

Management of Patients With Pancreatic Cysts

Analysis of Possible False-Negative Cases of Malignancy

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Goals: To examine the utility of integrated molecular pathology (IMP) in managing surveillance of pancreatic cysts based on outcomes and analysis of false negatives (FNs) from a previously published cohort (n = 492).

Background: In endoscopic ultrasound with fine-needle aspiration (EUS-FNA) of cyst fluid lacking malignant cytology, IMP demonstrated better risk stratification for malignancy at approximately 3 years' follow-up than International Consensus Guideline (Fukuoka) 2012 management recommendations in such cases.

Study: Patient outcomes and clinical features of Fukuoka and IMP FN cases were reviewed. Practical guidance for appropriate surveillance intervals and surgery decisions using IMP were derived from follow-up data, considering EUS-FNA sampling limitations and high-risk clinical circumstances observed. Surveillance intervals for patients based on IMP predictive value were compared with those of Fukuoka.

Results: Outcomes at follow-up for IMP low-risk diagnoses supported surveillance every 2 to 3 years, independent of cyst size, when EUS-FNA sampling limitations or high-risk clinical circumstances were absent. In 10 of 11 patients with FN IMP diagnoses (2% of cohort), EUS-FNA sampling limitations existed;

Fukuoka identified high risk in 9 of 11 cases. In 4 of 6 FN cases by Fukuoka (1% of cohort), IMP identified high risk. Overall, 55% of cases had possible sampling limitations and 37% had high-risk clinical circumstances. Outcomes support more cautious management in such cases when using IMP.

Conclusions: Adjunct use of IMP can provide evidence for relaxed surveillance of patients with benign cysts that meet Fukuoka criteria for closer observation or surgery. Although infrequent, FN results with IMP can be associated with EUS-FNA sampling limitations or high-risk clinical circumstances.

Key Words: disease management, endoscopic ultrasound-guided fine-needle aspiration, pancreatic cyst, risk

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Increased use of cross-sectional imaging, as well as advances in technology, has led to an increase in detection of pancreatic cysts. Magnetic resonance imaging and computed tomographic studies have estimated the prevalence of incidental pancreatic cysts to be 2% to 15% in populations excluding patients with known pancreatic

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disease or pancreatic symptoms,¹⁻³ with prevalence rising with increasing age.¹⁻⁴ Although incidental pancreatic cysts are associated with an increased overall risk of pancreatic adenocarcinoma,^{5,6} few are malignant at the time of diagnosis and the rest carry a very low risk of malignant transformation (0.4% per year of surveillance).⁶ The most reliable test for determining the presence of malignancy is cytological analysis of specimens obtained by endoscopic ultrasound with fine-needle aspiration (EUS-FNA). Cytology on cyst fluid aspirates has high specificity for the presence of malignancy (90% to 100%) but is often indeterminate due to low cellularity or variable levels of atypia that do not clearly indicate malignancy, leading to low sensitivity (< ~40%).⁷⁻¹¹ In the absence of overt malignancy, currently available first-line tests (imaging, EUS-FNA, cytology, and amylase/carcinoembryonic antigen in cyst fluid^{11,12}) cannot accurately determine which nonmalignant cysts will become malignant over time.^{11,13}

Guidelines for pancreatic cyst management have been published by several groups.^{11,12,14,15} The strategy for frankly malignant cysts is clearly one of resection, whereas the management of cysts in the absence of frank malignancy remains challenging. While a cautious approach (resection) makes sense when considering the mortality rate of pancreatic cancer,¹⁶ surgery has its own significant mortality and morbidity to consider, especially as most resected pancreatic cysts are found to be benign.¹⁷⁻²⁰ Currently, in the absence of definitive malignancy by cytology, indications for surgery are largely based upon particular morphologic features according to International Consensus Group 2012 (Fukuoka) guidelines. Absolute indications include suspicion of mucinous cystic neoplasm, main duct-associated intraductal papillary mucinous neoplasm, solid pseudopapillary tumor, or presence of “high-risk stigmata” (obstructive jaundice in a patient with a cystic lesion of the pancreatic head, enhancing solid component, main pancreatic duct ≥ 10 mm).^{11,12,14} For other cyst types that may have malignant potential but no worrisome morphologic features or high-risk stigmata (eg, branch-duct intraductal papillary mucinous neoplasms), the general recommendation is frequent surveillance at variable intervals based on cyst size.¹¹ These surveillance interval recommendations have generally been determined based upon physician gestalt, but lack supporting evidence regarding their ability to detect high-grade dysplasia (HGD) and early invasive malignancy.

