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Elevating pancreatic cystic lesion stratification: Current and future pancreatic cancer biomarker(s)

Joseph Carmicheal[a,1], **Asish Patel**[a,b,1], **Vipin Dalal**[a], **Pranita Atri**[a], **Amaninder S. Dhaliwal**[c], **Uwe A. Wittel**[d], **Mokenge P. Malafa**[e], **Geoffrey Talmon**[f], **Benjamin J. Swanson**[f], **Shailender Singh**[c], **Maneesh Jain**[a,g], **Sukhwinder Kaur**[a,*], **Surinder K. Batra**[a,f,g,h,*]

[a]Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE, USA

[b]Department of Surgery, University of Nebraska Medical Center, Omaha, NE, USA

[c]Department of Internal Medicine, Division of Gastroenterology-Hepatology, University of Nebraska Medical Center, Omaha, NE, USA

[d]Department of General- and Visceral Surgery, University of Freiburg Medical Center, Faculty of Medicine, Freiburg, Germany

[e]Department of Gastrointestinal Oncology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA

[f]Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE, USA

[g]Buffett Cancer Center, University of Nebraska Medical Center, Omaha, NE, USA

[h]Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, NE, USA

Abstract

Pancreatic ductal adenocarcinoma (PDAC) is an incredibly deadly disease with a 5-year survival rate of 9%. The presence of pancreatic cystic lesions (PCLs) confers an increased likelihood of future pancreatic cancer in patients placing them in a high-risk category. Discerning concurrent malignancy and risk of future PCL progression to cancer must be carefully and accurately determined to improve survival outcomes and avoid unnecessary morbidity of pancreatic resection. Unfortunately, current image-based guidelines are inadequate to distinguish benign from malignant lesions. There continues to be a need for accurate molecular and imaging biomarker(s) capable of identifying malignant PCLs and predicting the malignant potential of PCLs to enable risk stratification and effective intervention management. This review provides an update on the current status of biomarkers from pancreatic cystic fluid, pancreatic juice, and seromic molecular

*Corresponding authors at: Department of Biochemistry and Molecular Biology, Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, 985870 Nebraska Medical Center, Omaha, NE 68198-5870, USA., skaur@unmc.edu (S. Kaur), sbatra@unmc.edu (S.K. Batra).

[1]Equal contributors.

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analyses and discusses the potential of radiomics for differentiating PCLs harboring cancer from those that do not.

Keywords

Pancreatic cancer; Pancreatic ductal adenocarcinoma; Pancreatic cystic lesions; IPMN; Biomarker; Radiomics; Early detection

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a devastatingly lethal disease with a five-year survival of 9% [1]. It is currently the third leading cause of cancer-related deaths and is projected to replace colon cancer as the second leading cause within the next decade. Surgery remains the only possible curative option for those with PDAC, as long as it is performed in the early stages or prior to precursor lesion progression [2]. The high lethality is due to the discovery of PDAC at later stages, with metastatic disease present at the time of initial diagnosis [3]. Screening those at high risk of developing pancreatic malignancy can facilitate earlier diagnosis and life-saving surgical intervention, which still remains the most effective curative modality. Among the determined high-risk groups are those with a current or previous diagnosis of a pancreatic cystic lesion (PCL) [4]. Patients harboring a cystic lesion are more likely to progress to cancer than even those with a family history of PDAC [5] making them a prime target population for screening and surveillance modalities. However, complexities arise because PCLs present a variable risk for malignant progression: while some PCLs carry up to a threefold increased risk of developing PDAC [6,7], others present with a marginal risk or low probability of developing into PDAC [8].

The overall prevalence of all PCL types in the general population has increased in recent years due to the ubiquitous use of cross-sectional imaging modalities. According to the American Gastroenterological Association, the proportion of incidental PCL discoveries in patients who undergo magnetic resonance imaging (MRI) for an unrelated reason is 15% [9]. It is estimated that 0.7–2.6% of the general asymptomatic population harbors some form of PCLs [10]. The prevalence of these lesions increases with age; autopsies of 70 to 79-year-old patients revealed that 25% harbor a pancreatic cyst, increasing to 37% for patients older than 80 [11–13]. These proportions extrapolate to an estimated 3.5 million people with cystic lesions in the U.S. alone. The transformation potential of all cysts is minimal and an investigation utilizing the SEER database found that 33.2 per 100,000 pancreatic cysts will progress to PDAC [14].

Though screening and intervention are warranted in this high-risk group, the pervasiveness of PCLs leads to thousands of unnecessary medical and surgical interventions per year. These high-risk procedures carry great comorbidity, especially for the elderly. Even if surgery is not required, immense amounts of time and resources are utilized to monitor patients for years following initial discovery [15]. This monitoring is multimodal and can involve expensive sequential imaging methods along with invasive procedures such as repeat Endoscopic Ultrasound-guided Fine Needle Aspiration (EUS-FNA), culminating in eventual excision. This leads to an increased economic burden on the health care system. Importantly,

the anxiety and stress imparted on the patient after a PCL diagnosis cannot be understated, and the ability to ameliorate this burden would be immensely impactful [16].

The decades-old challenge still remains to identify the high-risk patients harboring early malignancy or precursor lesions that will eventually progress to PDAC. The goal of early cyst detection, characterization, and fluid analysis is not only to identify the high-risk patients but also to determine which pre-neoplastic lesions have the highest chance of progressing to PDAC, thereby accurately determining the necessity of surgery (Fig. 1). In effect, the task remains to identify the most at risk within this high-risk population. The use of novel biomarkers in conjunction with the current imaging and international consensus guidelines governing cystic clinical strategy can lead to improved utilization of limited resources and, more importantly, improved patient surgical stratification.

2. Classification of pancreatic cystic lesions

As per WHO classification (2000) [17–19] PCL's can be divided into; A. Serous tumors - including serous cystadenoma and serous cystadenocarcinoma B. Mucinous tumors - including mucinous cystadenoma, mucinous cystadenocarcinoma, intraductal papillary-mucinous adenoma and intraductal papillary-mucinous adenocarcinoma C. Solid pseudopapillary tumors.

2.1. Serous cystic neoplasms of the pancreas

Serous cystadenomas/Serious cystic neoplasms (SCAs/SCNs) are benign tumors that can be sub-divided into serous microcystic adenoma (SMA) and serous oligocystic adenoma (SOA) [17]. SMAs have a proclivity towards body or tail in 50–75% of cases while the rest involve the head of the pancreas [20,21]. SMAs account for 1–2% of all exocrine pancreatic tumors. Females (70%) are affected more than males with a mean age of 66 years (range 34–91 years) at presentation. SOAs are far less common than SMA with no sex predilection and are located mainly in the head and body of the pancreas [22,23]. On cross-sectional imaging with computed tomography (CT) or magnetic resonance imaging (MRI), SCAs are often multilocular with a honeycomb-like appearance and contain a central stellate scar [24] (Fig. 2A). This characteristic appearance can often lead to a definitive diagnosis via imaging. Histologically, the cysts are lined with glycogen-rich simple cuboidal epithelium, which is positive on periodic acid-Schiff (PAS) stain without diastase digestion [25]. If imaging results are inconclusive, EUS/FNA of cystic fluid can also be done but has low sensitivity though the addition of cytobrushing can improve the sensitivity of EUS-FNA [26,27]. SCNs have relatively lower carcinoembryonic antigen (CEA) levels than other PCL types with a higher risk of malignant progression [24]. However, there is no concrete evidence to support a direct correlation between CEA levels and risk of malignant progression [28].

SCAs are considered benign lesions that do not communicate with the pancreatic ducts [29]. Their progression to serous cystadenocarcinoma is exceedingly rare, with an incidence rate of < 1%, including the largest series that reported three cases of cystadenocarcinoma out of 2622 patients [30]. For this reason, the clinical recommendation is to observe serous cystadenomas, with or without serial imaging, to check on the growth rate of the tumor. Resection is warranted only if mass effect symptoms are present such as abdominal pain,

nausea, jaundice, or rapid cystic growth. Palliative resection can also be considered if the lesion transforms into a serous cystadenocarcinoma and becomes malignant [31].

2.2. Mucinous cystic lesions

2.2.1. Mucinous cystic neoplasms—Mucinous cystic neoplasms (MCNs) represent 2–5% of all exocrine pancreatic tumors [22]. The mean age at diagnosis is 49 years (20–82 years) and these lesions are predominantly found in women (F: M > 20:1) [22,32]. MCNs are mainly located in the body and tail of the pancreas and if present in the head of the pancreas, they are highly suspicious for mucinous adenocarcinoma [33,34]. MCN's are typically a single lesion, that can be unilocular or multilocular, which does not communicate with the pancreatic duct [35] (Fig. 2B). Morphologically, MCNs are characterized by a large, solitary, septated, thick-walled cyst with a pseudo-capsule containing either mucin or mixture of mucin and hemorrhagic material [36]. Histological analysis can reveal ovarian-like stroma in addition to columnar cells with abundant mucin production [37]. As per international consensus in 2004, the histological presence of unique ovarian-type stroma was necessary to confirm the diagnosis of MCNs [38]. In contrast to the aforementioned serous cystic lesions, MCNs have an increased propensity to be malignant or progress to a malignant state. Because of this, several studies have tried to identify a marker that can differentiate between the two (Table 1). The incidence rate of mucinous adenocarcinoma varies between 6 and 36% [39]. If the lesion is multilocular, contains papillary projections, and/or contains mural nodules the risk of malignancy drastically increases [40]. The spectrum of differentiation in terms of histology ranges from normal-appearing columnar epithelium to the atypical epithelium. Tumors can be classified as MCN with low/intermediate grade dysplasia, MCN with high-grade dysplasia, or MCN with an associated invasive carcinoma [41].

