Pancreatic Cytopathology
A Practical Approach and Review

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Context.—Pancreatic cytopathology plays an important role in the diagnosis and management of patients with solid and cystic lesions of the pancreas.

Objective.—To serve as a practical guide to pancreatic cytopathology for the practicing pathologist.

Data Sources.—A comprehensive assessment of the medical literature was performed.

Pancreatic cancer is the fourth leading cause of cancer death in the Western world, and in spite of tremendous efforts to advance our knowledge and the treatment of the disease, mortality rates have remained relatively unchanged for the last 40 years. Although harrowing, this reality stands in stark contrast to our ever-expanding understanding of pancreatic cancer. Indeed, our understanding of the clinical, radiologic, and pathologic aspects of pancreatic cancer has advanced greatly during the past 20 years and has allowed us to recognize at least a small fraction of preinvasive or early invasive disease, potentially leading to some reduction in the disease's overall mortality.

The marriage of cytology and radiology has allowed for minimally invasive, safe, accurate, and cost-effective diagnosis of pancreatic lesions, previously accessible only by laparotomy. As a result, cytologists are increasingly called upon to diagnose disease in specimens procured under image guidance. This review is intended as a practical guide for the practicing cytopathologist. We open with a discussion of the role of pancreatic cytopathology in patient management and continue with a discussion of specimen types and specimen processing, sensitivity and specificity of brush cytology and fine-needle aspiration (FNA), and the significance of "suspicious" and "atypical" diagnoses. We then describe an algorithmic approach to the diagnosis of pancreatic cytology specimens. A more detailed discussion of the diagnostic features of specific lesions then follows. Comments on routine ancillary techniques (eg, immunohistochemistry and cyst fluid analysis) will be interspersed where appropriate. Finally, we close with a discussion of advanced diagnostic techniques (eg, mutation analysis and fluorescent in situ hybridization [FISH]), as they apply to pancreatic cytology specimens.

Conclusions.—We review pancreatic cytopathology, with specific discussions of its role in patient management, specimen types and specimen processing, specific diagnostic criteria, and the use of ancillary testing and advanced techniques.

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THE ROLE OF PANCREATIC CYTOPATHOLOGY IN PATIENT MANAGEMENT

Pancreatic cytopathology is uniformly embraced in the setting of unresectable disease at presentation (including advanced locoregional and metastatic disease and disease deemed unresectable owing to comorbidities) and to confirm the clinical impression before instituting cytotoxic or radiation therapy. It has also found an application with a subset of patients with unresectable disease who are enrolled in treatment protocols that include neoadjuvant therapy. It is also frequently cited as useful for ruling out metastases in patients who have had prior malignancies.

Using cytology for a patient deemed to have a cancer that is clinically and radiologically resectable is somewhat more controversial. In a review of biopsy techniques for pancreatic neoplasms, Goldin and colleagues assert that "[g]enerally, pancreatic masses should not be biopsied prior to attempted resection." They argue that in seemingly straightforward cases, FNA increases costs, is associated with increased morbidity (including the risk of tumor seeding), delays diagnosis, and has an unacceptably modest negative predictive value. They concede, though, that "[i]f the results of FNA will change the management of the patient, FNA should be undertaken."

Surely, one could take issue with the claims of morbidity, "seeding," and diagnosis delay, and wonder furthermore about the overall relative increased cost associated with the procedure. Finally, the authors' concession that FNA should be performed if the results have the potential to change patient management seems almost paradoxical because cases are not always as straightforward as they might seem (Figure 1). For example, in Mesa and colleagues' series of metastatic lesions diagnosed by pancreatic endoscopic ultrasound–guided FNA (EUS-FNA), 4 of 11 metastases were presumed clinically to represent pancreatic primary neoplasms. There also is frequent clinical and radiologic overlap between chronic pancreatitis and...
ductal adenocarcinoma.4 Autoimmune pancreatitis (lymphoplasmacytic sclerosing pancreatitis), a newly described and increasingly recognized cause of chronic pancreatitis, is especially likely to mimic malignancy, with 50% and 63% of patients presenting with obstructive jaundice or a mass, respectively, in the series of Deshpande et al.5 While we acknowledge that a negative cytology result has modest predictive value, it is not equal to a positive cytology result. Such a result may often prompt additional testing (eg, determining serum immunoglobulin G4 [IgG4] concentration), which in some cases, may save the patient from having to undergo an unnecessary surgery. Even in the best of hands, surgery is associated with much greater morbidity than is any cytologic sampling technique.1

At our institution, rather than causing delay, cytology actually facilitates timely diagnosis and management and is an integral part of a multimodal approach to pancreatic tumor diagnosis. Depending on the clinical presentation, patients examined at or referred to our institution undergo a combination of computed tomography, ultrasound, magnetic resonance imaging (MRI), magnetic resonance cholangiopancreatography, endoscopic retrograde cholangiopancreatography (ERCP) (especially for patients with obstructive jaundice and bile duct stricture) with biliary brushing, and EUS (especially for patients with small mass lesions, including lesions in the body and tail, and for patients with cystic lesions) with FNA. We have close working relationships with our expert gastroenterologists, specially trained in these procedures and with sophisticated knowledge of pancreatic pathology. We provide on-site interpretation, which, as discussed below, increases the diagnostic yield of the procedure through our directive for additional needle passes as necessary.6,7 The process culminates in a multidisciplinary conference (ie, tumor board) where the results of clinical, radiologic, cytologic, and laboratory evaluations are discussed, and a treatment is planned.

SPECIMEN TYPES AND PROCESSING

In our practice, most pancreatic cytopathology specimens are procured either by EUS-guided FNA (EUS-FNA) or by ERCP. Most specimens obtained by ERCP are brush samples, although we occasionally evaluate ductal aspirates.8 At institutions without an active EUS program, percutaneous aspirates acquired under either computed tomography or ultrasound guidance may be more frequently seen.9 The number of intraoperative cytology specimens is inversely proportional to the prominence of preoperative cytology at a given institution and are now only rarely seen.9,10 Less often, cytologic specimen types include samples obtained for stent cytology and from peritoneal aspirates or washings.11-15

Endoscopic ultrasound–guided FNA is certainly emerging as the dominant modality for the morphologic assessment of solid and cystic pancreatic masses.16,17 In brief, endoscopists place powerful ultrasound transducers (echoendoscopes) against the gastric or duodenal wall, in close apposition to the lesion of interest, and are able to obtain a resolution superior to that of other techniques. Fine-needle aspiration then increases diagnostic accuracy. This technique addresses many of the aforementioned criticisms leveled by Goldin et al.3 Powis and Chang18 assert that EUS-FNA represents a cost-effective tool in the management of all patients with pancreatic tumors through its ability to image and sample small pancreatic lesions (<20 mm), to accurately and preoperatively stage pancreatic cancers (by imaging the neurovascular axis and by imaging and sampling peripancreatic lymph nodes), and to potentially deliver palliative celiac neurolysis. The complication rate is also very low compared with that of computed tomography–guided pancreatic FNA (1% to 2% vs 5%). Potential complications include bleeding, pancreatitis, and infection, the latter being more prominent from the aspiration of cystic lesions. The EUS-FNA needle traverses fewer structures and the use of color Doppler guards against the risk of vascular perforation.9,16 Regarding peritoneal seeding, in a retrospective study of patients with pancreatic cancer who were diagnosed by either EUS-FNA or percutaneously, the incidence of positive findings from peritoneal cytology (at restaging after neoadjuvant therapy) was 2.2% versus 16.3%, respectively.14 Also of note, for pancreatic head lesions (sampled through the duodenum), the EUS-FNA tract would be encompassed in a Whipple resection.3 The clinical significance of positive findings from peritoneal cytology is not straightforward, with Meszoely et al15 reporting a lack of statistically significant difference in time to recurrence and only a trend toward decreased survival (on the order of 4 months) in a group of 135 patients with resectable disease, 13 of whom had positive peritoneal cytology results.

