocytes, especially when automated systems are used, because these may miscount the latter as tumor cells. We therefore advocate the inclusion of a statement in cytology reports "that the grade and index may ultimately change on the resection specimen."

"Precision Medicine": Doing What We Do Better; Toward Improved Staging of Pancreatobiliary Cancers

In the pancreas, the discovery of small cancers arising in IPMNs and MCNs necessitated the creation of substages of T1 (T1a, T1b, and T1c).[11] For more advanced cancers, the lack of proper definition of "peripancreatic soft tissue" and "common bile duct involvement" (as to which part is meant) made T3 highly subjective, and thus a size-based staging system was devised and is under consideration for the upcoming American Joint Committee on Cancer revised manual.[24,25] For the ampulla, because of the region's anatomic complexity and the underappreciation of 3-dimensional tumor spread in this area (in particular its frequent extension into periduodenal soft tissues and duodenal serosa, which are not addressed in the current system and which require specific grossing approaches for accurate documentation), a revised T-staging system has been proposed, and therefore major revisions are needed.[26] Recently proposed refined definition and site-specific subclassification of ampullary tumors highlight the areas needing improvement.[3,27] In the gallbladder, it has become clear that many gallbladder cancers are clinically and grossly "unapparent," making the establishment of proper grossing protocols and adequate sampling crucial. "Early" gallbladder cancers confined above the mucosa/muscularis (Tis/T1) have a 10-year survival rate of 90%, provided that deeper invasion is excluded by total sampling. In fact, a recent proposal on substaging of T2 gallbladder cancers has revealed that even early T2 cancers have a very good prognosis, close to that of early gallbladder cancers and incomparably better than that of advanced T2 cancers.[28]

New Standardized Terminology and Nomenclature for Pancreatobiliary Cytology

In 2014 the Papanicolaou Society recommended standardized guidelines and nomenclature for signing out pancreatobiliary cytology specimens. There are now 6 diagnostic categories, including nondiagnostic, negative (for malignancy), atypical, neoplastic (benign or other), suspicious (for malignancy), and positive/malignant (Table).[29] The neoplastic category is subdivided into neoplastic; *benign* (such as serous cystadenoma, cystic teratoma, and schwannoma), and neoplastic; *other* (such as well-differentiated PanNETs, solid-pseudopapillary neoplasm [SPN], intraductal papillary neoplasms of the bile duct, and neoplastic mucinous cysts [IPMN, MCN]). The inclusion of PanNET in the "neoplastic; *other*" category is somewhat controversial because it implies that these tumors are not malignant, when in fact they all have the potential to metastasize. In fact, PanNET studies with more comprehensive follow-up have shown that the vast majority of tumors, even when small, eventually exhibit malignant



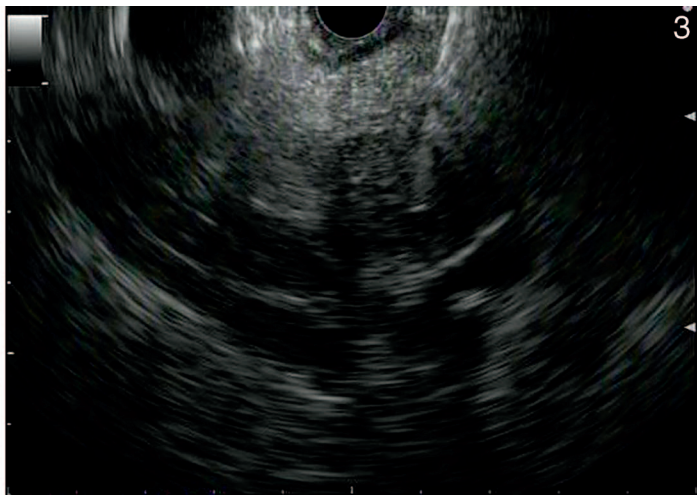


Figure 2. *Ki-67–stained slide of a well-differentiated pancreatic neuroendocrine tumor in which the Ki-67 index was calculated by counting a camera-captured photomicrograph of Ki-67^{+} positive brown tumor cells (circled in red) among 500+ (Ki-67^{-}) tumor cells (marked in black; original magnification ×400).*

Figure 3. *Echo-endosonographic image from a linear endoscopic ultrasound at 7.5 MHz shows a 25-mm hypoechoic pancreatic mass undergoing fine-needle aspiration. The needle can be seen passing into the mass during collection of cells for rapid on-site examination.*

behavior, albeit decades after original diagnosis in some cases.

New Perspectives on Serous Adenomas, Their Variants, and So-Called Serous Cystadenocarcinoma

Careful analyses have recently revealed that, in contrast to the impression held in guideline texts on the topic, the gross variants of serous cystadenomas, including macrocystic (oligocystic, unicystic) ones, are in fact clinicopathologically similar to their more common microcystic counterpart, but they are more prone to misdiagnosis radiologically because of their mimicry of other megacystic neoplasms. Solid serous cystadenomas (SCNs) are extremely rare (2%) and are often misdiagnosed radiologically as neuroendocrine tumors (NETs). Appraisal of the literature suggests that true serous "cystadenocarcinomas" do not truly exist because

Terminology and Nomenclature for Pancreatobiliary Cytology: Papanicolaou Society Guidelines

Diagnostic Category	Definition	Examples
Nondiagnostic	Provides no diagnostic/useful information about the solid or cystic lesion sampled	
Negative for malignancy	Adequately cellular to evaluate or define a lesion identified on imaging	Benign pancreatobiliary tissue Acute or chronic pancreatitis Autoimmune pancreatitis Pseudocyst Lymphoepithelial cyst Splenule/accessory spleen
Atypical	Cells with cytoplasmic, nuclear, or architectural features inconsistent with normal or reactive changes, and insufficient for classification as neoplastic or suspicious for high-grade malignancy	
Neoplastic		
Benign	Sufficiently cellular representative cytologic specimen diagnostic of a benign neoplasm (with/without clinical, imaging, or ancillary studies)	Serous cystadenoma Cystic teratoma Schwannoma
Other	Premalignant or low-grade malignant neoplasm	IPMN, MCN, IPN-B Well-differentiated PanNET Solid-pseudopapillary neoplasm
Suspicious for malignancy	Has some but insufficient typical features of a specific malignant neoplasm; qualitatively or quantitatively insufficient for conclusive diagnosis or ancillary studies	
Positive/malignant	Neoplasm with unequivocal malignant cytologic characteristics	PDAC and its variants Cholangiocarcinoma Acinar cell carcinoma High-grade PanNEC (small/large cell) Pancreatoblastoma Lymphoma Sarcoma Metastasis to pancreas

Abbreviations: IPMN, intraductal papillary mucinous neoplasm; IPN-B, intraductal papillary neoplasm of the bile duct; MCN, mucinous cystic neoplasm; PanNEC, pancreatic neuroendocrine carcinoma; PanNET, pancreatic neuroendocrine tumor; PDAC, pancreatic ductal adenocarcinoma.

there are virtually no tumor-related deaths or distant metastases that are directly attributable to dissemination. Most so-called "malignant" SCNs likely represent local adhesion or liver involvement by multifocal tumors (many of them unverified histologically). Secondary "infiltration" (direct adhesion/penetration) of spleen, stomach, colon, and/or lymph nodes occurs rarely (3%) in larger tumors (mean, 11 cm) but is seemingly innocuous, with no associated distant metastasis.[30]