DNA profiling of pancreatic cyst fluid has examined a number of molecular features that correlate with malignancy but do not individually determine malignancy risk when used alone.²¹⁻²³ Integrated molecular pathology (IMP) testing integrates DNA analysis with imaging, fluid chemistry, and cytological test information to categorize cysts as “benign (BEN),” “statistically indolent (SI),” “statistically higher risk (SHR),” or “aggressive (AGG)” for malignant potential in the absence of frank malignancy by cytological analyses.^{22,24} We have shown previously that BEN/SI IMP diagnoses reliably predict benign disease (97% probability of benign outcome for up to 7.7 years from initial IMP testing) and that SHR and AGG diagnoses are reliable predictors of malignancy (malignant outcomes in 47% and 88% patients, respectively, in up to 7.7 years’ follow-up; all detected within 12 months of IMP test).¹³ Comparison of IMP performance characteristics with those of a model of the Fukuoka (also called Sendai)

2012 management recommendations in the same patient cohort indicated better risk stratification for malignancy using IMP versus Fukuoka criteria.¹³ Only 21% (60/289) of patients who met Fukuoka 2012 surgery criteria had malignant outcomes at 7.7 years from initial IMP test, therefore comparatively, SHR and AGG IMP diagnoses identified malignant outcomes with fewer false-positive cases. Malignant outcomes [false negatives (FNs)] occurred in only 2% (3/144) and 3% (8/253) of patients with SI and BEN IMP diagnoses, respectively, and in 3% (6/203) patients in the Fukuoka 2012 model surveillance category. An independent study found that similar DNA analysis combined with EUS imaging, cytology, and fluid carcinoembryonic antigen results has value in aiding clinical decision-making in patients with pancreatic cysts,²⁵ and others have further validated this approach in another unrelated cohort using similar DNA markers and clinical characteristics to those incorporated into IMP testing.²⁶

In this study, we conducted additional analysis of previously published data¹³ to examine the clinical utility of adjunctive IMP testing when used alongside current guideline recommendations in the management of pancreatic cysts evaluated by EUS-FNA. FN cases are further analyzed and patient management recommendations for appropriate surveillance intervals and surgery decisions have been derived from the analysis. Possible sampling limitations and high-risk clinical circumstances that should be considered when determining the appropriate management approach are also discussed.

MATERIALS AND METHODS

Study Design and Patient Population

The study design and patient population have been reported previously.¹³ Briefly, adults with a pancreatic cyst and negative, nondiagnostic, indeterminate or acellular cytology results who therefore had cyst/duct fluid aspirate tested by IMP were included. Exclusion criteria included previous pancreatic cancer and any treatment for pancreatic lesions before IMP test. Clinical outcomes were determined by retrospective review of patient medical records documented in the National Pancreatic Cyst Registry (10 academic and private institutions in the United States) and categorized as “benign” or “malignant pancreatic adenocarcinoma.” Further information on data abstraction from medical records is provided in the Supplemental Methods (Supplemental Digital Content 1, <http://links.lww.com/JCG/A252>). Benign outcomes included surgical pathology demonstrating a benign cyst or low/intermediate-grade dysplasia, resolution of cyst on repeat imaging, and imaging follow-up for ≥ 23 months without evidence of malignant outcome. Patients with follow-up imaging of < 23 months were excluded unless clear benign or malignant clinical end points occurred within this time-frame.¹³ Malignant outcomes were malignant cytology results (unknown during IMP diagnosis), clinically confirmed pancreatic cancer, death attributed to pancreatic cancer, and HGD. Only cases where the malignant outcome related specifically to the lesion tested by FNA were included. The date of malignant outcome was defined as the earliest date at which a definitive diagnosis of malignancy could be made.

IMP Diagnosis

IMP diagnoses (PathFinderTG™ Pancreas; RedPath Integrated Pathology Inc., Pittsburgh, PA) were made before inclusion in this study as a component of clinical testing per the prescribing physician's standard of care, according to standard operating procedures at RedPath (now Interpace Diagnostics Corporation), and were made blinded to the patient's clinical outcome.¹³ IMP examines DNA-based aberrations present in cyst/duct fluid aspirates collected by EUS-FNA analyzed in the context of noteworthy clinical features described in the EUS-FNA imaging, cytology, and fluid chemistry reports. The IMP diagnostic algorithm is shown in Supplemental Table 1 (Supplemental Digital Content 1, <http://links.lww.com/JCG/A252>). Four molecular criteria that correlate with pancreatic malignancy or HGD are assessed: elevated level of high-quality DNA; a single high-clonality mutation (75% to 100% of DNA extracted from cyst fluid has mutation); multiple low-clonality mutations (50% to 75% of DNA extracted from cyst fluid has mutation); and a single low-clonality oncogene mutation.²² The presence and clonal expansion of mutations in the *KRAS* oncogene and in microsatellites next to tumor suppressor genes were included in the panel of mutations examined. Assessments for tumor suppressor gene loss of heterozygosity included the following chromosomal loci (associated genes in parentheses): 1p (*CMM1*, *L-myc*), 3p (*VHL*, *HoGG1*), 5q (*MCC*, *APC*), 9p (*CDKN2A*), 10q (*P TEN*, *MX11*), 17p (*TP53*), 17q (*NME1*), 18q (*DCC*), 21q (*TFF1* and *PSEN2*), and 22q (*NF2*). Each case was categorized according to the 4 IMP diagnostic categories based on the combination of DNA analysis, imaging, fluid chemistry, and cytological test information.¹³ The BEN and SI categories were considered negative and the SHR and AGG categories positive for malignant outcome.