Ideally, we attend the FNA procedures, both EUS-guided and computed tomography–guided, and perform on-site interpretation with air-dried, Diff-Quik–stained smears (Siemens Healthcare Diagnostics, Deerfield, Illi- nois). At the same time, we make smears and fix them in ethanol to be stained via the Papanicolaou method when we return to the laboratory. On-site interpretation is advantageous because it allows for both discont inuing the procedure once diagnostic material is obtained, and for the gathering and triaging of additional material when necessary.6,7 This type of participation generally increases the diagnostic yield with cytology specimens. Additional material can be triaged for cell block preparation, flow cytometry or other studies depending on what is seen on-site (the use of cell block with pancreatic cytology specimens will be detailed below). On-site interpretation also

Figure 1. A late-enhancing, 2.9-cm, well-circumscribed nodule within the pancreatic tail, initially believed to represent a pancreatic endocrine tumor. Patient underwent resection of the tail of the pancreas and spleen and was found to have an intrapancreatic splenic rest (splenunculus).
allows for direct communication between the pathologist and the person obtaining the sample, ie, the endoscopist in the case of EUS-FNA. We believe that detailed understanding of the clinical and radiographic impressions is paramount to providing the best service possible when interpreting pancreatic cytology specimens (this also will be discussed in more detail as we review the specific cytologic features seen with aspirates from various pancreatic lesions).

Cell blocks can be constructed by using a variety of methods, and we have described our method elsewhere. Briefly, we allow bloody material gathered by FNA to clot on a slide, scrape it into 10% buffered formalin, and process it by routine histology. Anecdotally, we have had better experience by using immunohistochemistry with this material than we have had by using immunocytochemistry with smears or other cytologic preparations. This result is likely to be laboratory dependent, however.

Brushing and intraductal aspirates procured by ERCP are usually processed via monolayer technology at our institution, that is, they are collected in Cytolyt and ThinPrep slides are made (Cytyc, Marlborough, Massachusetts). This is done for ease and cytoconcentration and to reduce artifact secondary to air drying, which is seen more frequently with these smears because cytotechnologists or cytopathologists are usually not present at the time of sample collection. Most studies have shown increased sensitivity for the diagnosis of malignant bile duct brushings when the ThinPrep (Cytyc) technique is used.

Sensitivity and Specificity of Brush Cytology and FNA for the Diagnosis of Pancreatic Malignancy

The sensitivity of brush cytology is usually around 50%, while the specificity approaches 100%. This low sensitivity is often a result of sampling, although interpretation certainly plays a significant role. For a brushing to be positive, malignant cells must be present endoluminally, a condition not always present when the duct is strictured secondarily to external compression. Furthermore, brushings are not performed under direct visualization, so focal lesions may be missed and incorrect areas may be sampled. The duct can also be lined by significantly reactive epithelia whose formation is secondary to whatever process induces the stricture, a fact that leads to greater difficulty for interpreting brushings than FNAs. Logrono et al analyzed the reasons for false-negative diagnoses in a series of 183 pancreaticobiliary brushing specimens (with 36 false negatives) and found the causes to be sampling errors (67%), interpretive errors (17%) due to missing focal tumor on the glass slides, and technical errors (17%) due to artifact caused by extensive drying. Despite its modest sensitivity, the procedure is widely accepted as an adjunct to ERCP because it adds little cost, does not increase morbidity, and has the potential to secure a definitive diagnosis.

The reported sensitivities for EUS-FNA vary widely, from 60% to 100%, with a mean of about 80%, while the specificity again approaches 100%,9,31–45 Factors affecting the sensitivity of a study include (1) the size of the lesions, (2) whether lesions are solid or cystic (many of the studies with the highest reported sensitivities have restricted their analysis to solid tumors), (3) the experience of the EUS endosonographer (one of the barriers to the universal adoption of EUS is its steep learning curve), (4) the number of needle passes, and (5) the availability of a cytopathologist for on-site interpretation (which has been shown to increase the diagnostic yield by 10%–15%). A retrospective analysis of the accuracy of EUS-FNA, percutaneous FNA, and intraoperative FNA has shown that the techniques are equivalent, although lesions sampled by EUS-FNA were often smaller.

Of note, variability in the sensitivities reported in the literature may be attributable to the presence of suspicious, atypical, and nondiagnostic cases in the calculation of test characteristics. For example, Eloubeidi and colleagues reported a sensitivity of 94.7% in a series of 101 EUS-FNAs of solid pancreatic lesions for patients with suspected pancreatic cancer. Five cases were interpreted as suspicious (all proved to be malignant on follow-up), and 6 cases were interpreted as atypical (5 proved to be malignant). Two cases (2%) were nondiagnostic (both proved to be malignant). Their reported sensitivity is based on a calculation that includes suspicious and atypical cases as true positives and excludes the nondiagnostic cases from the data analysis. Including the 2 nondiagnostic cases in the calculation decreases the sensitivity to 92.3%; furthermore, if the cases interpreted as atypical would have been interpreted as false negatives, as is not uncommon, the calculated sensitivity would be 85.9%.

This is not intended as a criticism of Eloubeidi’s group whose nondiagnostic rate of 2% is excellent and probably reflects the fact that their analysis was restricted to solid tumors and that an on-site cytopathologist was present at the EUS-FNA. Their inclusion of atypical cases in the true positive group seems clinically appropriate, given the outcomes in these 6 patients. Rather, this observation is intended as a caution to underscore the fact that a test characteristic is more than just a number, that varying sensitivities between studies must be thoughtfully compared, and that it may be instructive for an individual laboratory to analyze its own test characteristics.

We recently correlated the results of preoperative cytology with the final histologic results obtained for all pancrea resected during a 14-year period, from July 1991 through December 2005, at the University of Virginia (Charlottesville) in an attempt to understand better the causes of false-negative pancreatic cytology. We considered all cytologic diagnoses that would fail to prompt a resection for resectable lesions as false negatives. Overall sensitivity was 74%, with sensitivities for brush cytology (46%) and FNA (85%) approximating the values reported in the literature. Intraductal aspiration had a sensitivity of 66%. Sensitivity for the diagnosis of lesions smaller than 1 cm was 67%, while it was 82% for lesions between 1 and 3 cm. Sensitivities for specific diagnostic entities were as follows: 79% for ductal adenocarcinoma, 100% for acinar cell carcinoma, 75% for pancreatic endocrine tumor, 70% for intraductal papillary mucinous neoplasms (IPMN), and 42% for mucinous cystic neoplasms (MCN).