Recently discovered alternations of the von Hippel-Lindau (*VHL*) gene establish SCNs as prototypes of clear cell tumorigenesis and angiogenesis with both diagnostic and therapeutic potential.[31] The characteristic rich capillary network seen in SCN and other VHL-related clear cell tumors (including clear cell renal cell carcinoma and capillary hemangioblastoma) is likely related to a clear cell angiogenesis pathway fueled by *VHL* mutations and modified downstream effectors hypoxia-inducible factor (HIF1), glucose uptake and transporter-1 (GLUT-1), and vascular endothelial growth factor (VEGF); modified downstream effectors are a key factor in their striking capillarization.[32]

EVOLVING APPROACHES TO THE DIAGNOSIS OF PANCREATIC LESIONS

New Sampling Techniques

Computerized tomography and percutaneous ultrasound–guided fine-needle aspiration (FNA) have been superseded by endoscopic ultrasound–guided FNA (EUS-FNA) for sampling pancreatic lesions. EUS-FNA has greater sensitivity than cross-section imaging in detecting lesions, including those less than 1 cm,[33] and because the (transgastric/transduodenal) needle tract is within the resected area, the risk of seeding is low. The needle tip is visible during EUS-FNA, enabling detection and sampling of regional lymph nodes and ascites, as well as metastatic lesions (Figure 3). Rapid on-site evaluation in combination with EUS-FNA has improved diagnostic sensitivity and has now become the norm in the initial evaluation of pancreatic lesions.[34–36] As tissue sampling moves more toward EUS-FNA, design modifications in EUS needles and needle tips show preliminary diagnostic benefit in minimizing the number of passes.[37,38] Newer needles with back-facing bevels or multiple cutting surfaces facilitate the collection of sheared tissue and the acquisition of tissue cores. Newer endoscopic "fanning," "wet suction," and "slow pull" techniques have also enhanced tissue procurement.[39–41]

Cystic Pancreatic Lesions

With the increased sensitivity of newer imaging modalities, the number of incidentally discovered pancreatic cysts is on the rise.[42] In fact, more than 10% of elderly people undergoing abdominal magnetic resonance imaging receive a diagnosis of "incidental" pancreatic cysts. In evaluating cystic pancreatic lesions, the primary goal is to exclude malignancy and distinguish innocuous from mucinous cysts, because the latter are more prone to harbor or progress to malignancy. Conventional cytohistomorphology has now been integrated with cyst fluid biochemistry and molecular/genetic testing in order to accurately interpret radiologic findings and better stratify patients for surveillance or surgical intervention. Pancreatic cysts may be nonneoplastic or neoplastic. Nonneoplastic cysts include pseudocysts (which account for 75% of all cystic pancreatic lesions), lymphoepithelial cysts, and squamoid cyst of the pancreatic duct. Neoplastic cysts include SCNs, mucinous cysts (IPMN, MCN), SPN, and solid tumors that undergo cystic degeneration. Although most nonneoplastic cysts and SCNs do not require resection unless they are symptomatic, most neoplastic cysts should ideally be resected.

Cyst Fluid Analysis

Viscosity.—Viscosity levels greater than that of serum have been shown to correlate with mucinous cysts.[43] These cysts contain thick viscous, mucoid material that is difficult to express from the needle, smear as gelatinous material, and take a longer time to dry. By placing a drop of cyst fluid between thumb and index finger and measuring maximum length of stretch, one can indirectly measure viscosity. Values of 3.5 mm correlate with mucinous cysts; however, similar results may be seen with the nonmucinous cysts, lymphoepithelial cysts, and squamoid cyst of the pancreatic duct.[44]

Biochemical Analysis.—*Carcinoembryonic Antigen*.—Carcinoembryonic antigen (CEA) remains a mainstay of cyst fluid analysis, and values of 110 to 200 ng/mL are strongly associated with neoplastic mucinous cysts.[43,45–47] Carcinoembryonic antigen may have a higher sensitivity, specificity, and accuracy than cytology or EUS-FNA alone.[45,47] Although often higher in IPMN than MCN and in malignant versus benign cysts,[47,48] CEA alone cannot be used to accurately distinguish these cyst types,[49,50] and low levels do not exclude a mucinous cyst.[51] Elevated CEA may rarely be seen in nonmucinous cysts (pseudocysts, squamoid cyst of the pancreatic duct, SCN, and lymphoepithelial cysts).[48,49,52]

Amylase.—In addition to CEA, cyst fluid amylase is also helpful in distinguishing pancreatic pseudocysts from neoplastic mucinous cysts (IPMN and MCN). Amylase levels are frequently markedly elevated in pancreatic pseudocysts (>1000 ng/mL).[47,48] Although amylase levels are typically higher in IPMNs than MCNs (because of their connection to the duct), these levels cannot be used to distinguish between them, and nonmucinous cysts, such as squamoid cyst of the pancreatic duct, may also have elevated amylase.[47,49]

VEGF-A Assay.—Yip-Schneider et al[53] recently found markedly elevated VEGF-A in SCNs compared with pseudocysts, IPMNs, MCNs, and pancreatic ductal adenocarcinoma (PDAC). Mean VEGF-A in SCNs is incomparably higher than in other pancreatic neoplasms and has high sensitivity (100%) and specificity (97%).[53] Although very promising, verification in larger studies is needed.

Molecular Studies in the Diagnosis of Pancreatic Cysts.—Molecular alterations in cyst fluid can be extremely helpful in distinguishing cyst types, and in one study they had 95% concordance with fluid aspirated during surgery.[54] *KRAS* mutations and loss-of-heterozygosity (LOH) events involving the tumor suppressor genes *CDK2NA*, *RNF43*, *SMAD4*, *TP53*, and *VHL* are helpful in distinguishing nonmucinous from mucinous cysts.[54–59] Aneuploidy in mucinous cysts has been linked to malignant transformation.[54,60,61] These and other tests, including next-generation sequencing (NGS), are already becoming integrated into cyst fluid analysis, although they are not yet widely available.[51]

In the following section, the new developments in molecular carcinogenesis in different pancreatic cystic

lesions, and their application in daily practice will be discussed.