In an analysis of 163 patients with resected MCNs, the prevalence of adenocarcinoma was reported to be 17.5% by Crippa et al. [42]. The older patients with invasive adenocarcinoma in this cohort suggested a progression from adenoma to carcinoma. Thus, this group stated that resection should be considered in patients with high-risk MCNs and patients with low-risk MCNs, defined as size < 4 cm and no nodules, can be considered for non-radical resections [42].

2.2.2. Intraductal papillary-mucinous neoplasms—Intraductal papillary-mucinous neoplasms (IPMNs) are mucin-producing tumors arising from the main pancreatic duct or its branches [43]. These lesions are characterized by a dilation of the pancreatic duct resulting from immense mucus production and papillary growth of ductal epithelium (Fig. 2C, key differences between SCNs, MCNs, and IPMNs are presented in Table 2). IPMNs comprise 1–3% of exocrine pancreatic neoplasms, with an incidence rate of 1 per 100,000 per year [12,44,45]. and their frequency is higher in males than in females with a median age of diagnosis in the 6–7th decade [35,46]. IPMNs can be divided into low-risk and high-risk, with the latter defined as dilated main pancreatic duct > 5 mm or the presence of a mural nodule. The pooled cumulative incidence of high-grade dysplasia or pancreatic cancer for low-risk IPMNs is 0.02%, 3.12% and 7.77% at 1 year, 5, and 10 years, respectively. While that for high-risk IPMNs is 1.95%, 9.77% and 24.68% at 1 year, 5 and 10 years, respectively

[47]. IPMNs can involve the main duct (MD-IPMN) or side branches (BD-IPMN) or both known as mixed IPMN. BD-IPMNs are not only the most common IPMNs but also the most common pancreatic cyst. MD-IPMNs carry a higher risk of malignancy than BD-IPMN, with 38–68% of the resected specimens of MD-IPMNs showing high-grade dysplasia or cancer [48]. The relative risk of malignant transformation for multifocal IPMNs is not at higher risk as compared to a single cystic lesion [49]. Mixed-type IPMNs contain features of both, yet behave most similarly to MD-IPMNs in terms of progression and malignant potential and are clinically treated as such. Differentiating IPMNs that are malignant/invasive from those that are benign is a persistent and important problem to address. Recent studies that have identified molecular markers in various body fluids capable of distinguishing between invasive and non-invasive IPMNs are summarized in Table 3.

Based on the cytoarchitectural features and mucin immunohistochemistry (i.e. MUC1, MUC2, and MUC5AC), IPMNs have been classified into four histopathological types; gastric (49–63%), intestinal (18–36%), pancreaticobiliary (7–18%), and oncocytic (1–8%) [49–51]. Recent investigations showed benign EUS findings (cyst size < 5 mm and the absence of a mural nodule) are associated with gastric type IPMN [52]. Corroborating this finding, Furukawa et al. found prognostic relevance to IPMN classification, where patients with gastric type had a better prognosis than patients with intestinal-type IPMNs [53]. Gastric type is associated with the more indolent BD-IPMN, whereas intestinal type is often associated with MD-IPMN [54]. The pancreaticobiliary type has been regarded by some as a high-grade version of the gastric type. These lesions are uncommon, not well characterized, and the invasive carcinoma associated with this type is more aggressive [55,56]. Oncocytic type is relatively uncommon, tends to be large lesions with obscure intraductal appearance, and is less invasive [57]. A retrospective study of patients with cystic lesion type verified on pathology found that even though gastric type IPMN had a better prognosis than intestinal type, those with gastric type who developed invasive disease had worse outcomes when compared to those with progression arising from intestinal-type [53]. The reason for this phenomenon was that gastric IPMNs had the potential to develop a more aggressive tubular (ductal) carcinoma as opposed to the colloid (mucinous) carcinoma arising from intestinal IPMNs [53].

Pancreatic/cystic fluid has been investigated extensively for identifying biomarkers to classify IPMNs into histologic categories that dictate the need for resection. A study by Hara et al. consisting of 36 patients with surgically resected IPMNs found that mucin histology of pancreatic fluid cytology could differentiate the four subtypes of IPMNs with an accuracy of 89% [58]. The same study reported that a combination of cytology and MUC positivity had a sensitivity and specificity of 77% and 86% respectively, for distinguishing high-grade dysplasia/invasion from benign lesions [58]. Table 4 lists histological classifications of IPMN along with their respective mucin expression levels. This table is comprised of the World Health Organization (WHO) recommendations for mucin profiling to classify IPMN subtypes [41] along with the findings of various groups investigating specific mucin expression in the various subtypes (and low-grade vs high-grade dysplasia) including MUC1 [59], MUC2 [59,60], MUC5AC [59], MUC6 [59], MUC4 [61], and MUC13 [62]. EUS-FNA can preoperatively differentiate between the four subtypes of IPMNs, provided biopsy is adequately taken from the epithelial lining of the cyst [63].

2.3. Other pancreatic cystic lesions

Other cystic lesions of the pancreas include solid pseudopapillary tumors (SPT), lymphoendothelial cysts (LEC), and neuroendocrine neoplasms (PNENs). SPT is a rare tumor seen most frequently in young women in their 20's. < 10% of SPT's have aggressive tumor behavior pathologically with a 5-year disease-specific survival of over 98% [64]. The tumor is considered indolent given the high survival rates of patients even when metastases are present [65]. The diagnostic accuracy of preoperative imaging for SPT is high with a sensitivity of 95% [66]. LECs are extremely rare complex lesions that are often round and exophytic that predominately occur in males [67]. They are often anechoic or hypoechoic on EUS and can present with elevated CEA and amylase levels in the cyst fluid aspirate [67]. While these lesions can harbor malignancy, their rarity has prevented adequate pathological characterization [68]. PNENs are also rare and may be solid, cystic, or mixed in morphology. They are usually non-functioning and may occur sporadically or in individuals with multiple endocrine neoplasia type 1 (MEN1) and/or Von Hippel-Lindau (VHL) [69]. They usually present in the sixth decade and have equal gender predisposition. Unlike SPTs, the diagnostic accuracy of preoperative imaging for PNEN is low (sensitivity of 53.3%) [66]. However, EUS-FNA of PNENs has a 90% diagnostic accuracy [70].

Inflammatory pseudocysts are non-malignant fluid-filled sacs often filled with necrotic and hemorrhagic material along with pancreatic enzymes [71]. These are not true cysts as they do not have an epithelial lining. In the absence of any clinical symptoms, they can be monitored with imaging while symptomatic lesions can be effectively treated with steroids and/or surgical drainage [72]. Notably, pseudocysts are often associated with a history of chronic and/or acute pancreatitis [73], alcoholic pancreatitis [74], or autoimmune pancreatitis [75]. These studies found that 42–56% of pancreatitis patients harbor pseudocysts [73,74]. Unfortunately, some cystic neoplasms, including those with malignant potential, can initially present with pancreatitis or even cause recurrent bouts of pancreatitis [16,76]. Along with this, serum amylase and lipase levels, the standard metrics by which pancreatitis is assessed in clinics, are unable to differentiate between MCNs and pancreatitis without supplemental imaging and invasive procedures [77]. Up to 15% of IPMN patients present with pancreatitis [78] as well as 9% of those with MCNs [42] and some IPMNs can elicit an immune response thus inducing autoimmune pancreatitis [79]. BD-IPMNs, in particular, can be difficult to differentiate from pseudocysts in the setting of pancreatitis and thus, many pseudocysts are often mismanaged as IPMNs [16]. It has been reported that 10–15% of cystic lesions discovered with a background of pancreatitis can be malignant [75,80]. Large cyst size and poor response to steroids increase the likelihood of the presence of malignancy [75,80].

3. Imaging and diagnostic algorithms

Multiple imaging modalities have been utilized to differentiate between malignant and benign pancreatic cystic lesions. These include computerized tomography (CT), positron emission tomography (PET), endoscopic ultrasound (EUS), magnetic resonance imaging (MRI), and magnetic resonance cholangiopancreatography (MRCP). A systematic review including 19 studies showed that the accuracy of CT and MRI or MRCP to differentiate

benign from malignant cysts was 71–80% and 55–76%, respectively [81]. MRI or MRCP has a higher sensitivity of 96% as compared to CT, which has a sensitivity of 80% in diagnosing IPMN. The meta-analysis suggested the use of CT as an initial diagnostic modality followed by MRI or MRCP for further confirmation [81]. EUS imaging can differentiate between benign and malignant cysts in 65–96% of the cases, which is similar to the accuracy of MRI or MRCP, and CT scans [81]. However, EUS identifies high-risk features such as mural nodules, which could be missed on MRI and MRCP. Although, EUS is not recommended as a first-line modality for small pancreatic cysts with a clear diagnosis, as it is invasive and has comparable accuracy as CT and MRI or MRCP. De Jong et al. showed increased sensitivity with a combination of MRI and EUS as compared with either modality alone in identifying IPMNs, MCNs, pancreatic cancer, and cysts with high-grade dysplasia [82,83]. Combination PET-CT was found to be superior to CT alone in distinguishing benign and malignant lesions [81]. Kauhanen et al. in a prospective study reported the diagnostic accuracy of PET-CT to be 94% compared with 77% for MDCT and 87% for MRI/MRCP in identifying the pancreatic cysts [84]. However, PET-CT is currently not included in the standard diagnostic algorithm for PCL due to insufficient data.