That specificity approaches 100% in most series is a testament to the application of solid criteria for the diagnosis of pancreatic neoplasia and reflects pathologists’ understanding of the gravity of a positive diagnosis, which generally leads to pancreactectomy or chemotherapy. Potential sources for false-positive diagnoses include the overinterpretation of reactive atypia and the misinterpretation of contaminating nonneoplastic gastrointestinal material (ob-
tained by EUS-FNA) or pancreatic material. Contaminating material is especially problematic for the interpretation of paucicellular cystic lesions. Finally, rare cases of sampled pancreatic intraepithelial neoplasia can lead to an overdiagnosis of malignancy. It may be debated, however, whether such cases truly constitute false-positive diagnoses.

**SIGNIFICANCE OF SUSPICIOUS AND ATYPICAL DIAGNOSES**

Qualitative terminology found in diagnostic reports is certainly not used in a consistent fashion. That said, a diagnosis of “suspicious for adenocarcinoma” is generally applied in cases where the material is believed to be quantitatively insufficient for an outright diagnosis of adenocarcinoma. For this diagnosis to carry its full meaning, qualitative features of malignancy described below should be present. For example, this diagnostic category might also demonstrate the presence of a single group or a few cells with significant anisonucleosis, nuclear overlap, and chromatin and nuclear membrane irregularities. Several groups have analyzed the clinical significance of a suspicious diagnosis. In the review of Enayati et al. in which pancreatic cytopathology was performed for patients thought to have clinical signs of pancreatic cancer, the results from 10 of 145 FNAs were interpreted as suspicious; all cases showed malignancy on follow-up. In the previously mentioned EUS-FNA series of Eloubeidi et al., all 5 suspicious cases showed malignancy on follow-up. Furthermore, in this same study, suspicious cases were reviewed by a second pathologist, who rendered a diagnosis of adenocarcinoma in 2 cases (40%). This is not surprising as there is no consensus as to what constitutes quantitative sufficiency for reaching a diagnosis of malignancy. For example, Robins et al. proposed a minimum of 6 diagnostic groups, while Lin and Staerkel countered that, given the presence of minimum diagnostic criteria, fewer groups may be sufficient. Similar analyses have been performed with pancreatobiliary brushings, with Logrono and Wong reporting malignancy on follow-up in 8 of 9 suspicious cases. In our opinion, the category suspicious for adenocarcinoma has significant interobserver and intraobserver variability, should be applied within the clinical and radiologic context of a given case, and when applied judiciously, is a very powerful predictor of malignancy. As mentioned above, pancreatic intraepithelial neoplasia can be sampled in patients without true malignant disease. Such a case could easily be interpreted as suspicious for adenocarcinoma.

An atypical diagnosis is rendered in cases in which a definitive diagnosis of benignity or malignancy cannot be established and in which the qualitative features fall short of malignancy and instead overlap with those for benign processes. As with the category labeled suspicious, the material of interest is often limited. We believe that atypical diagnoses are best accompanied by a brief note to the clinician describing the cytologic findings and providing an assessment of one’s level of concern. In our experience, atypical diagnoses are much more common with brush cytology than with FNA specimens. As mentioned previously, moderate to severe reactive atypia is much more common with brush specimens such that it may overlap some of the criteria used to diagnose adenocarcinomas.

Furthermore, and not often discussed, is the fact that the procedure samples duct epithelium, which may be involved by intraepithelial neoplasia that, although cytologically atypical, may not have definitive features of malignancy.

The clinical significance of an atypical diagnosis must be carefully interpreted in the context of the clinicopathologic picture. While it is a less powerful predictor of malignancy, it should not be ignored. Logrono and Wong discussed the importance of reporting “significant atypia less than obvious carcinoma.” In their study, 7 of 11 patients (64%) ultimately had carcinoma. Similarly, in the series of Enayati et al., 12 of 22 patients (55%) for whom an atypical diagnosis was rendered were found to have malignancy on follow-up. A reasonable approach to some atypical diagnoses is close follow-up or rebiopsy, if clinically indicated.

**AN ALGORITHMIC APPROACH TO THE DIAGNOSIS OF PANCREATIC FNA SPECIMENS**

To interpret pancreatic FNA requires a working knowledge of each case’s clinical and cytologic findings (including cellular and extracellular). Additionally, one obviously needs to know pancreatic surgical pathology, which includes neoplastic and nonneoplastic lesions. The excellent classification systems for neoplastic endocrine and exocrine pancreatic lesions put forth by the World Health Organization recognizes more than 40 diagnostic entities. For practical use with cytology specimens, this list can be narrowed down to a little more than 10 lesions (Table 1) including ductal adenocarcinoma (and its variants), cellular epithelioid neoplasms (ie, acinar cell carcinoma, pancreatic endocrine neoplasm) specific functioning endocrine neoplasms are best defined clinically), solid-pseudopapillary neoplasm, and pancreatoblastoma) mucous-producing cystic neoplasms (ie, IPMN and MCN), serous cystadenoma, nonepithelial tumors, and metastases. The most common nonneoplastic entities that are encountered with FNA specimens are chronic pancreatitis (including autoimmune pancreatitis), pseudocyst, and benign, normal-appearing pancreas.

A simplified interpretive algorithm is presented in Figure 2. The pathologist first determines whether the lesion is solid or cystic. This information may be obtained through discussions with the radiologist or the endoscopist, either in person (on-site) or over the telephone, or it may be conveyed with the requisition form. With solid lesions, as with aspirates from all sites, the pathologist must first determine whether noncontaminant and abnormal tissue is present. If no such tissue is present,
these aspirates are generally interpreted as “nondiagnostic.” We usually provide a description of the tissue that is present, especially if normal pancreatic tissue is seen, because aspirations are sometimes performed when the clinician has little reason to suspect neoplasia, such as when a patient has a noted radiographic “fullness” or benign-appearing duct stricture. The pathologist must then determine if neoplastic tissue is present. Generally, this requires the recognition of a monocellular component distinct from inflammatory disease. Most cases without neoplastic cells will be interpreted as pancreatitis, usually chronic, rarely autoimmune. Most neoplasms will be typical ductal adenocarcinomas, and the neoplastic cell population will be obviously glandular, with sheets of malignant cells (defined below). Other neoplasms, variants of ductal adenocarcinomas, other uncommon primary pancreatic neoplasms, and secondary malignancies of the pancreas require attention both for determining specific cytologic features and for ancillary testing, usually cell block immunohistochemistry (Table 2).