Neoplastic Mucinous Cysts (IPMN and MCN).—Of 96 IPMNs recently studied by Springer et al,[54] 98% (94 of 96 cases) had at least 1 mutation, LOH, or aneuploidy, with *KRAS* mutations being most prevalent (78%). In another study, *KRAS* mutation had a sensitivity and specificity of 54% and 100%, respectively, for detecting PDAC, IPMN, and MCN.[57] A commercially available molecular kit was used to assess *KRAS* mutation, LOH, DNA quality, and DNA quantity in pancreatic cyst fluid and had 83% sensitivity and 100% specificity for malignancy.[62] Somatic *GNAS* oncogene mutations (codon 201) are seen in 58% to 60% of IPMNs,[46,54] with intestinal-type ones showing the highest prevalence (n = 9 of 11; 82%), followed by gastric (n = 39 of 64; 61%) and pancreatobiliary (n = 3 of 9; 33%), but not oncocytic IPMNs or MCN.[54] This mutation is even detectable in duodenal pancreatic juice.[54,63] More than 90% of IPMNs have either *GNAS* or *KRAS* mutations, with 47% to 50% showing both,[54,59] whereas SCNs show neither.[54,59] Therefore, assays containing both *GNAS* and *KRAS* amplicons could potentially distinguish between these 3 cyst types.[59] Somatic *RNF43* mutations are seen in IPMNs and MCN.[59] Other PDAC-related driver gene mutations (*TP53*, *p16/CDKN2A*, *SMAD4/DPC4*) may occur in IPMN and MCN[54,59] and are seen in cysts with high-grade dysplasia or invasion.[64–69]

Serous Cystadenomas.—*VHL* gene mutations have recently been identified in SCN cyst fluid,[54,59] but not in IPMN, MCN, or SPN.[54] However, these mutations may be seen in PanNETs, and are therefore not specific for SCN. In one study, 67% (8 of 12) of SCNs analyzed had either *VHL* mutation, LOH of chromosome 3, or aneuploidy of chromosome 3p.[54] However, KRAS, *GNAS*, and *RNF43* alterations have not been identified.[54,59] VEGF protein levels are also elevated in SCNs.

Solid-Pseudopapillary Neoplasm.—Solid-pseudopapillary neoplasms harbor the lowest genetic alterations of any pancreatic neoplasm, with activating somatic mutations of β-catenin gene (*CTNNB1*) being most frequent (95%–100%).[54] These mutations cause cytoplasmic and eventual nuclear β-catenin accumulation and result in nuclear (in addition to cytoplasmic) staining with β-catenin, a marker routinely used in their diagnosis. β-Catenin is a mediator of the Wnt signal transduction pathway.[59,65,70] Mutated nuclear β-catenin interacts with LEF1/T-cell factor transcriptional complex, leading to activation of other Wnt-responsive genes. LEF1 is overexpressed by SPN nuclei and is another helpful diagnostic marker.[71] *CTNNB1* alterations cause aberrant E-cadherin overexpression in tumor nuclei,[72] resulting in their characteristic and diagnostic cellular dyscohesion, and the pseudopapillary pattern that is now an integral part of this tumor's name. *VHL*, *GNAS*, and *RNF43* mutations have not been identified in SPNs.[54,59]

Summary

The frequency of somatic mutations in specific pancreatic cyst types strongly suggests that the future use of molecular assays containing a panel of these markers could prove useful in their presurgical diagnosis. Springer et al[54] recently developed a highly sensitive and specific assay that uses massively parallel sequencing of a host of molecular markers (*VHL*, *GNAS*, *CTNNB1*, and *RNF43*, chromosomal LOH as well as aneuploidy) and combined these with specific clinical findings (jaundice, abdominal pain, and main pancreatic ductal dilatation, as well as age), to robustly correlate them with different pancreatic cysts (IPMN, MCN, SPN, and SCN; Figure 4). *SMAD4* mutation, chromosome 17 LOH (*RNF43* gene region), or aneuploidy in chromosome 5p, 8p, 13q, or 18q was predictive of MCN, SPN, or IPMN with high-grade dysplasia or invasive carcinoma, thus identifying surgical candidates with 75% sensitivity and 92% specificity.[54] These composite markers have yet to be validated in clinical practice.

Histopathologic Diagnosis of Solid Pancreatic Lesions: An Algorithmic Approach

As more and more pancreatic resections are being performed in increasingly smaller institutions, experience with the diagnosis of pancreatic tumors has improved exponentially in the past decade. The vast majority of pancreatic lesions encountered in daily practice are solid tumors. Unlike cystic lesions, most of which are either benign or low grade and are often incidentally detected, most solid pancreatic tumors are symptomatic. Solid tumors are regarded in 2 broad categories: One comprises ill-defined, stroma-rich, scirrhous neoplasms represented by pancreatic ductal adenocarcinoma (75%) and chronic pancreatitis, whereas the other encompasses fleshy, stroma-poor, circumscribed neoplasms that correspond to nonductal tumors like NETs (3%–4%), acinar cell carcinoma (ACC; 2%), SPN (1%), and pancreatoblastoma (1%).

Solid-Scirrhous, Ill-Defined Tumors: PDAC and Chronic Pancreatitis

Most PDACs (>90%) are tubule-forming adenocarcinomas, the prototype of pancreatobiliary cancers. They may be deceptively bland, making distinction from reactive ducts of chronic pancreatitis a major diagnostic challenge on cytology and resection specimens (Figure 5). The use of key architectural and cytologic criteria can help with distinction. In terms of architecture, malignant ducts are often increased in number, lose their lobular configuration, and may be localized to the perineural, perivascular, or intravascular spaces (Figure 6). When PDACs invade the nerves and vessels, they become paradoxically well differentiated and start forming ductal units by lining these structures, closely mimicking native ducts or PanIN.

Recent observations have revealed other subtle but important criteria in the distinction of invasive from benign ducts. For example, the finding of a duct next to a thick-walled medium-caliber vessel, or of "isolated solitary ducts"[73] (ie, lying individually within adipose tissue without accompanying fibrosis, acini, or islets), is in fact diagnostic of PDAC, no matter how bland such ducts appear (Figure 6, A). Additionally, malignant ducts have open, round lumina, prominent cytoplasmic vacuoles with granular debris or mucin, and irregular contours, unlike reactive ducts, which are smooth contoured. The surrounding desmoplastic stroma in PDAC is abundant, dense, and myxoid in appearance, with malignant cells typically having enlarged nuclei with cellular disorganization, nuclear irregularity, cytoplasmic vacuolization, abnormal mitoses, and necrosis. A subset of PDACs (foamy gland variant) have abundant foamy/microvesicular cytoplasm, which can be very difficult to distinguish from benign ducts, especially on cytology. Cytoplasmic pallor, fine cytoplasmic vesicles, a luminal