The decision to operate or observe an IPMN is guided by the 2017 revised Fukuoka International Consensus Guidelines [15]. These guidelines elucidate specific radiological features, particularly worrisome and high-risk stigmata, which dictate clinical decision making. The presence of worrisome features requires continual sequential surveillance, while the presence of high-risk stigmata necessitates resection. Imaging high-risk stigmata include main pancreatic duct (MPD) dilation greater than or equal to 10 mm, an enhancing solid component within a BD-IPMN, cysts > 3 cm in size, thickened/enhancing walls, non-enhancing mural nodules, and/or abrupt change in the caliber of the MPD with distal pancreatic atrophy [15]. However, multiple analyses of IPMNs found that size may not accurately predict malignancy, and thus, the importance of size was downgraded in the most recent consensus guidelines [85–87]. An investigation of IPMNs resected according to the Fukuoka criteria found the overall prevalence of PDAC to be only 15.2% [88]. However, this study did conclude that these criteria, in comparison to the 2015 AGA guidelines, were far more sensitive for resection of IPMNs with pathology confirmed high-grade dysplasia [88]. A study with MCN patients found that the sensitivity and specificity of CT scan were 100% and 98% for malignancy if mural nodules were present [89]. Another study analyzing tumor markers and cyst size found that a size > 3.0 cm had greater than four times the risk of malignancy compared to smaller cysts but tumor markers (serum CEA, CA19.9, and cyst fluid CEA) had no predictive value for these circumstances [90].

Prior to the advent of the Fukuoka guidelines, a retrospective study was conducted on surgically resected PCLs that were discovered incidentally. It showed that while the majority of resections contained premalignant or malignant pathological changes, only the malignant group (20.4%) had diminished overall survival [91]. Thus, almost 80% of surgical interventions were unnecessary. After the implementation of the Sendai criteria and then Fukuoka guidelines, the stratification of PCLs into low-risk and high-risk groups lead to improved triaging of patients and refinement of surgical candidates [92]. Congruently, PCL patients with benign features are far less likely to be recommended for surgery when comparing Fukuoka guidelines to Sendai Criteria [92].

While these studies prove that Fukuoka guidelines are an improvement upon previous criteria, the overall sensitivity and specificity for lesions containing PDAC and for those which may progress to PDAC remain low. The increase in sensitivity for detecting malignancy, paired with the limited increase in specificity offered by the Fukuoka guidelines, has resulted in many unnecessary surgical interventions [93]. These criteria have been continually explored in retrospective studies and have been found to improve the association of PCL resection with malignancy when compared to non-adherence [94–96]. However, other studies trying to validate the consensus guidelines have yielded inconsistent results. A meta-analysis of 7 such studies found a positive predictive value of only 36% and a negative predictive value of 90% [87]. Other studies analyzing the cyst-malignancy correlation found that operative resection is undertaken in 30–32% of cystic cases with only 20–38% of those resected lesions found to be malignant on pathology [26,91]. Yet another study showed only correlation with malignancy in 41% of resected cases [97]. The variation among different studies calls into question the inherent validity of utilizing only Fukuoka guidelines to determine PCL malignancy potential and, by extension, clinical strategy.

4. Predictive biomarkers

4.1. Cellular and molecular profiling of cystic fluid

Historically, cystic fluid has been collected and assessed for myriad molecular markers for two primary reasons; discerning between mucinous and serous cysts (Table 1), and differentiating benign from malignant lesions (Table 3). Coupled with EUS imaging, fine-needle aspiration (EUS-FNA) is a procedure used to obtain cystic fluid for analysis. This fluid can be assessed for the presence of a myriad of diagnostically useful species such as cells, proteins, and nucleic acids.

4.2. Cytology

Patients harboring cysts with worrisome features, or those > 3 cm, are recommended to undergo EUS. Each cyst subtype has discreet characteristic cellular typology, morphology, and immunohistochemical staining. Pseudocysts contain inflammatory cells while serous mucinous neoplasms demonstrate glycogen-containing cells [29]. Mucinous cystadenocarcinomas demonstrate cells with malignant features (increased nuclear/cytoplasmic ratio, pleomorphism, anaplasia, hyperchromatism, prominent nucleoli, and mitoses) nearly 50% of the time, but the yield is usually low [98]. MUC2 and CDX2 are indicative of intestinal differentiation and can be used as immunohistochemical markers to identify intestinal-type IPMN [56,59]. A meta-analysis by Suzuki et al. showed a sensitivity and specificity of EUS-FNA cytology to be 65% and 91%, respectively, for distinguishing malignant and benign IPMNs [99]. There has always been a concern with peritoneal seeding secondary to EUS/FNA; however, a study found no significant increase in metastatic disease secondary to the procedure in patients with IPMNs who underwent EUS/FNA [100].

While cystic fluid cytology can help differentiate between various cyst types and can be highly specific, the cellular yield is usually too low to be diagnostically useful thereby greatly diminishing the sensitivity. In the Cooperative Pancreatic Cyst study (CPC study) that involved 341 patients with PCLs, the sensitivity of cyst fluid cytology for diagnosing

mucinous cysts was only 34% because of the low number of cells found in cystic fluid [101]. In conjunction, a multiloculated cystic lesion leads to the compartmentalization of fluid species, thus, FNA may not be representative of the lesion's gestalt.

4.3. Protein markers

4.3.1. CA19.9 and CEA—CA19.9 is a Sialyl Lewis A glycan present on multiple glycoproteins and is currently used in the clinic to monitor patients for PDAC progression and/or evaluation after surgical resection. Carcinoembryonic antigen (CEA) is a secreted glycoprotein involved in cell adhesion and has been used as a biomarker for various gastrointestinal malignancies [102,103]. These proteins are often aberrantly expressed in cystic fluid and much research has been conducted to discern their respective diagnostic and prognostic significance (Tables 1 and 3).

Cyst fluid CA19.9 levels have proven useful in diagnosing MCNs [104] as well as IPMNs [10]. CA19.9, however, can yield confounding results since it is often elevated in PDAC[66]. CEA is one of the most clinically useful cystic fluid biomarkers for PCLs and the prediction of malignancy. A multicenter prospective trial found that a CEA cystic fluid level > 192 ng/mL could distinguish MCNs and IPMNs from the other types of cysts with a sensitivity and specificity of 73% and 84%, respectively [26,54]. Congruently, a study involving 112 patients with histologically confirmed PCLs found CEA to have a sensitivity of 91% for correctly diagnosing a mucinous cyst prior to resection. Unfortunately, the specificity was 31% with an AUC of 0.61 [26].

Cystic fluid CEA and CA19.9 levels have also been shown to be higher in malignant compared to benign cysts [105]. In this study, the sensitivity/specificity for CEA and CA19.9 for predicting malignancy were 92%/64% and 81%/69%, respectively [105]. Another study found CEA levels > 800 ng/mL are also suggestive of premalignant or malignant lesions [98], while a different group determined that cystic fluid CA19.9 has the potential to detect cancer in PCLs [106]. However, other studies have shown that cystic fluid levels for either of these markers are not sufficient alone to specifically differentiate malignant vs nonmalignant cysts [107–109]. Interestingly, there is increasing evidence that these biomarkers are efficacious when measured in pancreatic fluid [110,111]. Pancreatic fluid offers many advantages over cystic fluid including facile and less invasive collection using FNA, the increased representation of the pancreatic gestalt, and less of the complexity introduced by multiloculated lesions.

A combination of serum CA19.9 and CEA was used to determine the presence of malignancy in resected IPMNs [112]. CA19.9 alone had a sensitivity/specificity of 74%/86%, CEA had a sensitivity/specificity of 40%/92%, and a combination of CA19.9 (cutoff > 37 U/mL) and CEA (cutoff > 5 g/mL) had a sensitivity/specificity of 80%/82% [112]. Hirono et al. examined CEA (cutoff > 30 ng/mL) in pancreatic juice in BD-IPMNs and reported sensitivity/specificity of 94%/85% for malignancy [110]. A meta-analysis looking at the ability of serum CA19.9 and CEA to identify invasive and malignant IPMNs found CA19.9 had a pooled sensitivity of 52% and 40% and specificity of 88% and 89%, respectively while CEA has a pooled sensitivity of 18% for both invasive and malignant IPMNs and a specificity of 95% and 93%, respectively [113].

CA19.9 is limited in its diagnostic utility as Le(a⁻b⁻) patients do not have the necessary fucosyltransferase enzyme to produce it and also, this phenotype is highly prevalent among different ethnic groups: Asian (7%), European (8%), African (19%) [114]. CEA can be expressed in serum resulting from other diseases including lung fibrosis, Alzheimer's disease, and a variety of other cancers [115]. With this, these studies demonstrate that clinically used cystic fluid, pancreas juice, and serum markers are variable in their diagnostic potential and that more accurate biomarkers are needed to determine malignancy in pancreatic cystic lesions.

4.3.2. Mucins—Mucins are large O-linked glycoproteins that are expressed on the cell membrane or secreted into ducts and function to protect the luminal surfaces in the liver, lungs, gastrointestinal tract, mammary ducts, and various other organs. In cancer cells, mucins vary in expression, glycosylation, localization, and splicing [116–118]. These mucin traits are exploited by tumor cells to interact with their microenvironment and proliferate and metastasize [116].

MUC1 is a transmembrane mucin that is expressed by most epithelial cells and plays a role in cell proliferation through its interaction with the growth factor receptor tyrosine kinase, ErbB [119]. Its expression is elevated in mucinous cystadenocarcinomas as well as malignant IPMNs [120,121]. MUC1 mRNA levels are increased in the pancreatic juice of malignant IPMNs with a sensitivity and specificity of 89% and 71%, respectively (Table 3) [122]. However, some studies found no correlation between cyst fluid MUC1 protein or RNA with the risk of malignancy in IPMNs or MCNs [60,123]. Further investigation into MUC1 expression in mucinous cysts is warranted based on the current conflicting data.