Aspirates of cystic lesions rarely are cellular, with features typical of an aspirate from a solid pancreatic lesion (eg, an aspirate from a cystic pancreatic endocrine tumor). Usually, such features are immediately apparent and the findings should be interpreted like those of an aspirate from a solid lesion (because all typically solid pancreatic neoplasms can present as cystic lesions).49

The presence of extracellular, atypical thick mucus is diagnostic of a mucus-producing cystic neoplasm and with such specimens, epithelial atypia should be described.50,51 In the absence of thick mucus, we generally describe the pertinent findings. Most other aspirates have either inflammatory or nonspecific features; rarely, features specific for uncommon entities are noted (eg, prominent keratinous debris with a pancreatic lymphop epithelial cyst).52,53 Findings from such aspirates are generally correlated with clinical findings and with the results of cyst fluid analysis (Table 3).

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**Table 2. Selected Immunohistochemistry of Pancreatic Epithelioid Neoplasms**

<table>
<thead>
<tr>
<th>Neoplasm</th>
<th>CK</th>
<th>CD56</th>
<th>SYN</th>
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<th>VIM</th>
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Abbreviations: ACC, acinar cell carcinoma; BCAT, β-catenin; CG, chromogranin; CK, (pan)cytokeratin; PB, pancreatoblastoma; PEN, pancreatic endocrine neoplasm; SPN, solid-pseudopapillary neoplasm; SYN, synaptophysin; TRP, trypsin; VIM, vimentin; +, positive; -/-, often positive; -/+e, occasionally positive; -, negative.

^1 Nuclear immunoreactivity.
^b Occasional ACCs demonstrate scattered reactivity with antibodies to neuroendocrine markers.
^c Rare cases of BCAT nuclear immunoreactivity in ACC have been described.
^d SPNs may show weak keratin reactivity, dependent on the makeup of a laboratory’s pancytokeratin.
^e SPNs also show occasional, weak reactivity with antibodies to neuroendocrine markers.
DUCTAL ADENOCARCINOMA

Pancreatic ductal adenocarcinoma represents 85% to 90% of pancreatic neoplasms. It is a disease of mature adults, is somewhat more common in men, with a male to female ratio of 1.6:1, and usually occurs in the pancreatic head. Patients may present with signs and symptoms attributable to the mass lesion (eg, abdominal pain, obstructive jaundice) or with associated loss of exocrine or endocrine function. On imaging, ductal carcinoma presents as an ill-defined, heterogeneous mass. Cytologists need to be comfortable diagnosing this malignancy as it is by far the most common diagnosis of malignancy in pancreatic cytopathology.

The cytologic features of ductal adenocarcinoma have been well described and are summarized (with reference to their overall frequency) in Table 4.1,40,45,55 Aspirates are generally cellular and are predominantly composed of neoplastic ductal cells. The background is highly variable and can be “clean,” inflammatory, necrotic, or mucinous. Sheets of neoplastic cells show a loss of the normal honeycomb pattern, with the long axes of nuclei arranged haphazardly within the sheet and with nuclear crowding and overlap. In moderately to poorly differentiated examples, markedly irregular 3-dimensional clusters may be appreciated (Figure 3). Atypical, single cells are present and again are more prominent in moderately to poorly differentiated cases. At higher power, nucleomegaly (ie, nuclei with a diameter at least 2 times that of a neighboring red blood cell), irregular nuclear contours, and clumped chromatin are present. Depending on the grade of the tumor, nucleoli and mitotic figures are variably prominent.

Diagnosing most moderately to poorly differentiated cases is generally straightforward, but although assigning the diagnosis of well-differentiated adenocarcinoma is usually not too difficult with cellular material, a more careful assessment of the cytologic material may be required. Lin and Staerkel recently described the features of well-differentiated adenocarcinoma: anisonucleosis (4 times), irregular nuclear contours, nucleomegaly, and loss of honeycomb pattern. These features were present in 92% to 99% of the 74 well-differentiated adenocarcinomas they examined, which represented 33% of all ductal adenocarcinomas (Figure 4). Not surprisingly, features less likely to be present included marked chromatin abnormalities, macronucleoli, mitotic figures, and background necrosis.

Several variants and histologic patterns of ductal adenocarcinoma have been described, including adenosquamous carcinoma, foamy gland adenocarcinoma, undifferentiated (anaplastic) carcinoma, and undifferentiated carcinoma with osteoclast-like giant cells. While some of these variants behave differently from typical ductal adenocarcinoma, their overall prognosis and treatment most resembles ductal adenocarcinoma.

Typical ductal adenocarcinomas have at least some areas of focal squamous differentiation. Adenosquamous carcinoma, as defined by the World Health Organization, should have areas of squamous differentiation in at least 30% of the lesion. Although adenosquamous carcinoma is described in the cytology literature, we generally defer making this diagnosis until a resection has been performed and instead render a diagnosis of “ductal adenocarcinoma with prominent squamous features” on FNA (Figure 5). Of note, atypical squamous cells may also be encountered in reactive conditions including chronic pancreatitis and with stent placement. Although an entirely squamous malignant lesion suggests metastasis, rare primary squamous cell carcinomas of the pancreas have been described.

Pancreatic adenocarcinomas can produce a variable amount of mucus and some are even termed colloid carcinomas, as determined by the percentage of tumor that is histologically composed of extracellular mucus. While colloid carcinomas may have a better prognosis, stage for stage, when compared to conventional pancreatic ductal adenocarcinomas, they are defined histologically, and such diagnosis is thus left to surgical pathologists. While fewer neoplastic cells are obscured by abundant mucus and debris, the cytologic features of typical pancreatic ductal adenocarcinoma are present. Also of note, mucinous adenocarcinomas of the pancreas (both colloid and noncystic) can be associated with signet ring cells, although these cells rarely predominate.

Foamy gland adenocarcinomas are characterized by abundant microvesicular cytoplasm (imparting a relatively low nuclear to cytoplasmic ratio) and basally oriented, hyperchromatic nuclei. Adsay et al formally described foamy gland adenocarcinoma in 2000, referring to it as a deceptively benign-appearing variant; the undercalling of this entity in surgical pathology material has represented a significant potential diagnostic pitfall. We screened cell block material from 52 pancreatic ductal carcinomas and found 12 cases with foamy gland features (23%); in half of these, the predominant pattern was that of foamy gland adenocarcinoma. We then reviewed all the cytology slides and assessed cytologic features. All 12 cases demonstrated loss of honeycomb pattern, significant anisonucleosis, and irregular nuclear contours. We concluded that the foamy gland pattern is not uncommon and that cytologists, accustomed to making the diagnosis of well-differentiated adenocarcinoma in the face of low nuclear to cytoplasmic ratios, would not struggle with this diagnosis in most cases.