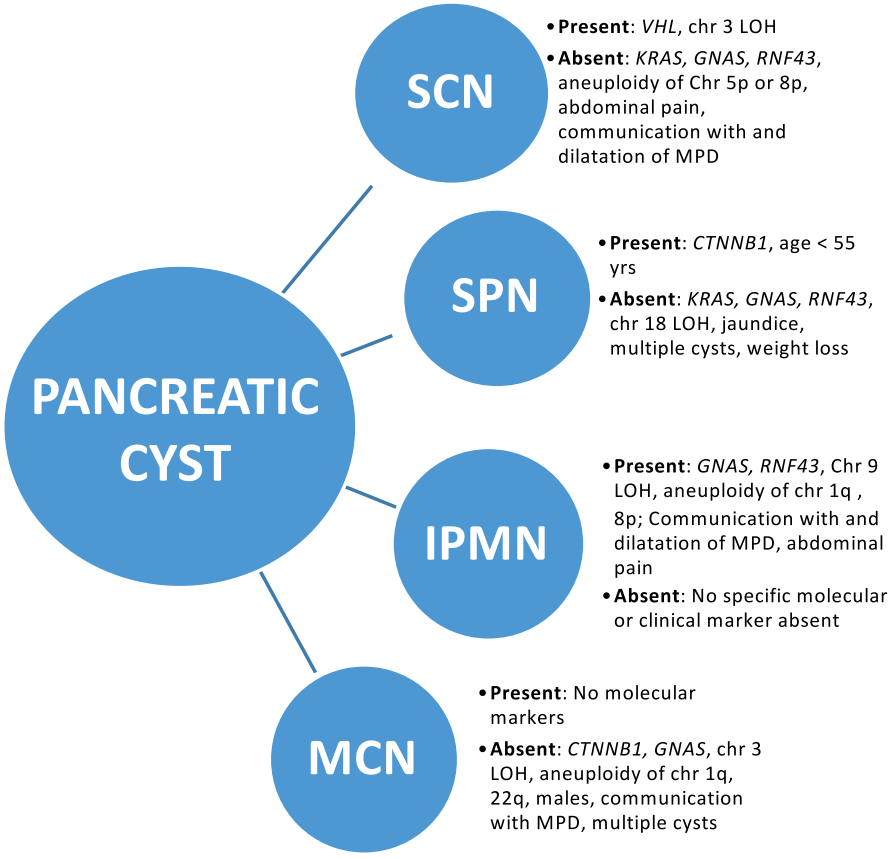


Figure 4. *Flow chart of the most frequent molecular alterations seen in the most commonly encountered pancreatic cysts. Abbreviations: chr, chromosome; IPMN, intraductal papillary mucinous neoplasm; LOH, loss of heterozygosity; MCN, mucinous cystic neoplasm; MPD, main pancreatic duct; SCN, serous cystic neoplasm; SPN, solid-pseudopapillary neoplasm; yrs, years.*

brush-border–like cuticle, and raisinoid nuclei are diagnostic of this subtype (Figure 5).[74]

Molecular and Immunophenotype of PDAC.—There are 4 key driver gene mutations (*KRAS*, *TP53*, *p16/CDKN2A*, and *SMAD4/DPC4*) that orchestrate PDAC development, and their identification in tissues, fluid, and cytologic samples can be very helpful diagnostically. The p53 and Dpc4 immunostains (IHC) are surrogate markers for their respective gene mutations (Figure 7).[58] Somatic missense mutations of *TP53* lead to strong nuclear positivity for p53 protein in PDAC.[75] Wild-type p53 may cause weak to moderate nuclear labeling in reactive ductal cells and gastrointestinal epithelium, a potential diagnostic pitfall.[76,77] *SMAD4/DPC4* inactivation (55% of PDACs) leads to loss of Dpc4 staining in tumor nuclei,[65] but staining is preserved in reactive ductal cells.[76] Additionally, Dpc4 loss is associated with widespread tumor metastasis.[69] Although p53 is not reliable, Dpc4 can be helpful in daily practice, but it should be interpreted cautiously.

Other promising IHC markers that require further scrutiny include placental S100 (S100P) and insulin-like growth factor II messenger RNA–binding protein-3 (IMP-3; Figure 7, D). Mammary serine protease inhibitor (Maspin), IMP-3, S100P, and pVHL may help to distinguish well-differentiated PDAC from reactive ducts. This 4-panel stain was found to identify more than 90% of PDACs (IMP-3, S100P [nuclear and/or cytoplasmic], and Maspin-positivity and pVHL negativity/loss, unlike reactive ductal cells, which

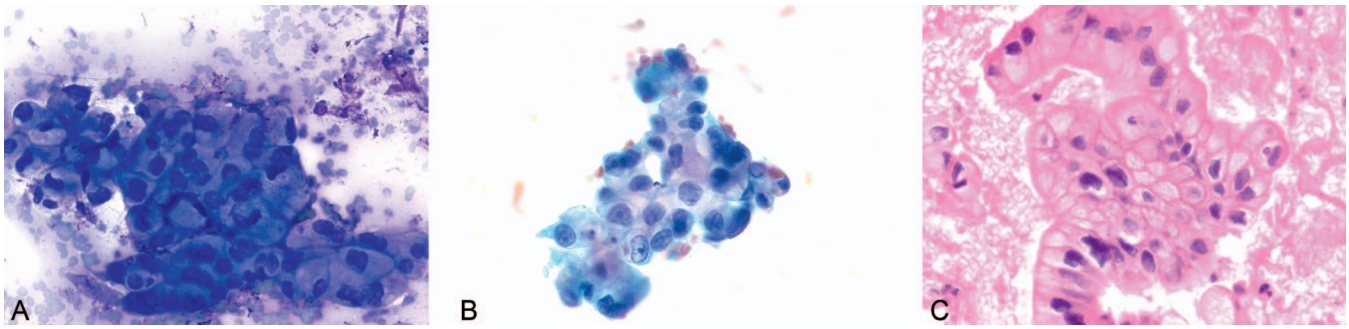


Figure 5. *Fine-needle aspiration of pancreatic ductal adenocarcinoma, foamy gland variant. A, Tumor cells form a relatively flat sheet with nuclear crowding (drunken honeycomb sheets), well-defined cell borders, and voluminous foamy cytoplasm, resembling foamy macrophages. B, Corresponding Papanicolaou-stained smear shows similar cells with nuclear irregularity, hypochromasia, and prominent nucleoli. C, On cell block, tumor cells have pale "foamy" or frothy cytoplasm, well-defined cell borders, and a distinct luminal brush-border–like zone (Diff Quick, original magnification ×400 [A]; original magnification ×400 [B]; hematoxylin-eosin, original magnification ×400 [C]).*

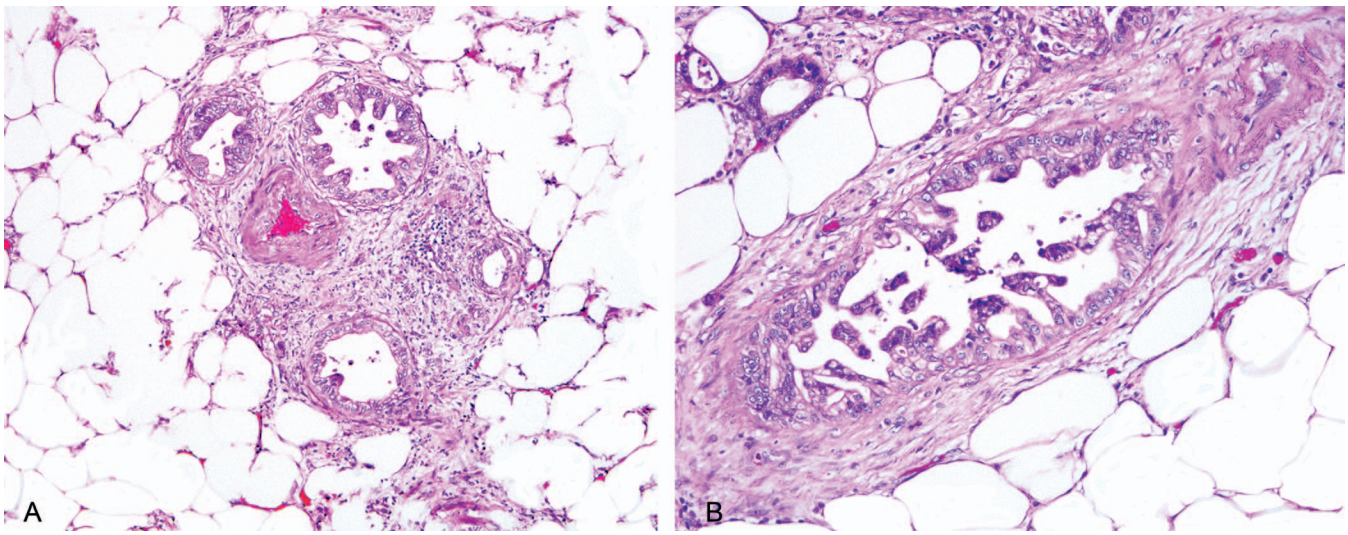


Figure 6. *A, Malignant ducts are located next to a thick-walled, medium-caliber vessel and are diagnostic of adenocarcinoma, despite their bland appearance. B, Well-differentiated pancreatobiliary adenocarcinoma disposed as a ductal unit lining a vessel lumen, a close histologic mimic of pancreatic intraepithelial neoplasia (PanIN; hematoxylin-eosin, original magnification ×200).*