MUC2 is a secreted, gel-forming mucin that was found to be elevated in cystic fluid from noninvasive MCNs [120]. It is also found in intestinal-type IPMN as it is secreted by goblet cells in normal physiology. Multiple studies have found that MUC2 detected by IHC was associated with an increased risk of malignant IPMNs [56,124]. MUC2 has also been found to be predictive of malignancy specifically in mixed-type IPMNs [125]. The usefulness of MUC2 as a marker in mixed-type IPMNs can be attributed to their predominant grouping in the intestinal category and propensity for goblet cell formation. While MUC2 levels have been found to be significantly elevated in the fluid of highly dysplastic/malignant intestinal IPMNs [60], in other histological types of IPMNs MUC2 is either absent or at a low level, thus limiting its usefulness in diagnosing malignancy in other IPMN categories [41,125,126] (Table 4).

MUC4 is a transmembrane mucin that is not normally expressed in the pancreas. It has been discovered in early pancreatic intraepithelial neoplasms as well as adenocarcinoma and also mediates its functional effects through the growth factor family member ErbB [119]. Kitazono et al. conducted a tissue analysis on 142 IPMN samples comparing gastric vs. intestinal IPMNs and found a significantly higher expression of MUC4 in intestinal-type [61] (Table 4). In addition, this study found that MUC4 expression increased as the level of dysplasia advanced, regardless of IPMN type [61]. MUC4 is also increased in the cystic fluid of dysplastic and invasive IPMNs [60]. In an elegant study, Maker et al. utilized various target types including mRNA, miRNA, and DNA to develop a qPCR based assay to discern

high-risk (high grade dysplasia to invasive cancer) from low-risk (low to moderate grade dysplasia) IPMNs [127] A combination of Interleukin 1β, MUC4, and prostaglandin E synthase 2 mRNAs proved to be the most accurate at discerning between low-risk and high-risk cysts with an AUC of 0.86 [127]. These studies did not verify their findings in external validation sets, thus further investigation is warranted.

MUC5AC is a secreted mucin that is a marker of gastric epithelium and has been found to be present in all MCNs based on immunohistochemistry of tissue specimens as well as in all four IPMN categories [128] (Table 4). MUC5AC was significantly increased in the serum of high-risk IPMN patients [60] and is also present in the tissue of pancreatic ductal adenocarcinoma, thus making it a malignancy marker [129]. A glycan variant of MUC5AC, when measured in cystic fluid along with CA19.9, provided a sensitivity and specificity of 87% and 86% respectively, for differentiating MCNs and IPMNs from SCNs [130]. In a clinical trial evaluating the effect of neoadjuvant erlotinib (a tyrosine kinase inhibitor) on IPMN growth, researchers hypothesized that MUC5AC production is dependent upon the EGFR pathway, and inhibition of the EGFR effector molecules may reduce growth. A decrease in growth and a reduction in MUC5AC expression was observed in the subsequent resected tissue [131]. Recently, variation in the glycoforms of MUC5AC with a concurrent assessment of endorepellin was proven to be a highly efficacious biomarker for mucinous cysts [132]. Another combinatorial panel of MUC5AC, MUC2, and CEA was tested and provided up to 99% accuracy to discriminate malignant from premalignant lesions in a cohort of 24 patients [133]. While this finding is quite encouraging, predictive efficacy is still in question as the patient number was limited.

MUC6 is expressed in IPMN lesions and has a slight proclivity towards the gastric subtype [59]. However, MUC6 has an intermittent expression in the other types, thus rendering it not the most effective for IPMN delineation alone, but still useful when assessed in combination with other mucins [59].

MUC13 has been shown to be moderately differentially expressed in the fluid of the various IPMN types [62]. In this same study, the MUC13 expression levels were greater in high-risk IPMNs as compared to low-risk [62]. Taken together, this suggests that while MUC13 may not be applicable to IPMN differentiation per se, it may offer valuable insight into the presence or absence of malignant progression.

MUC16 (CA125), is a membrane-bound mucin that is ubiquitously used as a biomarker in the surveillance of ovarian cancer [134] as well as advanced PDAC [135]. It has been found in the cystic fluid of pancreatic lesions, however, its levels were not useful in accurately distinguishing between the various types of cysts [26].

4.3.3. Other proteins—Proteomic analysis of cystic fluid using mass spectrometry has been employed for discovering biomarkers for differentiating mucinous cysts and defining the risk of malignancy with greater accuracy [128]. Ke et al. identified amylase isoenzymes, mucins (MUC1, MUC5AC, MUC5B, and MUC16), CEACAM family members (CEACAM 5, 6, and 7), and S100 homologs that provide valuable information on the invasive potential of a pancreatic cyst [136]. Another mass spectrometry analysis identified olfactomedin-4 as

associated exclusively with MCN and IPMNs [137]. Mass to charge (m/z) ratio was found to be different between samples from malignant and benign IPMNs. Five protein peaks were identified that were highly accurate in discerning a malignant IPMN [138]. These studies are limited by their sample numbers; however, they are useful for identifying novel proteins for further individual analysis.

General inflammatory markers have been measured in cystic fluid to differentiate between the cystic subtypes and categories and determine malignant potential. Maker et al. analyzed cystic fluid from 40 patients consisting of predetermined high risk and low-risk cysts. They examined the fluid for IL-1, 2, 4, 5, 8, 10, 12, 13, INFα, and TNFα. Only IL-1 was predictive for high-risk IPMNs with a sensitivity and specificity of 79% and 95% [139]. Additionally, markers of specific pancreas damage/inflammation have also been utilized to determine the presence of cancer within a cystic lesion. For example, a significant correlation was found between increased serum pancreatic enzymes (amylase and lipase) and malignancy/invasiveness of IPMNs in patients without a history of pancreatitis [140]. However, these conclusions may be complicated by the fact that a cystic fluid amylase level > 250 U/mL correlates with a pseudocyst [98].

In a study consisting of 87 patients with pathology confirmed PCLs, vascular endothelial growth factors VEGF-A and VEGF-C were found to be significantly increased in fluid from benign SCNs compared to MCNs. Although these markers failed to distinguish between benign and malignant mucinous lesions, a diagnostic algorithm combining VEGF-A, VEGF-C, and CEA as biomarkers to distinguish serous from mucinous cysts may help increase overall diagnostic capabilities [141].

Tissue polypeptide antigen (TPA) is produced by rapidly proliferating tissue. Its levels in cystic fluid distinguished mucinous cystadenocarcinomas (MCAC) from benign lesions with a sensitivity and specificity of 75% and 97% at a cutoff value of 100,000 U/mL [142]. IPMNs and PDAC control cases were not included in this study, so further analysis of this protein will be required in fluid from IPMNs to observe any effects on its accuracy.

SPINK1, a protease inhibitor also known as pancreatic secretory trypsin inhibitor, has previously been utilized for the diagnosis and risk assessment of hereditary pancreatitis [143,144]. SPINK1 has also been used to identify IPMNs based on pancreatic juice analysis with a specificity of 98% [145]. Recently, it has been measured in cystic fluid and distinguished between benign and potentially malignant lesions in addition to differentiating MD-IPMN from BD-IPMN [146]. These studies highlight SPINK1 potential for cystic lesion stratification, but validation is required as the utility could be limited by the aforementioned association with pancreatitis [147].

Claudins are transmembrane proteins that are a component of tight junctions and have also been analyzed in the tissues of PCLs. Claudins 2, 4, and 18 have been found to be elevated in mucinous lesions. Their relative tissue profiles can also help distinguish between the different histologic subtypes of IPMNs [148]. Increased claudin 4 expression in pancreaticobiliary IPMNs and increased claudin 18 in gastric IPMNs was reported. They also found increased expression of claudins 4 and 18 and decreased expression of claudin 2

with tumor progression. Claudin 18 expression has been found to be increased in the tissues of other premalignant lesions such as Pan-IN (97%) and MCNs as well as PDAC [149]. There is a lack of studies assessing claudins in cystic fluid but should be encouraged based upon tissue data, as they may prove to be a useful marker of the malignant potential of pancreatic cystic lesions.

Ubiquitin and thymosin-4 (an actin sequestering protein) were found to be significantly over-expressed in FNA samples of IPMNs with high-grade dysplasia [150]. Plectin-1 was also found in 100% (4/4) of malignant cystic fluid samples from IPMNs and no measurable Plectin-1 was detected in the three benign IPMNs studied [151]. mAb Das-1 is a monoclonal antibody against colonic epithelium and reactive to premalignant upper gastrointestinal conditions. It was able to differentiate cystic fluid from high risk/malignant IPMNs vs. low-risk lesions with a sensitivity of 89% and specificity of 100% in a study consisting of 27 patients undergoing resection of their IPMNs [152]. The same group recently validated these results in a larger patient cohort and were able to detect high-risk IPMNs (i.e. with cancer, high-grade dysplasia, or intestinal-type histology with low-grade dysplasia) with 88% sensitivity and 99% specificity [153]. This finding is limited because it was a retrospective analysis but the group is currently beginning a prospective study.

Prostaglandin E2 (PGE2) has shown to have increased expression in the pancreatic cancer tissue as compared to the normal tissue [154]. PGE2 concentrations in the pancreatic cyst fluid can help in distinguishing between different types of mucinous cysts. Schmidt et al. did fluid analysis of 58 resected cystic lesions which showed higher PGE2 concentrations in IPMNs versus MCNs. However, the utility of this biomarker is limited in the clinical setting due to the overlap of PGE2 concentrations in benign MCNs and SCNs [155].

Sonic hedgehog (SHH) is a signaling peptide that plays a role in organogenesis. It is abnormally expressed in pancreatic intraepithelial neoplasia (PanINs) and is increased in pancreatic juice obtained from patients with IPMNs. Thus it may aid in the differentiation between IPMN and chronic pancreatitis, which is a distinction that is difficult to make based on imaging alone [156].