Undifferentiated (anaplastic) carcinoma of the pancreas is a pleomorphic malignant neoplasm. Its morphologic spectrum includes predominantly spindled lesions (sometimes referred to as sarcomatoid carcinoma), as well as lesions composed of polygonal large cells (pleomorphic large cell carcinoma). These tumors are frequently punctuated by bizarre giant tumor cells (Figure 6). Aspirates are obviously malignant. The main challenge is separating this lesion from other pleomorphic malignant neoplasms, including melanoma, sarcoma, metastatic carcinoma, and lymphoma. The presence of focal keratin immunoreactivity supports the diagnosis of carcinoma. Occasional foci of more typical ductal adenocarcinoma may be present, supporting the designation of primary pancreatic cancer, although clinical information or ancillary testing is usually necessary for making a clear distinction.

Undifferentiated carcinoma with osteoclast-like giant cells is extremely rare and its nature is somewhat contested. As the name implies, scattered osteoclast-like giant cells with numerous canal-appearing nuclei are present in the background (Figure 7). The malignant population is poorly differentiated and is epithelioid and may react focally with antibodies to cytokeratin. Again, foci of more typical ductal adenocarcinoma may be present.

Given the presence of multinucleated giant cells, the differential diagnosis includes various infectious and inflam-
Figure 3. Typical cluster of malignant glandular cells seen with an aspirate of pancreatic ductal adenocarcinoma. Anisonucleosis is present with nuclear overlap; nuclear membrane irregularity, clumpy chromatin, and prominent nucleoli are observed (Papanicolaou, original magnification ×400).

Figure 4. Loss of honeycomb architecture, nuclear overlap, and anisonucleosis are seen in this smear of a well-differentiated ductal adenocarcinoma (Diff-Quik, original magnification ×400).

Figure 5. Prominent squamous change is frequently seen with aspirates of pancreatic ductal adenocarcinoma (Papanicolaou, original magnification ×400).

Figure 6. Multinucleated tumor giant cells are seen with aspirates of undifferentiated pancreatic carcinoma (Diff-Quik, original magnification ×400).
It potentially affects the choice of chemotherapy. As such, important for patients whose tumor is not resectable because cell carcinomas will stain strongly with antibodies directly when dealing with a cellular epithelioid neoplasm. Acinar cell carcinomas on imaging. Generally, in the setting of hepatic metastasis, 10% to 15% of patients manifest the lipase hypersecretion syndrome, characterized by subcutaneous fat necrosis and polyarthralgias. The 5-year survival for ACC is minimally better than that of ductal adenocarcinoma, on the order of 5% to 10%.

Smears are cellular and composed predominantly of variably sized loose clusters and single cells (Figure 8). Acinar formation is common and is indistinguishable from the pseudorosette formation seen with aspirates of pancreatic endocrine neoplasms (PENs). The background is generally cleaner than in ductal adenocarcinoma, and numerous naked nuclei are typically seen. At higher power, cells are noted to have abundant granular cytoplasm and indistinct cell borders. Chromatin is typically clumped and, although anisonucleosis is slight, prominent nucleoli are typically present. Although nuclei are generally centrally placed, occasional plasmacytoid and binucleate forms are seen that resemble the neoplastic cells from as- trally placed, occasional plasmacytoid and binucleate forms are seen. At higher power, cells are noted to have abundant granular cytoplasm and indistinct cell borders. Chromatin is typically clumped and, although anisonucleosis is slight, prominent nucleoli are typically present. Although nuclei are generally centrally placed, occasional plasmacytoid and binucleate forms are seen that resemble the neoplastic cells from as- trally placed, occasional plasmacytoid and binucleate forms are seen. At higher power, cells are noted to have abundant granular cytoplasm and indistinct cell borders. Chromatin is typically clumped and, although anisonucleosis is slight, prominent nucleoli are typically present. Although nuclei are generally centrally placed, occasional plasmacytoid and binucleate forms are seen that resemble the neoplastic cells from as- trally placed, occasional plasmacytoid and binucleate forms are seen. At higher power, cells are noted to have abundant granular cytoplasm and indistinct cell borders. Chromatin is typically clumped and, although anisonucleosis is slight, prominent nucleoli are typically present. Although nuclei are generally centrally placed, occasional plasmacytoid and binucleate forms are seen that resemble the neoplastic cells from as- trally placed, occasional plasmacytoid and binucleate forms are seen. At higher power, cells are noted to have abundant granular cytoplasm and indistinct cell borders. Chromatin is typically clumped and, although anisonucleosis is slight, prominent nucleoli are typically present. Although nuclei are generally centrally placed, occasional plasmacytoid and binucleate forms are seen that resemble the neoplastic cells from as- trally placed, occasional plasmacytoid and binucleate forms are seen. At higher power, cells are noted to have abundant granular cytoplasm and indistinct cell borders. Chromatin is typically clumped and, although anisonucleosis is slight, prominent nucleoli are typically present. Although nuclei are generally centrally placed, occasional plasmacytoid and binucleate forms are seen that resemble the neoplastic cells from as- trally placed, occasional plasmacytoid and binucleate forms are seen. At higher power, cells are noted to have abundant granular cytoplasm and indistinct cell borders. Chromatin is typically clumped and, although anisonucleosis is slight, prominent nucleoli are typically present. Although nuclei are generally centrally placed, occasional plasmacytoid and binucleate forms are seen that resemble the neoplastic cells from as- trally placed, occasional plasmacytoid and binucleate forms are seen. At higher power, cells are noted to have abundant granular cytoplasm and indistinct cell borders. Chromatin is typically clumped and, although anisonucleosis is slight, prominent nucleoli are typically present. Although nuclei are generally centrally placed, occasional plasmacytoid and binucleate forms are seen that resemble the neoplastic cells from as-
Figure 9. A smear from a solid-pseudopapillary tumor. Single, uniform epithelioid cells are arranged around a metachromatic papillary core (Diff-Quik, original magnification × 200).

Figure 10. Aspirates of pancreatic endocrine tumors are often cellular with loose clusters and single cells that are frequently “plasmacytoid” in appearance (Papanicolaou, original magnification ×200).

Figure 11. Pseudorosettes, seen in aspirates of pancreatic endocrine tumors, are indistinguishable from acinar structures. Also of note, prominent nucleoli can sometimes be seen with pancreatic endocrine tumors (Papanicolaou, original magnification ×1000).

Figure 12. Calcific debris is seen in an aspirate obtained from a case of well-developed chronic pancreatitis (Diff-Quik, original magnification ×200).
Table 4. Frequency of Specific Cytologic Features of Pancreatic Ductal Adenocarcinoma: Summary of Data From Literature Reviewa

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<td>Mitotic figures</td>
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*a Ellipses indicate numbers not reported in study.

Pancreatoblastoma is an extremely rare tumor, with the number of reported cases at less than 75 at the time of writing of the World Health Organization's latest gastrointestinal tumor book. It is typically a pediatric tumor, with a median patient age at time of diagnosis of 4 years; rare cases have been reported in adults. There is a slight male predominance, and although cases of tumor throughout the gland have been described, there is preferential involvement of the head. Patients have symptoms associated with the mass (eg, pain), weight loss, or that present incidentally. Several cases have been described in association with Beckwith-Wiedeman syndrome and FAP. Pancreatoblastomas are polyphenotypic tumors with evidence of acinar, endocrine, and ductal differentiation, with acinar differentiation generally predominating. Interestingly, while tumor resection is often curative for pediatric patients without metastasis, tumors in adults (even low-stage disease) more closely pattern traditional ACCs.