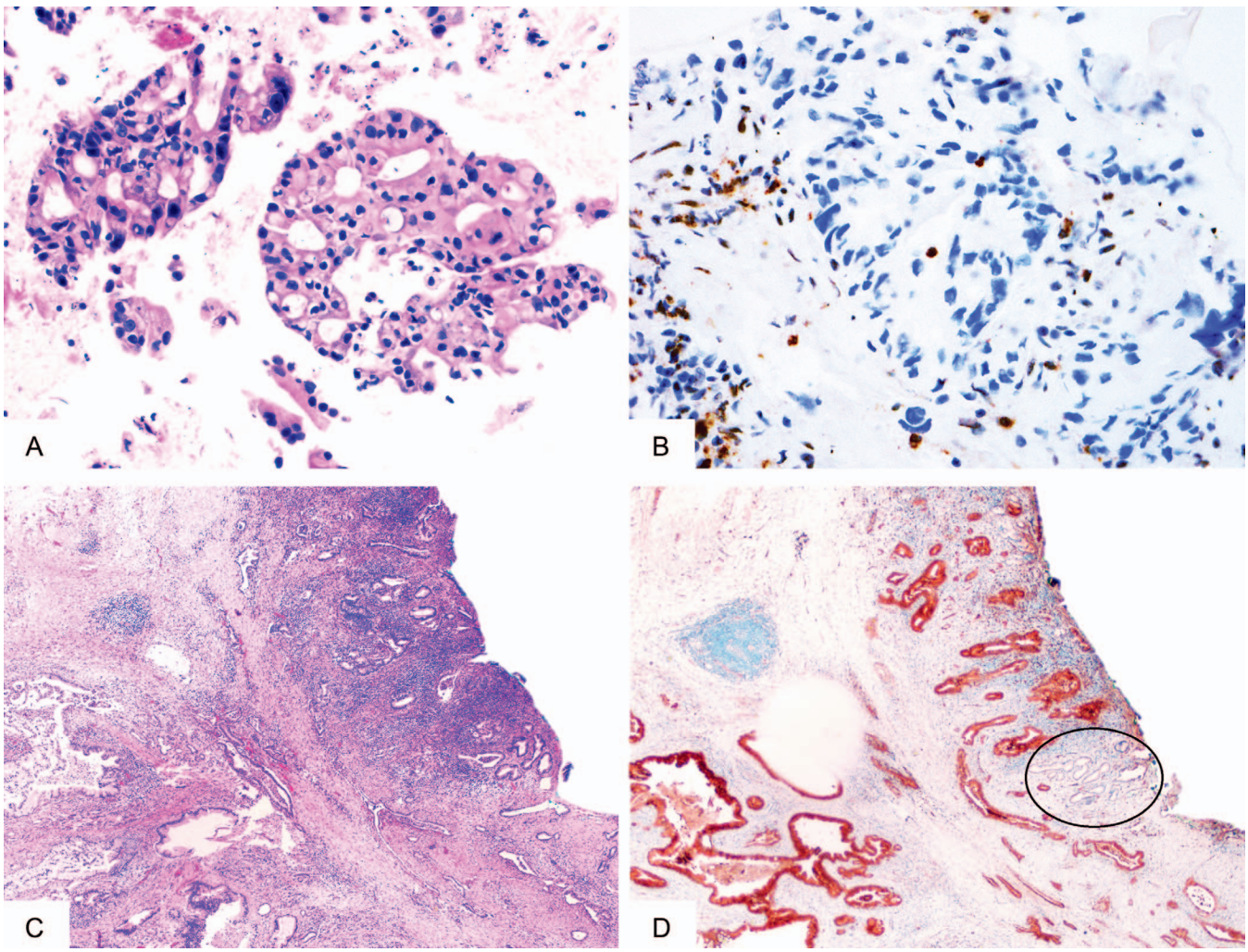


Figure 7. *Fine-needle aspiration of pancreatic ductal adenocarcinoma. A, Cribriforming malignant glands on cell block have prominent vacuolated cytoplasm, and (B) their tumor nuclei show loss of Dpc4. C, The malignant glands in this pancreatic ductal adenocarcinoma infiltrate the wall of the common bile duct, and are positive for S100P (D), in contrast to (circled) benign native peribiliary submucosal glands (hematoxylin-eosin, original magnification ×200 [A and C]; Dpc4, original magnification ×200 [B]; S100P, original magnification ×200 [D]).*

show the opposite results).[70] Maspin and S100P may cross-react with gastrointestinal tract epithelium and should be cautiously interpreted.[70] After extensive literature review, Lin et al[78] recently proposed pVHL, Maspin, S100P, IMP-3, cytokeratin 17, MUC5AC, and Dpc4 as the best IHC panel for diagnosing PDAC in surgical and cytology material. However, further testing is needed to verify this impression. Although annexin A10 and plectin 1 IHC are both positive in (>75%) PDACs, they are not used routinely.[79,80] Similarly, microRNA alterations (MiR-21, MiR-155, and MiR-221) are not yet considered reliable.[81,82]

Demarcated, Cellular, Stroma-Poor Neoplasms: Nonductal Tumors or Secondary Neoplasms.—In the differential diagnosis of solid pancreatic neoplasms, the second major category is demarcated, cellular, and stroma-poor tumors. These nonductal, nonmucinous tumors are relatively rare, solid, fleshy, demarcated neoplasms with sheetlike growth pattern. Tumors with this pattern include acinar carcinomas, SPNs (discussed above), pancreatoblastomas, NENs, and metastatic tumors.

NENs of the Pancreas.—On cytology, well-differentiated PanNETs are typically composed of bland, singly dispersed, or loosely cohesive plasmacytoid cells with eccentric nuclei and salt-and-pepper chromatin. Poorly differentiated pancreatic neuroendocrine carcinoma (PanNEC) of small cell type forms clusters of small to intermediate-sized malignant cells with high nuclear to cytoplasmic ratio, nuclear molding, crush artifact, brisk mitotic activity, and single-cell and/or confluent necrosis. Large cell carcinoma has larger cells with more abundant cytoplasm and prominent nucleoli.