The analysis of proteins in cystic fluid and serum has expanded in recent years, which speaks to two primary notions. The first is that proteomic analysis of PCLs holds incredible potential for differentiating patients with malignant versus benign lesions as well as classifying those within the PCLs, which have the potential to progress to PDAC. The second is that the multitude and variation in proteins tested displays a lack of one definitive marker capable of accurate diagnoses. Further investigation is required to discover a possible multi-marker means of proteomic PDAC screening for PCL patients.

4.4. Genomic markers

4.4.1. DNA—Genomic data has also been studied and recognized as a highly valuable target in cystic fluid. It was recently demonstrated that cell-free supernatant of pancreatic FNA samples does contain DNA that can be used for analysis [157].

KRAS mutations have been well characterized in the pathogenesis of pancreatic cancer [158,159]. Alterations in KRAS are thought to be an early event in IPMN biogenesis as it is found in all types without significant differences [160,161]. Mutant KRAS in cystic fluid was found to be highly specific (92–96%) for mucinous cyst diagnosis but with low sensitivity (33–45%) [120]. It was observed in 4 out of 5 cystic fluid samples from malignant mucinous cysts [162] and these mutations have also been found in a majority of tubular carcinomas arising from intestinal-type IPMNs [160]. In conjunction, cysts without high-grade dysplasia were found to have fewer KRAS mutations as well as a lower risk of progression [160]. Thus, the potential of KRAS as a biomarker for poor prognosis is feasible yet not necessarily the best for lesion stratification and cancer detection.

A large and comprehensive investigation, termed the PANDA study, was a prospective multicenter study consisting of 113 patients that analyzed DNA in the cystic fluid. High amplitude KRAS mutations were able to detect malignancy with high specificity (96%) but low sensitivity (45%) [163]. This study most importantly concluded the use of DNA analysis in highly selected circumstances to detect the presence of malignancy when cyst cytology is negative. In addition to KRAS analysis, higher DNA quantity and allelic loss in amplitude over 82% in cystic fluid were found to have diagnostic efficacy for malignancy [120,163]. There was a confirmed association between the patient requirement for operative intervention and survival rates, however, there was selection bias as no DNA analysis data was provided on those who did not have surgery. Thus, further investigation of KRAS DNA analysis with long-term follow-up is needed before making it a routine testing method [164].

GNAS codes for the alpha subunit of a stimulatory G protein and a gain of function mutation results in uncontrolled cellular signaling via continuous cAMP production [165]. An activating GNAS mutation is detected in the tissues of 61% of IPMNs, but its individual presence does not correlate with clinical outcome [166]. When analyzed in conjunction with mutations in KRAS, 96% of IPMNs were positive for at least one of the oncogenes, and the GNAS mutation was not present in other types of cystic lesions or in PDAC [167]. Singhi et al. found that a mutation in either GNAS or KRAS in cystic fluid had a sensitivity and specificity of 65% and 100%, respectively, for mucinous differentiation [168]. This finding was further corroborated by next-generation sequencing, which confirmed that mutations in GNAS and KRAS supported an IPMN with non-mucinous CEA in 71% of the cysts. Furthermore, 19% of the non-malignant cysts with non-mucinous CEA were reclassified as mucinous by the presence of KRAS mutation [162]. Recently, Singhi et al. in another study showed that NGS detection of KRAS/GNAS mutations was 100% specific for IPMN and in combination with TP53/PIK3CA/PTEN alterations, was able to distinguish between IPMNs with low and high-grade dysplasia. However, KRAS/GNAS mutation was detected in < 50% of cyst patients [169]. GNAS mutations have recently been associated with colloid carcinoma, which is less aggressive than PDAC which can also arise from intestinal-type IPMN [160]. These findings highlight the potential GNAS has in the detection of early precancerous lesions. This could prove invaluable in the challenge of detecting PDAC at a resectable stage.

A recently developed technique called the multivariate organization of combinatorial alterations (MOCA) was utilized to determine the diagnostic efficacy of a combination of

patient characteristics and cystic fluid genomic data [170]. Cystic fluid samples were analyzed for mutations in genes shown to be involved with pancreatic cystic lesions (BRAF, CDKN2A, CTNNB1, GNAS, KRAS, NRAS, PIK3CA, RNF43, SMAD4, TP53, and VHL), to indicate loss of heterozygosity at CDKN2A, RNF43, SMAD4, TP53, and VHL tumor suppressor loci, and to indicate aneuploidy in samples. A composite of these molecular markers distinguished cysts requiring resection (SPN, MCN, IPMN with high-grade dysplasia or cancer) with a sensitivity and specificity of 75% and 92%, respectively. However, when combined with clinical factors such as age, pain, communication with the main pancreatic duct (MPD), and dilation of the MPD, the sensitivity increased to 89%; however, it came with a substantial decrease in specificity to 69%.

Of note, integrated molecular pathology (IMP) testing (PancraGen/PathFinder TGR, Interspace Diagnostics) is a validated DNA mutational analysis platform for cystic fluid analysis. It measures various oncogene activation and loss of heterozygosity markers to determine the malignant potential of cystic lesions. This was utilized in a multicenter retrospective study that analyzed cystic fluid from 492 patients with IMP and then followed them for at least 23 months. IMP had similar sensitivity compared to the 2012 international consensus guidelines with imaging as the primary diagnostic modality (83% vs. 91%). However, IMP outperformed the guidelines with a specificity of 91% versus 46%, $p < 0.0001$ [171]. Epigenetic modification of DNA also holds promise as a novel biomarker for PCL characterization and progression. One such study investigated the methylation of various DNA markers found in cystic fluid and found the methylation patterns of four genes (*SOX17*, *SLIT2*, *EYA4*, *SFRP1*) were distinguished high-risk from low-risk cystic neoplasms with 88% overall accuracy [172]. Taken together, these data show novel DNA markers that can offer a method to individualize and target PCL therapy, in addition to imaging [173].

4.4.2. miRNA—MicroRNAs (miRNAs) have been increasingly investigated as potential biomarkers for pancreatic cancer in recent years [174]. Multiple studies have used resected tissue to determine miRNA profiles that are differently expressed between low and high-risk IPMNs [175–177]. A four-member miRNA panel (miR-21–5p, miR-483–3p, miR-708–5p, and miR-375) was observed to differentiate between IPMN and PDAC with a sensitivity and specificity of 95% and 85% respectively [178]. These findings in tissue have led to further analysis of cystic fluid. Cystic fluid miR-21 was able to differentiate between mucinous and non-mucinous cystic lesions with 80% sensitivity and 76% specificity [179]. Corroborating the value of miR-21, another group found a combination of miR-21 and miR-221 are indicative of malignancy in pancreatic cystic lesions [180].

Through logistic regression analysis of a set of 65 cystic fluid samples, Matthaei et al. found nine miRNAs were able to accurately identify cysts requiring resection versus observation, with a sensitivity/specificity of 89% and 100%, respectively [181]. In an interesting validation study, Utomo et al. utilized the Matthaei miR panel in an attempt to accurately stratify cystic lesions in a prospective manner [182]. They were unfortunately only able to detect lesions requiring resection with 10% sensitivity but 100% specificity and concluded that this panel was unlikely to improve clinical management of cystic lesion patients [182].

Next-generation sequencing in surgical specimens and EUS/FNA samples from PDAC and IPMN patients showed miRNA expression overlap between the two sets and specifically miR-93 was capable of identifying PDAC and IPMN patients from healthy controls with sensitivity/specificity of 100%/96% and 89%/88%, respectively [183]. Additionally, next-generation sequencing from cystic fluid from low-risk and high-risk IPMNs yielded a panel of 13 miRNAs that were differentially expressed and further, complexed with targets involved five canonical pathways that are implicated in cancer [184].

Of note, miRNA studies have also been conducted on various biofluids and with other non-coding RNAs. For example, MiR-155 expression specifically has been shown to be increased in the pancreatic juice from surgically resected IPMNs [120,185]. Plasma miR-4830–3p was able to discern PDAC from IPMN and miR-21 was significantly correlated with advanced disease [186]. Another study involving plasma miRNAs was able to determine the cancer status of IPMNs with 81%/53% sensitivity/specificity [187]. Permuth et al. investigated the diagnostic utility of a different type of seromic non-coding RNA, long non-coding RNAs (lncRNAs: RNA molecules > 200 nucleotides in length that resemble coding sequences but lack open reading frames), and combined these with imaging features and miRNA data for the early detection of PDAC [188]. They were able to show that eight lncRNAs were capable of differentiating malignant and non-malignant IPMNs (sensitivity/specificity, 79%/76%) and the specificity was dramatically improved when combined with imaging features and miRNA data (sensitivity/specificity, 71%/100%) [188] (Table 3).

These studies demonstrate that miRNA may be a powerful predictor of malignancy in EUS-FNA and serum/plasma samples. However, a limitation could be imposed by inadequate quantities of miRNA isolated from various biofluids. For example, only 58% of pancreatic fluid samples yielded enough miRNA to be analyses in one study [189]. Another caveat in the use of miRNA is the fact that so many studies have been undertaken and have subsequently elucidated hundreds of miRNA targets with few overlapping targets between groups.

4.4.3. Single-cell RNA sequencing—Single-cell RNA sequencing is a next-generation sequencing technology developed in recent years. It involves isolating single cells, extracting their transcripts, and mapping these transcripts to each individual cell. As a result, it provides a higher resolution of cellular differences as compared to conventional RNA sequencing methods, provides detailed insight into the existence and behavior of different cell types, and elucidates regulatory relationships between genes [190,191].