Smears are cellular and reveal 2 cell populations, epithelioid and immature mesenchymal. The epithelioid component may contain areas of identifiable acinar differentiation, with granular cytoplasm and prominent nucleoli. In other areas, the epithelioid component is relatively undifferentiated, composed of large cells in a syncytial arrangement. Heterologous elements (cartilage) may be seen. In one report, squamoid corpuscles, a diagnostic hallmark, were best appreciated on cell block preparations.

In the pediatric population, the differential diagnosis would include small round blue cell tumors that may involve the pancreas, including Wilms tumor, neuroblastoma, and lymphoma. Wilms tumor is the closest morphologic match, because it also is characterized by a mixture of mesenchymal and epithelial components. Identifying the characteristic acinar differentiation and squamoid corpuscles should help secure the diagnosis. In adult patients, the chief differential diagnosis is ACC and other epithelioid neoplasms. The presence of a significant mesenchymal component and squamoid corpuscles would argue against ACC, although, as discussed above, the distinction in this population may not be clinically significant. The lesion's immunophenotype reflects its polyphenotypic nature, and tumor cells can react with antibodies directed against pankeratin, neuroendocrine antigens, and pancreatic enzymes. Like SPN and ACC, it can exhibit β-catenin nuclear immunoreactivity.

Pancreatic endocrine neoplasms are also relatively uncommon, representing approximately 2% of pancreatic neoplasms. Although they occur over a broad age range, most develop in mature adults of either sex. Tumors occur throughout the gland and have a propensity for involving the tail. Although historically most patients had symptoms attributable to hyperfunction (eg, insulin-induced hypoglycemia with insulinoma), today, most are nonfunctioning endocrine tumors, and many lesions are discovered incidentally on radiologic examinations performed for other reasons.

As with ACCs and SPNs, smears of PENs are cellular with loosely cohesive cell clusters and single cells (Figure 10). Pseudorosette formation is typical and is impossible to distinguish from acinar formation (Figure 11). The background may be clean or bloody. At higher power, one usually notes the classic salt-and-pepper neuroendocrine chromatin; however, cases vary greatly and more clumpy chromatin and prominent nucleoli are sometimes seen. The presence of plasmacytoid and binucleate forms suggests a diagnosis of PEN, as does the presence of scattered atypical large cells (so-called endocrine atypia). Occasionally, a case may manifest a more spindled morphology.
and variants of PENs with prominent clear cell or oncocytic cell morphology have been described. Although we do not typically grade PENs on FNA, necrosis or prominent mitotic figures are sometimes present and may portend an aggressive course.83–87

Along with ACC and SPN, the morphologic differential diagnosis in select cases of PEN may include other tumors producing prominent plasmacytoid or epithelioid forms and spindled smear patterns, including plasmacytoma and melanoma. Immunohistochemistry easily resolves these diagnostic dilemmas, as PENs should react strongly with antibody to at least 1 specific neuroendocrine antigen (eg, chromogranin and synaptophysin) along with pan-keratin. Again, ACCs will typically not react with antibodies against endocrine antigens but will react instead with antibodies directed against pancreatic exocrine enzymes. Solid-pseudopapillary neoplasms show limited, if any, reactivity with antibodies to keratin, chromogranin, and synaptophysin. Plasma cell neoplasms are immunoreactive with antibodies to CD138 and MUM1 and should show light-chain restriction by in situ hybridization. Melanomas are immunoreactive with antibodies to S100 protein and (depending on the case) for other melanocytic markers including Melan-A and HMB-45.

Small cell undifferentiated (neuroendocrine) carcinoma of the pancreas is genetically distinct from typical PENs, and is mentioned here for the sake of completeness. These are incredibly rare lesions, cytologically indistinguishable from pulmonary small cell carcinomas, the distinction resting with clinical correlation.88 Thyroid transcription factor 1 immunoreactivity supports the diagnosis but is not itself diagnostic of a metastatic pulmonary carcinoid.89

**PANCREATITIS**

Not uncommonly, we encounter aspirates of chronic pancreatitis from patients with solid-appearing lesions by radiography that sometimes mimic pancreatic cancer. Although the cytologic features of chronic pancreatitis are straightforward, aspirates are potential diagnostic pitfalls, because ductal adenocarcinoma may arise in the setting of chronic pancreatitis, and pancreatic cancers may induce chronic pancreatitis in the surrounding pancreas.90

We reviewed the cytologic features of 20 consecutive cases of chronic pancreatitis, as defined by cell block morphology, diagnosed at Hennepin County Medical Center (Minneapolis, Minnesota).4 Cases were invariably cellular, composed of fibrotic stromal fragments with a variable amount of splayed-apart acinar tissue, at least the presence of rare ductal cells, and a mixed inflammatory infiltrate with numerous macrophages. The background contained prominent chalky, calcific debris (Figure 12). Not surprisingly, the amount of debris was generally inversely proportional to the amount of acinar tissue present. In half the cases, the degree of cytologic atypia in the ductal epithelium was judged to be mild, with evidence of anisocytosis (some nuclei were 3 to 4 times larger), focal nuclear crowding and overlap, granular chromatin, and prominent nuclei. In retrospect, this case could have been diagnosed as suspicious for adenocarcinoma. The other 2 cases were indistinguishable from the rest, emphasizing the importance of close clinical follow-up in presentations of “tumefactive” lesions interpreted as chronic pancreatitis in aspirates.

As mentioned earlier, autoimmune pancreatitis often presents as a mass lesion or with strictured common bile and pancreatic ducts. Deshpande et al9 recently reviewed the cytologic features of this uncommon entity in a group of 16 patients who had been previously evaluated by EUS-FNA. Although there was significant morphologic overlap with chronic pancreatitis, aspirates from cases of autoimmune pancreatitis tended to have a greater number of stromal fragments and were more cellular. In a minority of cases (37.5%), the stromal fragments contained greater than 30 lymphocytes per high-power field. These findings need to be confirmed. Given numerous, cellular, lymphocyte-rich stromal fragments in a case otherwise typical of chronic pancreatitis, suggesting the possibility of autoimmune chronic pancreatitis and recommending a test to measure serum IgG4 levels could result in the initiation of steroid treatment (if one’s clinical suspicion is confirmed) and could possibly spare the patient a needless resection.