Immunohistochemistry in the Diagnosis of Pancreatic NENs.—Neuroendocrine neoplasms are positive for pancytokeratin and CAM5.2, and they variably express neuroendocrine markers (synaptophysin, chromogranin A, and CD56). CD56 is the most sensitive but least specific neuroendocrine marker, whereas chromogranin A is the most specific but least sensitive neuroendocrine marker. Cytokeratin 19 (a pancreatic ductal lineage marker) and CD117 are sometimes expressed by PanNETs, and although they are proposed as adverse prognostic markers, they are not used routinely.[83–86] Ki-67 is now a required parameter in the grading of all pancreatic NENs (see discussion above).

Molecular Studies in the Diagnosis and Prognosis of Pancreatic NENs.—The most common, recurring mutation in PanNETs involves the death domain–associated protein (*DAXX*) and the α-thalassemia/mental retardation X-linked (*ATRX*) genes, and is detectable in almost half the cases.[87] These nuclear proteins are involved in telomere maintenance, and mutation results in loss of/negative tumor cell staining with retention in nonneoplastic tissue.[87] DAXX and ATRX loss is associated with increased metastasis and shorter survival in PanNETs but has not been identified in PanNECs.[88,89] Germ line *MEN1* mutations occur in multiple endocrine neoplasia 1 syndrome patients, 65% of whom develop PanNETs. Up to 45% of sporadic PanNETs also show somatic *MEN1* mutations or LOH at the *MEN1* locus.[87,90] The mammalian target of rapamycin (mTOR) cell signaling pathway is also rarely mutated in PanNETs (15%) and involves somatic mutations in *PIK3CA*, *PTEN*, and *TSC2*.[87] The mTOR pathway alteration is a potentially exploitable therapeutic target, because target-specific therapeutic drugs have recently been developed.[91] PanNETs do not show *KRAS*, *CDKN2A*, or *SMAD4* mutations but may rarely show *TP53* mutation. Up to 17% of patients with VHL syndrome develop PanNETs. *VHL* gene deletion occurs in up to 25% of sporadic PanNETs.[75] Chromosomal gains and losses have also been identified in NENs, and when frequent are associated with worse prognosis.[75]

PanNECs (high-grade neuroendocrine carcinomas of small cell or large cell type), on the other hand, are infrequently associated with MEN1 and retain DAXX and ATRX immunoreactivity.[89,92] They are positive for p53 (95%), consistent with mutated *TP53*. Retinoblastoma (*RB-1*) gene is also frequently mutated (>50%) in PanNEC and is associated with Rb protein loss in 60% to 90% of cases. Rb-positive PanNECs often show concurrent p16 loss, suggesting mutually exclusive roles in pathogenesis.[89] Conversely, PanNETs retain p53, Rb, and p16. BCL2 protein, which is overexpressed in pulmonary small cell carcinoma, is also overexpressed in PanNECs (100% in small cell and 50% in large cell tumors), but it is negative in grade 1 and is variably expressed in grade 2 PanNETs.[89] Platinum-based chemotherapy used to treat small cell lung carcinoma induces apoptosis via a BCL2-regulated pathway, suggesting that BCL2 antagonists could similarly be used in the treatment of PanNECs.

Acinar Cell Carcinoma.—Acinar cell carcinoma is a rare pancreatic tumor of acinar lineage. It occurs in older adults but may rarely be seen in children. Tumors have characteristic morphology (acinar or rosettelike pattern, or diffuse monotonous round cells with single prominent nucleoli and basophilic cytoplasm) and IHC features (expression of enzyme markers, especially trypsin)[93,94]; however, cytohistologic misdiagnosis (particularly as NEN) is not uncommon.[95]

Immunohistochemistry in the Diagnosis of ACC.—Immunohistochemistry is critical to the accurate diagnosis of ACC because it often closely resembles neuroendocrine tumors, SPN, and pancreatoblastoma, as well as normal pancreatic acini. Tumor cells are positive for pancytokeratin and the enzymes trypsin, amylase, lipase, elastase, and phospholipase 2, trypsin being the most sensitive and widely used in daily practice.[94] BCL10 is also positive in most ACCs, including trypsin-negative ones, and may be positive in adenosquamous carcinoma, but it is negative in PanNETs, PDAC, and SPN.[96] A major pitfall in the diagnosis of ACC is its IHC and morphologic resemblance to NETs, for which it is frequently mistaken.[94] Additionally, scattered neuroendocrine cells are not uncommon in ordinary ACCs. Moreover, mixed acinar-neuroendocrine carcinoma, which by definition has more than 30% neuroendocrine differentiation, must be distinguished from ACC. Although this diagnosis has been made on cytologic samples (due to coexpression of acinar and neuroendocrine markers), it is best reserved for resections.[95,97]

Molecular Studies in the Diagnosis of ACC.—Acinar cell carcinoma is genomically distinct from other pancreatic cancers and shows *APC*, *TP53* alterations,[98,99] as well as *RAF* gene fusions (23% of pure and mixed cases) and mutually exclusive inactivation of DNA repair genes (in 45%).[100] *SND1-BRAF* fusions are the most frequent and cause activation of the MAPK pathway, which is abrogated by inhibition of MEK. *SND1-BRAF*–transformed cells are sensitive to the chemotherapeutic MEK inhibitor trametinib. Acinar cell carcinomas lacking *RAF* rearrangements have numerous genomic alterations that cause inactivation of DNA repair genes, and increased sensitivity to platinum-based therapies and PARP inhibitors, thus representing potential new therapeutic targets in these tumors. Acinar

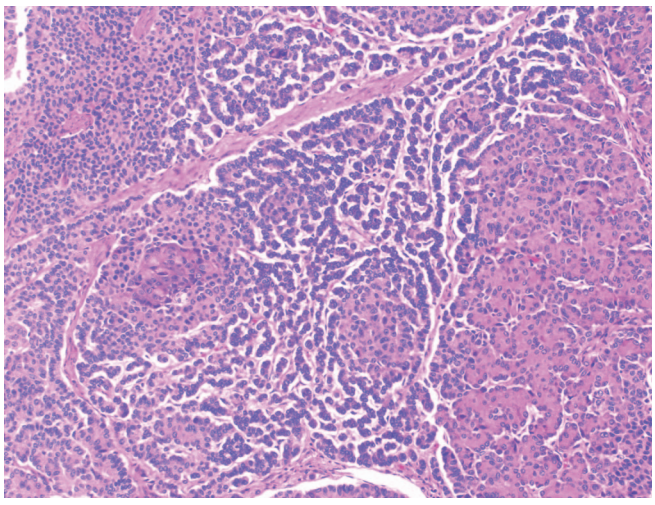


Figure 8. *Pancreatoblastoma tumor cells are arranged in sheets and show extensive acinar differentiation with focal central, ill-defined squamoid morules surrounded by more neuroendocrine cells with arborizing ribbonlike pattern (hematoxylin-eosin, original magnification ×200).*

cell carcinomas show allelic loss on chromosome 11p. *APC*/β-catenin mutations are rarely described and result in nuclear and cytoplasmic staining with β-catenin, a potential source of confusion with SPN.[101] The common genetic mutations of PDAC (*KRAS, p16/CDKN2A, p53* and *DPC4/SMAD4*)[65] and NENs (*DAXX/ATRX*) have not been described in ACC.[75]