In a first reported single-cell transcriptomic study on precursor cystic lesions of pancreatic cancer, Bernard et al. performed RNA sequencing on 5403 cells from two low-grade IPMNs, two high-grade IPMNs and two PDAC cases, obtained from surgically resected specimens [192]. Their prime objective was to understand the intralesional epithelial heterogeneity and tumor microenvironment heterogeneity that could provide insight into the progression of IPMN to PDAC. They found that the epithelial component of IPMNs and PDAC had similar as well as different transcriptomic elements. Notably, KRT1 and MUC1 were found in all samples, CEACAM6 was found only in IPMNs with high-grade dysplasia and cancer, and

MUC5AC was only present in IPMNs with low-grade dysplasia [192]. They also found tumor suppressor genes such as RAP1GAP to be expressed in lesions with low-grade dysplasia and silenced in those with high-grade dysplasia along with a concomitant increase in transcripts of oncogenes including S100P and S100A10 [192].

Their predominant finding described regarding the evolution of the tumor microenvironment during the progression of low-grade IPMNs to PDAC was alteration in the immune profile. Notably, pro-inflammatory immune cells, predominantly cytotoxic T-cells (measured by granzyme-and perforin-related transcripts), $CD4^+$ T-cells (CD69 expression) and cDC2-type dendritic cells (antigen-presenting cells), were seen in low-grade IPMNs but depleted during neoplastic progression. PDAC exhibited infiltration of immunosuppressive pro-tumorigenic CD11b+, S100A9+, CCL3+, APOE+ myeloid-derived suppressor cells (MDSCs) reaching 51% of stromal cells profiled, while IPMN stroma, with both low-grade and high-grade dysplasia, was comprised of 2.3% and 3.5% MDSCs, respectively. Interestingly, this MDSC population has previously been shown to be associated with cancer progression [193]. Thus, they postulated that in the future, single-cell analyses would be able to establish a threshold which foretells the invasive nature of a cystic lesion even in the absence of radiologically detectable features [192]. While intriguing and a first of its kind, limitations of the present study were the extremely small sample size for each cohort (2 cases each) and the lack of wet-lab validation of the virtual dissection data.

In another report, Beatty et al. observed higher infiltration of pro-inflammatory cells (CD8+, CD4+) in IPMNs with high-grade dysplasia as compared to those with low-grade dysplasia and normal pancreas tissue [194]. Other studies have also observed that infiltrating and/or circulating neutrophil-to-lymphocyte ratio could be a better indicator of IPMN malignancy [195–197]. Thus, the immune microenvironment and effects on PCL progression garner further attention.

4.5. Metabolomics

Metabolic reprogramming has long been associated with the transformation and growth of cancer [198]. The assessment of metabolic alterations in the setting of cancer for use as early detection biomarkers or therapeutic target delineation has gained high attention in recent times [199–202]. A serum-based panel comprised of 10 metabolites was observed to differentiate patients with cancer from normal controls with AUC of 0.92 on a ROC curve [203]. Another study utilized six metabolites panel (five of which were involved in lipid metabolism) to classify pancreatic cancer patients with a sensitivity/specificity of 90%/85% [204]. Branch chain amino acids were found to be increased in the serum of cancer patients up to ten years prior to the time of detection in another study [205,206]. While these findings have immense potential for cystic lesion stratification and malignancy determination, these studies utilized chronic pancreatitis, type 2 diabetes mellitus, and healthy patients as controls and did not test their findings in PCL patients or used PCL patients as benign controls.

However, some groups have started implying metabolomics to predict cystic lesion stratification. The initial study conducted in 2015 found that glucose and kynurenine are significantly lower in MCNs as compared to non-MCNs [207]. Glucose sensitivity and specificity for differentiating between MCN and non-MCNs was 94% and 64%, respectively,

at a cutoff level of 66 mg/mL. Kynurenine was likewise able to discern between MCNs and non-MCNs with a sensitivity/specificity of 90%/100% [207]. Further other studies have also highlighted the utility of glucose to distinguish between MCNs and non-MCNs [208–210]. Though promising for cystic lesions classification, neither glucose nor kynurenine was differentially expressed in malignant cysts.

In a comprehensive study by Gaiser et al., a combination of metabolomic and lipidomic profiling was applied to cystic fluid or serum samples to discriminate the malignancy status of various cystic lesions [211]. The cystic fluid IPMN metabolites represented many pathways associated with lipid metabolism (as compared to SCNs) including phosphatidylethanolamine synthesis, phosphatidylcholine synthesis, taurine metabolism, oxidation of branch-chain fatty acids, and sphingolipid metabolism [211]. Principal component analysis (PCA) was able to discriminate between IPMNs and SCNs with 100% accuracy utilizing cystic fluid or plasma. Interestingly, PCA of cystic fluid metabolites was able to differentiate between PCLs with high-grade dysplasia/cancer and all other cysts with a sensitivity and specificity of 89% and 92%, respectively. When plasma was used, the sensitivity dropped to 64% but the specificity increased to 100% [211].

These metabonomic data are supported by the D'Alessandro group's recent work that elucidated a "metabolic timeline" of PDAC by PCA analysis of 215 metabolites in healthy, IPMN, local disease, and advanced disease patient plasma samples [212]. Relevant to cystic lesion stratification, they found that 10 metabolites were different between IPMN and local disease (early stage) patient plasma and PCA analysis was able to predict patient status. From this, they concluded that metabolomics may provide a means of assessing PCL patient progression along the metabolic timeline (i.e. IPMN vs local disease), but may not provide a benefit to healthy patient screening [212]. These findings were further corroborated in another study on a rat model of PDAC where serum metabolic differences were observed to higher across non-malignant precursor lesions (PanINs) and invasive disease in comparison to normal pancreata and PanINs [213].

4.6. Radiomics

Recent advancements in image acquisition and analysis have led to the development of a novel field in cancer research called radiomics. With this new technique, it has now become possible to convert images into mineable data through high-throughput extraction of quantitative features by computers. The extracted data can then be combined with patients' clinical attributes to contrive a model that will improve the accuracy of diagnosis and prognosis for cancer as well as other diseases [214]. The core premise of radiomics is that the differences in size, shape, texture, and greyness of a tumor contoured from a radiological image can reflect the variations in histological phenotype and genotype of the tumor [215]. The workflow of radiomics involves the acquisition of images (i.e. USG, CT, MRI or PET), segmentation (defining boundaries) of the region of interest and the organ involved, extraction and analysis of features, and statistical modeling to predict outcome [216]. A primary benefit of radiomics is that the data can be calculated from already acquired images through freely available tools, so it bears no extra cost or burden on the patients. This method could provide us with a better, non-invasive tool for diagnosis and prognosis, as

compared to biopsies which harbor inherent limitations including increased risk to patients, limited sample amounts, and fail to incorporate lesion heterogeneity leading to sampling errors.

In recent times, radiomics has been used for tumor diagnosis, prognosis, survival time prediction, guiding therapy selection, response to therapy, and potential areas for physical biopsy [214]. Texture analysis and machine learning, which are integral parts of radiomics, have been used in multiple studies to either predict the presence of a tumor or to accurately differentiate between benign or malignant tumors in various cancers including breast, lung, thyroid, bladder, prostate, liver and pancreas [217–223]. It's only recently that radiomics, particularly texture analysis, has been used in PCLs and more specifically IPMNs [43].

Dmitriev et al. described an automatic classification algorithm to classify four common pancreatic cysts into their respective groups using CT images [224]. They utilized intensity, shape, and fine texture features to achieve 84% accuracy. This was the first CAD (computer-aided diagnosis) algorithm to classify common types of pancreatic cysts [224]. A similar study was performed by Chu et al. to classify PCLs into five types and segregate cystic lesions into IPMN and non-IPMN with approximately 78% and 82% accuracy, respectively [225]. Chakraborty et al. went a step further and developed a pre-operative model to differentiate between low risk and high-risk BD-IPMN by combining novel radiographically inspired features, standard texture features, and clinical variables [226]. They used pretreatment CT images of 103 patients with pathology-confirmed BD-IPMN to extract and combine features to predict malignant potential and achieved an accuracy of 81% [226].

In one of the initial studies employing radiomics on PCLs, Hanania et al. developed a model to predict the histopathological grade of IPMN in CT images of 53 patients [227]. Using a cross-validation design with logistic regression, their model yielded an impressive 96% accuracy. The study identified 14 imaging biomarkers to differentiate IPMNs with low-grade dysplasia from those with high-grade dysplasia [227]. During the same time, Permuth et al. utilized a unique plasma-based miRNA genomic classifier (MGC) with quantitative radiomics CT features of pathologically proven IPMN patients (n = 38) [228]. They concluded this noninvasive radio-genomic approach is more accurate in predicting IPMN pathology than using standard 'high-risk' or 'worrisome' radiologic features for malignancy considered in the Fukuoka consensus guidelines [15,228]. A recent study included 260 patients who underwent pancreatic resection for a PCL, with the aim to differentiate serous cystic neoplasms from other pancreatic cystic neoplasms and achieved a remarkable accuracy of 84%. Importantly, it was the first study to divide the patient cohort into two groups, a cross-validation cohort to extract features and an independent validation cohort to test the model [229].

These initial studies involving radiomics have shown promise but due to its recent genesis, the approach has its own challenges and difficulties. There is a lack of set protocols and guidelines for image acquisition and feature extraction, along with a lack of standards for validating results. Additionally, almost all of the studies are retrospective single institutional with limited patient cohorts [214,216]. A logical next step is to formulate reproducible, robust, and repeatable metrics capable of producing consistently high-quality results with

different scanners, software, and time points [216]. The end goal should be to develop a model capable of automated identification of regions of interest with the ability to precisely characterize the lesion in question [230]. This burgeoning field is poised to prove invaluable in the future for the identification of cancer and its precursor lesions, including PCLs. The diagnostic and prognostic capabilities of this technology could be further enhanced via astute integration with molecular/genomic markers obtained from the cystic fluid, pancreatic juice, or serum in addition to the incorporation of clinical data. Heretofore only one group has done so, as described above [228]. This ability of radiomics data to be combined with other molecular and clinical data makes it a prime candidate for developing models to accurately predict the presence of PDAC within IPMNs and even defining the risk for malignant progression.