**MUCUS-PRODUCING CYSTIC NEOPLASMS**

Intraductal papillary mucinous neoplasm (IPMN) and mucinous cystic neoplasm (MCN) represent the mucin-producing cystic neoplasms. They are considered here together because their histologic features and biologic potentials are largely overlapping (although not identical) and because their distinction, based solely on cytologic features, may not be possible. Historically, each was considered to be relatively rare, accounting at most for a small percentage of all exocrine pancreatic tumors, but their incidence appears to be increasing, coincident with increased utilization of diagnostic abdominal imaging. Intraductal papillary mucinous neoplasms, by definition, involve the main and/or side branch pancreatic ducts. They tend to occur in older men, who may present with abdominal pain, jaundice, pancreatitis, or diabetes. The resulting ductal dilation can often be appreciated radiologically.91 As a general rule, MCNs do not involve the ductal system (with rare reported exceptions), and they tend to occur in middle-aged women (mean age, 49 years) and involve the body and tail of the pancreas. Small tumors frequently present incidentally. Radiology may reveal a unilocular or oligolocular cyst with a thick wall and associated calcifications.92 Intraductal papillary mucinous neoplasms and MCNs are each lined by mucinous epithelium of varying types (although IPMNs tend to show greater heterogeneity, both may have gastric, pancreatobiliary, or intestinal-type epithelium) and grades (ie, low-grade dysplasia, moderate dysplasia, high-grade dysplasia/carcinoma in situ). Both lesions have the potential to give rise to in situ or invasive carcinomas. Despite their reported low incidence, aspirates from IPMNs and MCNs have come to represent approximately 10% of EUS-FNAs at some institutions.

Cytology plays a role in triaging patients for surgery, especially those with asymptomatic branch duct IPMNs. Indeed, many patients are now triaged directly to surgery because of main duct involvement or lesion size (Figure 13). According to the International Consensus Guidelines for Management of IPMNs and MCNs of the Pancreas, side branch IPMNs that are 1 to 3 cm in size should be
assessed by EUS and either magnetic resonance cholangiopancreatography or ERCP. The following findings should lead to a recommendation for resection: mural nodule, main duct dilatation, and atypical cytology.93

The diagnostic hallmark on FNA is the presence of thick, gelatinous mucus (Figure 14).50,51,94-97 In our recently published series of pancreatic cystic lesions, we noted diagnostic extracellular lesional material in 28 of 31 IPMNs (90%) and 4 of 7 MCNs (57%).51 Although papillary clusters formed by mucinous epithelium and goblet cells are characteristic of some IPMNs, they may only be identifiable in a minority of cytology specimens. In a series of aspirates from 18 IPMNs, we identified each of these features 17% and 33% of the time, respectively.50

Because it is the cyst contents and not the lining or wall that is sampled by FNA, aspirates are sometimes acellular and are frequently paucicellular; the ovarian-type stroma essential for a diagnosis of MCN is not, in our experience, represented. In fact, we observed diagnostic cellular material in only 22 of 38 aspirates (58%) of mucus-producing cystic neoplasms (Figure 15).51 Michaels and colleagues97 have reported a correlation between cytologic features and histologic grade. They found that the presence of 3-dimensional cell clusters with hyperchromasia predicted the presence of at least moderate dysplasia and that parachromatin clearing and necrosis predicted the presence of at least in situ carcinoma. Several investigators, including ourselves, have noted that smear cellularity and cellular atypia correlates with histologic grade. We would caution, though, that the absence of these atypical features does not exclude a higher-grade lesion. In our IPMN series, the cytology specimens from 2 noninvasive intraductal papillary mucinous adenocarcinomas were paucicellular and without demonstrable atypia.

The principal differential diagnosis is often gastrointestinal contamination (especially if a different type of cystic lesion is aspirated). In the series of Recine et al,94 2 of 19 tumors believed to represent mucus-producing cystic neoplasms on FNA proved to be serous cystadenoma on resection. In our series of cystic lesions, the combination of thick extracellular material (which in retrospect most likely represented keratinous debris) and gastrointestinal contamination led to the false impression of mucous-producing cystic neoplasm with an aspirate from a lymphoepithelial cyst.17% and 33% of the time, respectively.50 In our experience, the recognition of duodenal contamination in EUS-FNA of pancreatic head lesions is relatively straightforward. Contaminating mucus is thin and “dirty-appearing” or bile-stained. Duodenal mucosa presents as 2-dimensional, honeycomb sheets without nuclear atypia; clear-appearing goblet cells are regularly interspersed. Gastric contamination, occurring in the setting of EUS-FNA of body and tail lesions, may be more problematic. In the absence of diagnostic extracellular mucus, this bears directly on the diagnosis of MCNs and some IPMNs. Pitman and Deshpande98 have observed that, while contaminating foveolar-type epithelium exhibits an “apical cap” of cytoplasmic mucin, in low-grade MCNs, the mucin does not exhibit the same compartmentalization, instead filling and expanding the cells. This subtle distinction can be treacherous. In our experience, compared with IPMNs, MCNs are less likely to produce diagnostic imaging results, diagnostic mucus, and a cellular population—features that could easily separate MCNs from gastrointestinal contamination. This observation speaks to the sensitivity we observed (42%) in making this prospective diagnosis.46

An array of adjunctive techniques may assist in this differential diagnosis. Nagle and colleagues99 and Nawgiri and colleagues100 have advocated the use of B72.3 immunohistochemistry for distinguishing gastrointestinal contamination from neoplastic mucinous epithelium, with contaminating epithelium demonstrating punctate perinuclear staining and neoplastic epithelium demonstrating diffuse cytoplasmic staining. Although promising, the paucicellular nature of the most diagnostically challenging cases may limit this adjunct’s utility. Al-Haddad et al101 have recently reported the use of a new EUS cytology brush that can be deployed through a 19-gauge EUS needle. In 7 of 10 cases, the specimens procured by EUS brushing were superior in terms of cellularity. Two of 10 patients, though, experienced complications, including 1 major incidence of intracystic bleeding. In addition, for technical reasons, the new cytobrush could not be used with lesions in the pancreatic head. 

Figure 15. Atypical glandular cells were seen in an aspirate of a pancreatic cystic lesion. This finding is seen with more advanced mucin-producing pancreatic neoplasms and is generally considered a reason for resection. (Papanicolaou, original magnification ×200).

Figure 16. This scrape preparation from a microcystic serous cystadenoma shows bland, somewhat cuboidal glandular cells. With a transgastric fine-needle aspiration, it would be difficult to exclude contaminant gastric material (Diff-Quik, original magnification ×400).
Cyst fluid analysis plays a complementary role in the diagnosis of pancreatic cystic lesions.\textsuperscript{102–104} Van der Waa\textsuperscript{i} and colleagues\textsuperscript{105} pooled data from 12 studies and concluded that (1) a concentration of amylase of less than 250 U/L in fluid is 98% specific for serous cystadenoma or mucus-producing cystic neoplasm, (2) a carcinoembryonic antigen (CEA) concentration of less than 5 ng/mL is 95% specific for serous cystadenoma or pseudocyst, (3) a CEA concentration greater than 800 ng/mL is 98% specific for mucus-producing cystic neoplasm, and (4) a carbohydrate antigen 19-9 concentration of less than 37 U/mL is 98% specific for serous cystadenoma or pseudocyst (Table 3). Unfortunately, these cutoffs lack sensitivity for the diagnosis of their respective lesions, with sensitivities ranging from 19% to 50% only. This results in a significant overlap between types of lesions that require disparate management strategies. For example, most pseudocysts and mucus-producing neoplasms will have CEA levels between 5 and 800 ng/mL, and, not uncommonly, mucus-producing neoplasms, especially IPMNs, will exhibit amylase concentrations greater than 250 U/L. Despite its drawbacks, we consider cyst fluid analysis to be a valuable complement to EUS-FNA in the evaluation of cystic lesions.