Pancreatoblastoma.—Pancreatoblastoma (PB) is an extremely rare, malignant primary pancreatic tumor that is often seen in children but may be seen in adults. Tumors show multiple lines of differentiation, particularly acinar but also ductal and neuroendocrine. Tumors may be associated with familial adenomatous polyposis or related syndromes. Squamoid morules are a characteristic feature but are difficult to identify in cytologic samples except on cell block material (Figure 8).[102,103]

Immunohistochemistry in the Diagnosis of Pancreatoblastoma.—Immunolabeling in PB is related to the tumor's line of differentiation. Acinar differentiation, which is the most common, results in trypsin, chymotrypsin, lipase, and BCL10 positivity, making distinction from ACC challenging unless one identifies pathognomonic squamoid morules, and even primitive (blastemal) elements. The squamoid morules in PB contain "biotin-rich optically clear nuclei" and are believed to be a surrogate manifestation of (aberrant) nuclear/cytoplasmic β-catenin coupled with overexpressed estrogen receptor-β (ER-β) as a part of the "BROCN" group of tumors.[104] ER-β and β-catenin can be diagnostically helpful by highlighting squamoid morules. Typical squamous marker cytokeratin 5/6 is, interestingly, negative in squamoid morules.

Molecular Studies in the Diagnosis of Pancreatoblastoma.—The most common genetic abnormality in PB is allelic loss of chromosome 11p.[105,106] Cases associated with FAP and related syndromes show the molecular stigmata of these syndromes. Somatic alterations in the APC/β-catenin pathway (*CTNNB1* and *APC*) are also described, but PDAC-related mutations are not.[105]

BILIARY LESIONS: EVOLVING APPROACHES TO DIAGNOSIS

New Sampling Techniques

Endoscopic retrograde cholangiopancreatography provides fluoroscopic images of the biliary tree and is a mainstay of diagnosis and intervention in patients with biliary pathology. Worrisome cholangiographic features include stricture length greater than 14 mm, irregularity, abrupt shelflike borders, intraductal nodularity, and simultaneous common bile duct and pancreatic duct dilation (double duct sign).[107] The biliary tract is frequently sampled by brush cytology, allowing for cytologic analysis as well as fluorescent in situ hybridization (FISH) and molecular studies (see section below). The triple-modality approach (brush cytology, forceps biopsy, and FISH) has shown significantly increased sensitivity.[108] Interpretation of biliary cytology is extremely challenging, with poor sensitivity rates of 6% to 64%.[108–112] When combined with forceps biopsy there is some improvement in sensitivity of detection of malignancy.[113] We recently showed a concordance rate of 61% between brushing and concurrent biopsy diagnosis, with biopsies having higher sensitivity (69%) and accuracy (80%) than bile duct brush cytology (42% and 69%, respectively) in detecting malignancy (18 of 30 on biopsy versus 8 of 30 on brushing).[114] EUS-FNA has also been used for assessing biliary lesions; however, there is concern for needle track seeding, especially in proximal bile duct lesions. Prior needle sampling of biliary tumors can be an exclusion criterion for orthotopic liver transplant; therefore, intraductal sampling is indicated if patients are being considered for transplantation under a cholangiocarcinoma protocol.[107,115]

Direct cholangioscopy allows for endoscopic visualization of the bile duct lumen during endoscopic retrograde cholangiopancreatography. During the procedure, a second small-caliber cholangioscope is passed through the working channel of the duodenoscope and advanced into the bile duct over a wire. Strictures can be directly visualized, and visually directed biopsies can be performed (Figure 9). Compared with standard forceps and brush cytology, miniforceps biopsies have the highest sensitivity, accuracy, and negative predictive value.[116] Intraductal ultrasound can be performed by passing a small-caliber EUS probe through the working channel of the duodenoscope and into the biliary tree. Radial endosonographic images can then be obtained by imaging the duct wall layers and visualizing intraductal mass lesions and irregularities; however, no tissue diagnosis is obtained with intraductal ultrasound.[117] Confocal laser endomicroscopy is an emerging imaging modality for the biliary indications that uses a low-power laser to detect reflected fluorescent light through high-resolution contact imaging of the biliary epithelium. The procedure provides real-time imaging at cellular and subcellular resolutions. The imaging probe is passed through an endoscopic retrograde cholangiopancreatography catheter or cholangioscope. Researchers are evaluating the ability of the procedure to distinguish normal/inflammatory changes from neoplasia.[118,119] Further studies and more detailed evaluation of interobserver agreement may refine the role of this technology.[120]

Because of the notoriously low sensitivity rate of biliary brushings in diagnosing malignancy (6%–64%),[112] numerous cytomorphologic features have been proposed to improve the test's performance. We recently had 7 pathologists (with and without cytopathology and/or

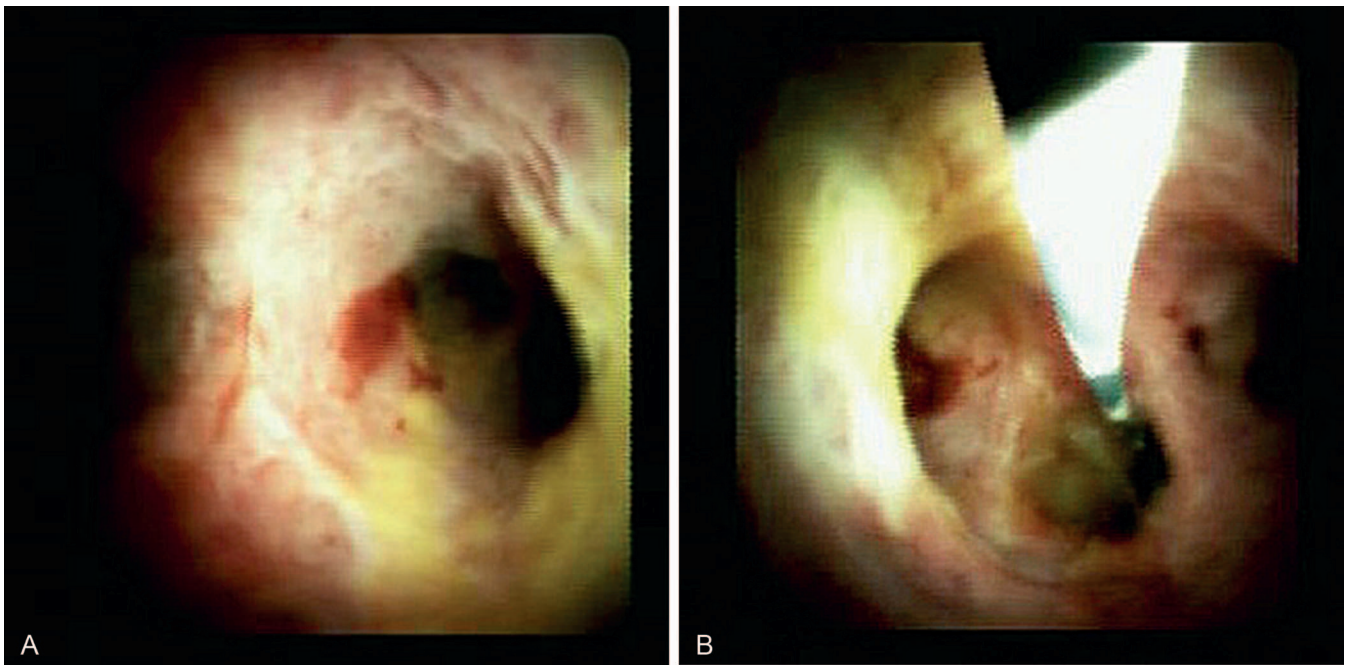


Figure 9. *A, High-resolution digital cholangiographic image of the biliary tree in a female patient with an indeterminate biliary stricture. The biliary epithelium is erythematous and friable, with some pale regions suggestive of chronic scarring. No frank mass lesion was visualized, and visually directed biopsies were negative for malignancy. B, High-resolution digital cholangiographic image of the biliary tree in a patient with indeterminate biliary stricture. In this image, the guide wire can be seen passing proximally into the biliary tree. A trifurcation of bile ducts is seen. The biliary epithelium is mildly erythematous and friable, without a mass lesion.*