5. Conclusions and perspective

The ability to determine the need for surgical resection of premalignant and malignant pancreatic lesions depends upon an accurate diagnosis. Comprehensive research has been carried out to try and uncover biomarkers for identifying malignant cystic lesions using cystic fluid, pancreatic juice, imaging, and blood samples. However, all these approaches have been unable to attain high enough specificity and sensitivity, due to some inherent limitations associated with each method. While tissue-based cyst classification can be incredibly accurate and allow for true histological analysis and verification of disease by a pathologist, sample acquisition can carry significant comorbidities due to the invasive nature of the procedure. Further, single-site biopsies do not account for the entirety of the lesion, thus increasing the possibility of missing malignant disease. Conversely, the use of imaging for classification is non-invasive and can take into account the holistic state of the lesion. Unfortunately, current imaging modalities are expensive, are susceptible to observer variation, and the respective guidelines (i.e. Fukuoka) have limited accuracy in differentiating malignant vs non-malignant cysts. Cystic fluid offers a less invasive means (i.e. EUS/FNA) of cytological analysis than biopsy. It can also be used for molecular profiling including assessment of protein, RNA, DNA, and metabolites. Yet many FNA samples have scant cellularity leading to inconclusive results and multiloculated cysts can partition fluid, thereby preventing gestalt assessment. Also, many of the biomarker studies discussed herein have limitations including low patient sample numbers and the lack of blinded external validation sets. Further, although some markers have been able to determine PCL cancer status, the majority of these studies have focused on mucinous vs nonmucinous cyst differentiation and have not shown robust efficacy in elucidating malignancy. This has led to a lack of uniformly agreed-upon cystic fluid molecular markers in the field.

MD-IPMNs and MCNs have a higher risk of malignancy and are often resected upon diagnosis, thus there continues to be a need for a reliable biomarker for surveillance [97]. The current guidelines for predicting the risk of malignancy status of PCLs require expensive imaging as well as invasive tests. The goal of cyst fluid analysis is to supplement the information gained from imaging. An intense investigation into biomarkers for the noninvasive characterization of PCLs is required. These biomarkers must consider the diversity in the genetic origins of PDAC [231].

Biomarkers could be used in conjunction with the current consensus for imaging guidelines to determine the need for surgical resection. These include options such as the assessment of circulating endothelial cells [232], cell-free DNA measurements [233], improved imaging/biopsy modalities [101], and extracellular vesicle (EV) analysis [234,235]. Continued analysis of the EV contents and membranes may provide unique biomarkers that may also be found in serum.

Radiomics could offer a means of determining the malignancy status of pancreatic cysts in a noninvasive manner. Future improvements, including guideline standardization for imaging acquisition, discernment of repeatable analysis metrics, and integration with molecular markers will increase the diagnostic and prognostic efficacy of this new technology. Although radiomics has already begun to enhance the power of existing imaging modalities and helps identify diagnostic and differentiating features that are otherwise not discernable, algorithms need to be further optimized to realize its full potential for surgical stratification.

Basturk and colleagues have proposed a revised classification system for neoplastic precursor lesions of the pancreas that will utilize the subtle molecular differences that have been found between PCL types [236]. Such a revision could facilitate appropriate clinical decision making and continue to evolve with a deeper understanding of the machinery that transforms these precursor lesions into cancer. Continued individualization is necessary for improved prediction of the future of the disease for patients.

While today's medicine lacks the noninvasive tools to accurately differentiate benign from malignant (or potentially malignant) PCLs [237], there is great potential for the development of novel biomarkers in the near future. If an inexpensive, noninvasive, and accurate screening modality can be discovered with high sensitivity and specificity, quality of life could be increased for the thousands of people who suffer from pancreatic cystic lesions and improve our ability to detect pancreatic cancer at a treatable stage.

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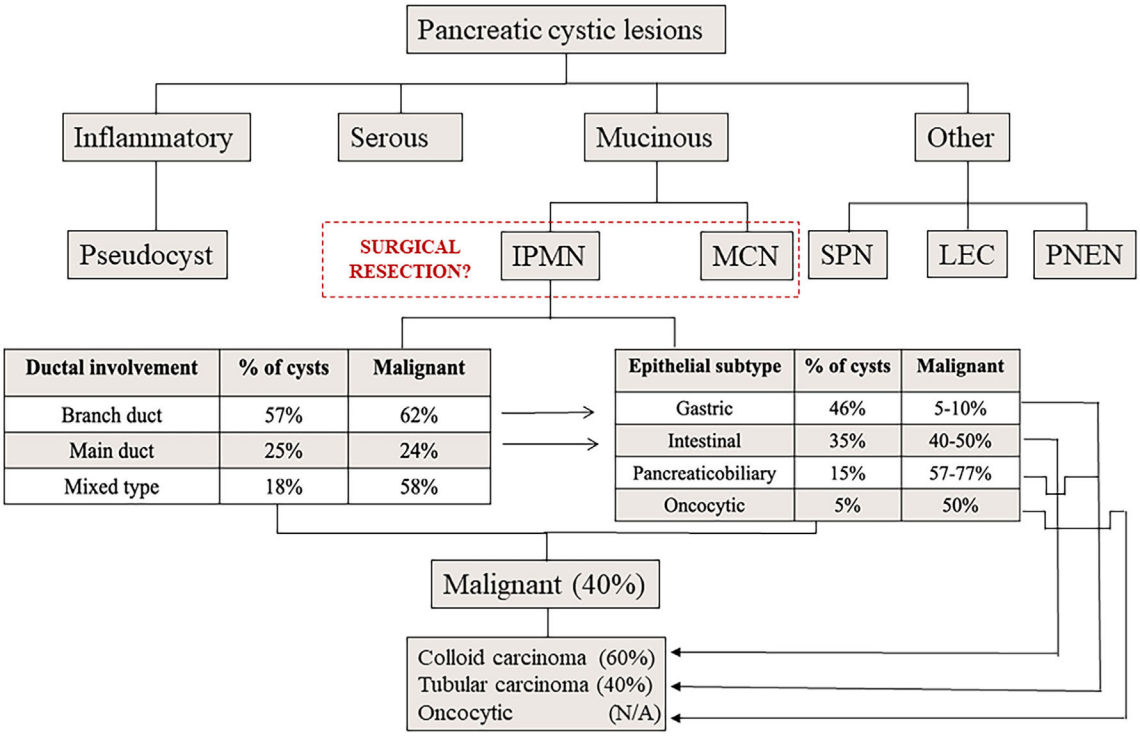

Fig. 1.

Chart depiction of pancreatic cystic lesions (PCLs) types. All PCLs are divided into three main types, inflammatory, serous and mucinous. Those grouped under the “Other” heading are rare cyst types that include pseudopapillary tumors (SPT), lymphoendothelial cysts (LEC), and pancreatic neuroendocrine neoplasia (PNEN). The inflammatory type is predominately comprised of pseudocysts. Importantly, serous type cysts are rarely malignant. Mucinous cysts, however, divided into intraductal pancreatic mucinous neoplasms (IPMN) and mucinous cystic neoplasms (MCN), harbor a greater potential for malignancy. Thus, this is the primary PCL population that undergoes surgical resection. The prevalence of IPMN types (branch, main, and mixed duct), as well as sub-classifications (gastric, intestinal, pancreaticobiliary, and oncocytic), are shown along with their respective proportions that harbor concurrent malignancy.