**OTHER CYSTIC LESIONS OF THE PANCREAS**

An array of other cystic lesions of the pancreas may be sampled including pseudocysts, serous cystadenomas, retention cysts, congenital and enteric duplication cysts, infectious cysts and squamous-lined cysts (ie, lymphoepithelial cysts, dermoid cysts, accessory splenic-epidermoid cysts).\textsuperscript{32,35,106–107} We frequently provide “descriptive” diagnoses for these aspirates. The presence of contaminating gastrointestinal epithelium in all aspirates potentially can lead to an overcall of “mucus-producing cystic neoplasm” and represents a significant potential diagnostic pitfall. A description of pseudocyst and serous cystadenoma follows.

Pseudocysts are generally diagnosed by radiologists, but occasionally, those with unclear diagnostic features on imaging or with atypical clinical presentations will be aspirated. Pseudocysts typically occur clinically in the backdrop of pancreatitis. Aspirates are dominated by an admixture of granular debris and a mixed inflammatory infiltrate including foamy histiocytes.\textsuperscript{17} Although, by definition, these cysts lack an epithelial lining, gastrointestinal contamination may be introduced through EUS-FNA. Cyst fluid analysis reveals high amylase concentration and generally low CEA concentration.

Serous cystadenomas are rare, benign neoplasms, representing up to 2% of pancreatic exocrine tumors. They are typically diagnosed in older adults, who often present with symptoms associated with the mass. The lesion is found more often in females, and although serous cystadenomas can be found throughout the pancreas, they tend to involve the body and tail. Radiology classically reveals a well-circumscribed, multicystic, spongelike mass with a central scar and associated “sunburst” calcifications (typical microcystic serous cystadenoma).\textsuperscript{108} Lesions with these typical radiographic features are less likely to be aspirated than oligocystic lesions.\textsuperscript{109} The aspirated material is nonspecific; it is composed of thin and watery fluid and is generally paucicellular, with a few loose clusters of nonmucinous epithelium without atypia (Figure 16).\textsuperscript{110–115} Although the lining epithelium in serous cystadenomas is generally flat, occasionally it is raised in a papillary configuration that creates potential confusion with an aspirate from an IPMN.\textsuperscript{116} Cyst fluid analysis generally reveals low amylase, CEA, and carbohydrate antigen 19-9 concentrations. If material is present in a cell block, the epithelium is composed of optically clear cells that are periodic acid-Schiff–positive, diastase-sensitive (glycogen).

**ADVANCED TECHNIQUES AND THE DIAGNOSIS OF PANCREATIC NEOPLASIA WITH CYTOLOGY SPECIMENS**

Neoplasia occurs via the stepwise accumulation of genetic changes conferring increased proliferative capacity and survivability to tumors and culminating in malignant disease because of the tumor’s ability to invade and cause metastasis. With tumors of the pancreas, this sequence of events has been best defined for ductal adenocarcinomas and, to a lesser extent, for other pancreatic tumors including acinar cell carcinomas, pancreatic endocrine tumors, solid-pseudopapillary tumors, pancreatoblastomas, mucus-producing cystic neoplasms, and serous cystadenomas. Implicated tumorigenic events include mutations in oncogenes, overexpression of oncogene products, and the silencing of tumor suppressor genes through mutation, hypermethylation, and deletion. Additionally, mRNA profiling has demonstrated that certain genes and proteins are overexpressed with pancreatic tumors. A number of ancillary techniques that take advantage of such changes at the genetic and protein level have been proposed to assist with the diagnosis of pancreatic neoplasia in cytology specimens.

**Ductal Adenocarcinoma**

The most common genetic alterations identified in pancreatic ductal adenocarcinoma include activating point mutations in codon 12 of KRAS (\textasciitilde90% of ductal carcinomas), the silencing of \textit{p16} through deletion, mutation, or hypermethylation (95%), the silencing of TP53 through deletion and mutation, and the silencing of DPC4 through deletion and mutation.\textsuperscript{117–120} These changes have also been detected in pancreatic intraepithelial neoplasia.\textsuperscript{54,121}

Several investigators have tried to exploit this knowledge diagnostically. Because of the near ubiquity and genetic homogeneity of KRAS mutations in ductal adenocarcinoma, KRA\textit{S} mutation analysis has been frequently explored.\textsuperscript{122–125} In general, the sensitivity of this analysis (performed on brushings, ductal aspirates, or FNA material) has paralleled the sensitivity of cytologic analysis, with a few cases being positive for a KRAS mutation but considered negative for ductal adenocarcinoma (or more generally atypical) by cytologic analysis, and vice versa. Unfortunately, for many of these studies, the specificities reported are inferior to those achieved with cytologic analyses, perhaps because KRAS mutations are seen with pancreatic intraepithelial neoplasia. For example, in the series of Uehara et al,\textsuperscript{122} KRAS mutations were identified in 50% of pancreatic cancers, 31% of chronic pancreatitis cases, and in 20% of healthy controls. Van Heek and colleagues\textsuperscript{124} used a panel of molecular studies, including KRAS mutation analysis and p53 and DPC4 immunohistochemistry, and looked for overexpression or loss of protein, respectively. At least 1 molecular test achieved a sensitivity of 67%, compared with 76% for cytologic analysis, for an abnormal result leading to a diagnosis of adenocarcinoma.
cystic lesions, specifically to identify higher-grade MCNs and IPMNs that are associated with invasive malignancies. Studies often include analysis of the KRAS2 gene (mutated in many MCNs and IPMNs, but at a lower incidence of mutation than that of ductal adenocarcinoma). Loss-of-heterozygosity studies have also been performed to identify those lesions whose neoplastic progression has advanced more significantly. In addition, the use of the telomeric repeat amplification protocol assay has been advocated with aspirates of pancreatic cystic lesions.

CONCLUSIONS

Pancreatic cytology has recently attained its heyday owing to advanced and more sophisticated imaging techniques and the development of EUS. Mostly, it relies on standard morphologic assessment (the diagnosis of ductal adenocarcinoma) and ancillary techniques such as immunohistochemistry, which have been discussed in depth, are not frequently needed. The diagnosis of cystic lesions, while problematic, is certainly not unique to the pancreas, and cytopathologists face similar difficulties with aspirates from other organs (eg, paucicellular aspirates with mucus from salivary glands). More advanced techniques eventually may prove to be helpful, not just for the diagnosis of disease, but also for the direction of patient-specific care. As things stand, pathologists, with their unique understanding of disease, their ability to diagnose pancreatic disease by using traditional as well as advanced methods, and their skills in dealing with limited tissue samples, are poised to participate in the future care of patients with pancreatic neoplasia.

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