Model of Fukuoka 2012 Patient Management Criteria

As reported previously,¹³ we determined the performance of a model of the Fukuoka (Sendai) 2012 criteria for managing this cohort of patients based on first-line test results. Cysts were determined as having high malignant potential ("surgery" category) if they met ≥ 1 of the Fukuoka 2012 "high-risk stigmata" (presence of obstructive jaundice in a patient with a cystic lesion of the head, enhancing solid component, main duct dilation ≥ 1 cm) or "worrisome features" [definite mural nodule confirmed by EUS, main duct involvement, severe (ie, suspicious) cytology, abrupt changes in duct caliber, a presumptive diagnosis of mucinous cystic neoplasm based on indications of mucin in records, cyst size > 3 cm]. Although we included all cysts > 3 cm in diameter in the surgery category, the Fukuoka 2012 algorithm suggests that in patients with cysts > 3 cm and no other high-risk stigmata or worrisome features, surgery is strongly considered only in young, fit patients. Patients were categorized as meeting Fukuoka 2012 "surveillance" criteria (low malignant potential) if they lacked all of the above features. Because the cohort comprised only patients with negative, non-diagnostic, indeterminate, or acellular cytology results (not all patients with a pancreatic cyst), this model does not exactly correspond with the published Fukuoka 2012 guidance. The number of cases for which information was not specified for each of the above features was

reported in the supplementary section of our previous report (Table e3).¹³

Definitions of Possible Sampling Limitations and High-risk Clinical Circumstances

It is recognized that EUS-FNA sampling has certain limitations, which are considered in diagnostic testing of other types of cancer.^{27,28} Any suspicion of possible EUS-FNA sampling limitations due to pancreatic cyst type or location should be taken into consideration when determining appropriate management based on EUS-FNA results. We consider possible EUS-FNA sampling limitations to include the following: multilocular cysts and cysts with solid components (also a concerning clinical feature for IMP diagnosis), field defects (as the site of malignancy may be difficult to discern in such cases), presence of multiple cysts, and cysts communicating with or associated with the main duct, especially if the main duct is dilated (eg, > 1 cm; also a concerning clinical feature for IMP diagnosis). In this cohort, features that may pose possible FNA sampling limitations were noted as part of the prospectively designed protocol/database, with the collection of multilocular and unilocular cyst configurations, duct dilation, communication of cysts with main or side ducts, and the number and location of cystic lesions from patient medical records. The analysis for the presence of possible sampling limitations was performed after the study was unblinded to the outcome of the patient.

High-risk clinical circumstances include presence of symptoms (eg, pancreatitis, steatorrhea, diarrhea, nausea, bloating, upper abdominal discomfort, jaundice, weight loss, abdominal pain, back pain, loss of appetite, stool or urine changes) suspicious clinical history or comorbidities, and presence of known risk factors for pancreatic cancer, including family history of pancreatic cancer and genetic disorders associated with an increased risk for pancreatic cancer (eg, Peutz-Jegher's syndrome, familial atypical mole malignant melanoma syndrome, Lynch Syndrome, and BRCA mutations²⁹⁻³⁴).^{11,12} The presence/absence of symptoms and family history of pancreatic cancer in this cohort was abstracted from patient records, per the prospectively designed protocol/database.

Statistical Analysis

Statistical analysis was performed using R statistical software (r-project.org). The proportion of malignant outcomes as a percentage of all outcomes was plotted by time from initial IMP diagnosis according to Fukuoka 2012 model category (surveillance or surgery) and IMP category (BEN, SI, SHR, or AGG).

RESULTS

Subjects

As reported previously, clinical outcomes were available for 492 patients who lacked frank malignancy upon initial cytological analysis.¹³ All were included in this analysis: 338 women, 154 men; mean age at IMP testing 64.9 years. Pancreatic cyst clinical characteristics and classifications were reported previously.¹³ Patient outcomes were classified as benign or malignant based on subsequent surgical pathology (n = 209) or subsequent clinical course (n = 283).¹³ Pancreatic cyst classifications by surgical pathology with corresponding IMP diagnoses in patients with surgical outcomes is shown in

Supplemental Table 2 (Supplemental Digital Content 1, <http://links.lww.com/JCG/A252>). In patients with outcomes determined by imaging, the median follow-up time was 3 years (range, 2 to 7.7 y).