gastrointestinal pathology training) review 60 biliary brushings (30 with histologically proven malignancy and 30 benign, with ≥18 months of follow-up) and identified several statistically significant malignant characteristics, including nuclear hypochromasia/hyperchromasia (70% versus 10% in benign), nuclear irregularity (67% versus 13% in benign), pleomorphism (62% versus 3% in benign), 2-cell population (57% versus 0% in benign), 3-dimensional groups (52% versus 3% in benign), high (>50%) nuclear to cytoplasmic ratio (48% versus 3% in benign), cytoplasmic mucin vacuoles (43% versus 13% in benign), cellular discohesion (38% versus 3% in benign), hypercellularity (23% versus 0% in benign), and prominent nucleoli (21% versus 0% in benign), with pleomorphism carrying the greatest odds of malignancy (odds ratio, 47.46; 95% confidence interval, 5.64, 399.29).[121] When malignant characteristics were each given numeric weight of "1", only 1of 22 samples (4.55%) with 3 or more malignant characteristics turned out to be benign. For every 1-unit score increase, the odds of malignancy increased 1.82-fold.

New Perspectives on the Classification of Bile Duct Neoplasms

The changes in classification and terminology of preinvasive lesions, including better characterization of intraductal papillary neoplasms of the bile ducts and the recognition of ITPN, were discussed previously. The diagnosis of invasive adenocarcinoma of biliary tract or cholangiocarcinoma continues to be challenging at both clinical and pathologic levels. Most tumors are tubule forming (well-differentiated) and closely mimic reactive changes, particularly in patients with primary sclerosing cholangitis, inflammation, or stents. Complicating this differential is the fact that there is a well-established inflammation-carcinoma sequence in the biliary tract, but it is often difficult to distinguish where one ends and the other begins. This overlap extends not only to morphologic findings but to molecular findings as well. But for management purposes, regenerative atypia must be distinguished from well-differentiated adenocarcinoma, and cytomorphology alone is often insufficient for distinction, particularly in specimens with low cellularity. Some have used ancillary diagnostic tools, such as forceps biopsy and FISH, in conjunction with brush cytology (triple-modality testing), which has better sensitivity (82%) than brush cytology alone (sensitivity 27%).[108]

FISH in the Diagnosis of Bile Duct Carcinoma.—FISH is an established adjunctive tool for diagnosing pancreatobiliary carcinoma and differentiating it from mimics.[108,122] The commercial UroVysion FISH probe kit (Abbott Molecular Inc, Des Plaines, Illinois) consists of 4 probes directed against chromosomes 3, 7, 9p21, and 17, and has a higher sensitivity (34%–53%) than cytology (27%–40%).[108,123] For UroVysion FISH–positive brushings, independently predictive morphologic criteria of malignancy include single cells, irregular nuclei, and nuclear enlargement.[124] UroVysion is also positive in nonurothelial tumors, including some lung, colon, renal, and gynecologic cancers.[125–127]

More recently, a pancreatobiliary-specific FISH probe set (PB FISH) was created for cholangiocarcinoma and PDAC in the hopes of identifying more cancers than UroVysion.[128] PB FISH increased the rate of cancer detection by 19% compared with UroVysion, and had 93% sensitivity and 100% specificity in tissue samples. It also had a higher sensitivity than UroVysion on brushings (65% versus 46%; $P < .001$) and cytology (19%; $P < .001$), but it had a specificity (93%) similar to both (UroVysion, 91%; cytology, 100%).[128] The PB FISH probes target the oncogenes *MCL1* on chromosome 1q, *EGFR* on chromosome 7p, and *MYC* on

chromosome 8q, all frequently gained in these tumors.[129] The *p16/CDKN2A* tumor suppressor gene located on the 9p21 locus is often deleted in dysplastic and invasive pancreatobiliary cancers.[130,131] Both the PB FISH probe set and UroVysion need to be validated in larger studies, although they are already being used by some gastroenterologists.

Immunohistochemistry in the Diagnosis of Bile Duct Carcinoma.—Although FISH is an established diagnostic tool in pancreatobiliary specimens, IHC is not routinely used. A 4-marker IHC panel for distinguishing cholangiocarcinoma from reactive epithelium that includes S100P, pVHL, mCEA, and IMP3 has been advocated but needs further scrutiny. Gallbladder and extrahepatic cholangiocarcinoma are $pVHL^{-}/S100P^{+}/IMP3^{+}/mCEA^{+}$, whereas reactive gallbladder/biliary epithelium is $pVHL^{+}/S100P^{-}/IMP3^{-}/mCEA^{-}$.[132–134] GLUT1, P-cadherin, CD24, and p53 are other potential distinguishing markers under investigation for the diagnosis of gallbladder adenocarcinoma and are positive in this tumor but negative in reactive gallbladder mucosa.[135,136] p16 is positive in almost half of gallbladders with high-grade dysplasia and a quarter of invasive gallbladder cancers but is negative in normal or reactive gallbladder epithelium.[137] However, none of the latter stains are used routinely for diagnosis.

Molecular Studies in the Diagnosis of Bile Duct Carcinoma.—Dudley et al[138] recently compared targeted NGS of 81 biliary duct brushings to UroVysion and cytology for the detection of higher-risk neoplasia or malignancy. The NGS identified driver gene mutations in 30% ($n = 24$ cases), including mutations in *KRAS* (21 of 24; 88%), *TP53* (14 of 24; 58%), *CDKN2A* (4 of 24; 17%), and *SMAD4/DPC4* (6 of 24; 25%). Cytology had 67% sensitivity and 98% specificity for cancer detection. When NGS and cytology were combined, NGS increased the sensitivity of cancer detection to 85%, whereas UroVysion only increased sensitivity to 76%.[138]

CONCLUSIONS

The frequent discovery, sampling, and resection of lesions of the pancreatobiliary tract have led to a rapid rise in the number of such specimens, now a routine part of pathologists' practice. Accordingly, advances in our knowledge of the underlying molecular events driving the pathogenesis of many of these neoplasms have led to new classification schemes, diagnostic terminologies, and highly sophisticated diagnostic modalities, some of them complementary and others superior to those in current use. The pathologist's role is crucial in the judicious incorporation of these updates into routine practice and reporting, which provides new opportunities for improvements in diagnostic accuracy and precision medicine, as well as potential therapeutic targets for these tumors.

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