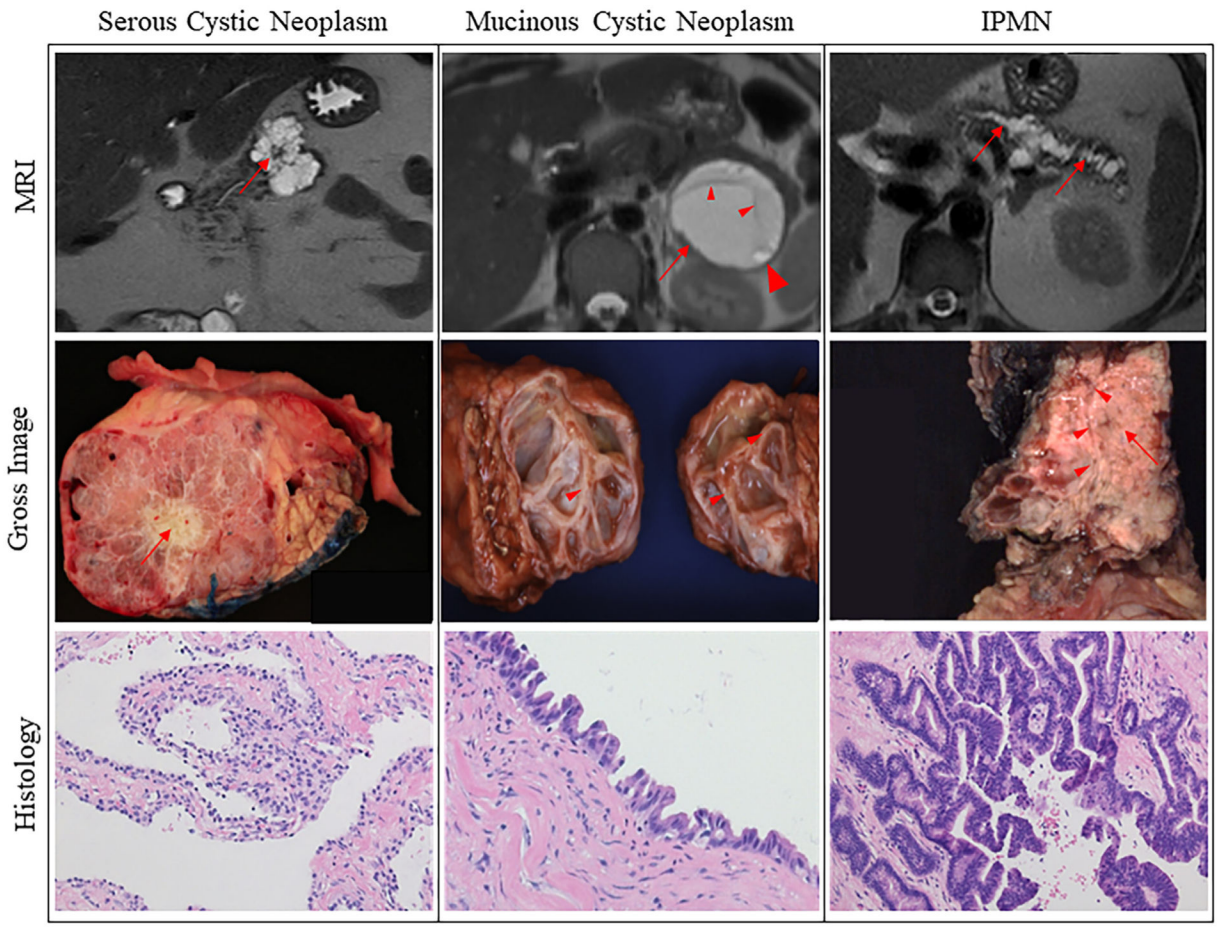

Fig. 2.

MRIs, gross images, and histology of various cystic lesion types resected from patients. The left column has serous cystic neoplasm (SCN) examples. A characteristic stellate scar is appreciated on the MRI and the gross specimen demarcated by red arrows. SCN histology is characterized by the presence of simple cuboidal epithelial cells with clear cytoplasm. The middle column has mucinous cystic neoplasm (MCN) examples. The MRI of an MCN is demarcated by a red arrow that is septated (small arrowheads) and contains an enhanced mural nodule (large arrowhead). The gross specimen contains a large septated lesion with a thick-walled pseudocapsule. Typical MCN histologic presentation with dense ovarian type stroma and epithelial cells lining the cyst that form papillae. The right column has examples of intraductal papillary mucinous neoplasms (IPMNs). Dilation of the main pancreatic duct is appreciated on MRI as demarcated by red arrows. The image of a gross IPMN specimen shows communication between the main pancreatic duct (arrowheads) and the solid/papillary lesion (arrow). Histologic presentation is typical of IPMNs with large papillary structures lined by epithelial cells harboring various degrees of atypia. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1

Cystic fluids markers that identify mucinous and non-mucinous cysts.

Marker	Cyst type	Cut-off	Sn/Sp (%)	Reference
CEA	Mucinous	> 192ng/mL	73/84	[26]
Amylase	Pseudocyst	> 250 U/mL	44/98	[98]
CA19–9	Mucinous	> 50,000 U/mL	75/90	[104]
MUC5AC + endorepellin[a]	Mucinous	NA	92/94	[130]
MUC5AC + CA19–9	Mucinous	NA	87/86	[132]
VEGF-A	Serous	> 8500 pg/mL	100/97	[141]
VEGF-C	Serous	> 200 pg/mL	100/90	[141]
SPINK1[b]	IPMN	25,000 ng/mL	48/98	[145]
GNAS/KRAS mutation	Mucinous	NA	65/100	[168]
Glucose	Non-Mucinous	66 mg/mL	94/64	[207]
Kynurenine	Non-Mucinous	185,650[c]	90/100	[207]

Sn - sensitivity, Sp - specificity, NA - not available, CA19–9 - cancer antigen 19–9, CEA - carcinoembryonic antigen, MUC - mucin, VEGF - vascular endothelial growth factor.

[a]Glycan variant of MUC5AC that reacts with a wheat germ lectin.

[b]From pancreatic juice, not cystic fluid.

[c]LC/MS determined abundance cutoff.

Table 2

Key differences between serious cystic neoplasms (SCNs), mucinous cystic neoplasms (MCNs), and intraductal papillary mucinous neoplasms (IPMNs).

Cyst type	SCN	MCN	IPMN
Decade of presentation	Seventh	Fifth	Sixth to seventh
Gender ratio	F > M	F > M	F < M
Location	Body/tail	Body/tail	Head
Imaging features	Microcystic	Macrocystic	Macrocystic
	Honeycomb-like	Unilocular/multilocular	Ductal involvement with accompanying duct dilation
	Stellate scar	No duct communication	Thickened/irregular walls
	Central calcification	Peripheral calcifications	
Viscosity[a]	Low	High	High
Amylase[a]	Low	Low	High
CEA[a]	Low	High	High
Epithelial lining	Simple cuboidal	Ovarian-like columnar with mucus production	Papillary structures with large quantity of atypia
Malignancy potential	Very low	High	Branch duct IPMN-low
			Main duct IPMN-high
Treatment	Observation unless symptomatic, then resection	Resection	Fukuoka criteria guided observation or resection

[a]Cystic fluid characteristics.

Table 3

Markers for malignant/invasive cystic lesions.

Marker	Sn/Sp (%)	Fluid source	Reference	Features
				Cytology
Cell morphology	55–65/91	Cystic fluid	[99]	Cells from EUS/FNA with nuclear atypia, dysplasia, and abnormal morphology are suggestive of PDAC
				Protein
CA19–9	40/89	Serum	[113]	Present on the surface of cells and glycoproteins. Only clinically used serum marker for monitoring PDAC
	90/43	Pancreatic juice	[111]	
	81/69	Cystic fluid	[112]	
CEA	18/93	Serum	[113]	Normally found in embryonic tissue. Increased levels are associated with MCNs and PDAC
	94/85[a]	Pancreatic juice	[110]	
	92/64	Cystic fluid	[112]	
MUC1 (mRNA)	89/71	Pancreatic juice	[122]	Mucins are a family of high-molecular-weight glycoproteins that are aberrantly expressed in the setting of PDAC
MUC2	NA	Cystic fluid	[60]	
MUC4	NA	Cystic fluid	[60]	
MUC5AC	NA	Serum	[60]	
IL-1	79/95	Cystic fluid	[139]	Cytokine with a role in the regulation of immune and inflammatory response
Ubiquitin[b]	NA	Cystic fluid	[150]	Regulatory molecule involved in protein degradation
Plectin-1	NA	Cystic fluid	[151]	Links microfilaments, microtubules, and intermediate filaments
mAb Das-1	89/100	Cystic fluid	[152]	Monoclonal antibody against colonic epithelium and reactive to premalignant upper gastrointestinal conditions
PGE2	NA	Cystic fluid	[155]	Mediates inflammation and is a product of COX2 conversion of arachidonic acid
				DNA
KRAS	45/96	Cystic fluid	[163]	Known driver mutation in PDAC
GNAS	NA	Cystic fluid	[166]	Encodes the a subunit of G Protein
MOCA	75/92	Cystic fluid	[170]	Diagnostic combinaton of patient characteristics and genomic data
PathFinderTG®	83/91	Cystic fluid	[171]	Integrated Molecular Pathology Assay
				Non-coding RNA
miRs-210, 223, 221, 155, 187	NA	Cystic fluid	[176]	miRs are small (19–24 nucleotides) non-coding, single-stranded RNA. These molecules regulate gene expression and have been implicated in various cancers including PDAC.
miRs-24, 30a-3p, 18a, 92a, 342–3p, 106b, 142–3p, 532–3p	89/100		[181]	
miR-155	NA	Cystic fluid	[185]	

Marker	Sn/Sp (%)	Fluid source	Reference	Features
lncs- ADARB2-AS1, ANRIL, GLIS3-AS1, LINC00472, MEG3, PANDA, PVT1, UCA1	79/76 71/100[c]	Plasma	[188]	lncRNAs are non-coding RNAs larger than 200 nucleotides in length. They resemble coding transcripts but lack open reading frames.

Sn - sensitivity, Sp - specificity, NA - statistically significant but Sn/Sp not reported, CA19–9 - cancer antigen 19–9, CEA - carcinoembryonic antigen, MUC - mucin, IL - interleukin, PGE2 - prostaglandin E2, KRAS - Kirsten rat sarcoma viral oncogene homolog, GNAS - guanine nucleotide-binding protein, MOCA - multivariate organization of combinatorial alterations, miR - microRNA, lnc - long-noncoding RNA.

[a] Malignancy in BD-IPMNs only.

[b] Sn/Sp of FNA samples was combined with tissue data.

[c] SN/SP value of eight lncRNA panel when combined with miRNA and imaging features.

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Table 4

Expressional status of mucins across various histology based subtypes of IPMN.

Mucin	Gastric	Intestinal	Pancreaticobiliary	Oncocytic	Reference
MUC1[a]	−	−	+	+	[41]/[59]
MUC2[a]	−	+	−	−	[41]/[60]
MUC5AC[a]	+	+	+	+	[41]/[59]
MUC6[a,b]	+	±	±	+	[59]
MUC4[b]	+ +	+ + +	−	ND	[61]
MUC13[b]	−	+ +	+ +	ND	[62]

ND - not determined.

[a]MUCs recommended by the WHO for classification of histological subtypes of IPMN in combination with morphology of papillae, cell differentiation pattern and staining for CDX2.

[b]Differential expression was observed in high-grade dysplastic lesions.