Clinical Features of IMP and Fukuoka Model FN Diagnoses

Noteworthy clinical features of the 11 FN cases are shown in Table 1. Patients varied in age (46.7 to 82.9 y), gender, and cyst size among both Fukuoka and IMP FN cases.

Of the 6 cases that were FN per the Fukuoka model, all were ≤ 2.0 cm, where indicated in the records. One patient who was FN by Fukuoka had obstructive jaundice but the cyst was located in the neck not the head of the pancreas. IMP correctly identified that there was a high risk for malignancy (ie, an IMP diagnosis of SHR or AGG) in 4 of 6 cases that were FNs by the Fukuoka model up to ~ 7 months before the actual diagnosis of malignancy. If cyst size > 3 cm had not been considered an indication for surgery in our Fukuoka model, there would have been 9 FN cases by Fukuoka 2012 criteria (and 183 false-positive cases versus 229 when cyst size was included).

Eleven cases were FN by IMP testing; 10 of these cases had possible EUS-FNA sampling limitations, including 9 patients who had main pancreatic duct-associated lesions and 1 patient who had a complex multilocular cyst. Of the main duct lesions, 3 of 9 had duct dilation of ≥ 1 cm. Where noted in the patient records, patients had cysts ranging from 0.7 to 6 cm in size and duct dilation ranging from 0.5 to 2.1 cm, both of which were often coincident with symptoms including pancreatitis, steatorrhea, nausea, bloating, and upper abdominal discomfort.

Two patients were FN by both Fukuoka 2012 criteria and IMP testing. One had a complex multilocular cyst and

the other had a family history of pancreatic cancer. Malignant outcomes in these patients were determined at 17.9 and 11.6 months post-IMP testing, respectively.

Derivation of Appropriate Surveillance Intervals and Surgery Decisions According to IMP Diagnosis

The finding that 97% of patients with BEN/SI IMP diagnoses had confirmed benign outcomes at a median of 3 years' follow-up (Fig. 1A), with the exception of the patients with possible EUS-FNA sampling limitations and/or high-risk clinical circumstances, supported long (2 to 3 y) surveillance intervals for 81% of patients in this cohort (397/492) based on follow-up time (Table 2). By contrast, the majority of malignant outcomes in patients with SHR and AGG IMP diagnoses were confirmed < 3 months after IMP testing (Fig. 1A). Such diagnoses supported the need for close surveillance (every ~ 3 mo) or a decision with regard to potential surgical resection.

While IMP supported 2- to 3-year surveillance intervals in 81% of patients, Fukuoka 2012 criteria supported such intervals in only 10% of patients (49/492) based on small (< 1 cm) cyst size (Table 2). In this 10% of patients, BEN/SI IMP diagnoses also supported long surveillance (2 to 3 y) intervals in most instances (45/49; Table 2). More importantly, BEN and SI IMP diagnoses supported longer surveillance intervals (every 2 to 3 y) for the majority (352/443) of patients for which Fukuoka suggested closer surveillance or even surgery. These patients included those who fit Fukuoka criteria for surveillance yearly ($n = 106$) or every 3 to 6 months ($n = 48$) and those who fit Fukuoka criteria for surgery ($n = 289$; Table 2). In addition, Fukuoka criteria recommended surveillance intervals of 1 year in 3 small cysts (1 to 2 cm) (Table 1), which had high-risk (SHR) IMP diagnoses and subsequent malignant

TABLE 1. Noteworthy Characteristics of Patients With False-negative Diagnoses by Fukuoka 2012 Model ($n=6$) and/or IMP ($n=11$) Testing (Recorded at Time of Initial IMP Test)

Fukuoka 2012 Category	IMP Diagnosis	Age (y)	Sex	Cyst Size (cm)	Duct Dilation (cm)	Symptoms	Family History of Pancreatic Cancer	Possible FNA Sampling Limitations	Time From IMP to Outcome (mo)
Surveillance	Aggressive	66.0	M	N	N	N	N	N	7.4
Surveillance	SHR	46.7	F	1.6	N	N	N	N	0.0*
Surveillance	SHR	74.7	M	1.1	N	N	N	N	0.8
Surveillance	SHR	75.7	F	2.0	N	Y†	N	N	2.2
Surveillance	SI	82.9	F	0.7	0.5	N	N	Y‡	17.9
Surveillance	Benign	82.3	F	1.2	N	N	Y	N	11.6
Surgery	SI	69.4	F	N	0.7	Y§	N	Y	1.6
Surgery	SI	71.0	M	3.7	N	N	N	Y	1.6
Surgery	Benign	81.0	F	N	0.9	Y§	N	Y	22.5
Surgery	Benign	68.0	M	6.0	N	Y§	N	Y	1.3
Surgery	Benign	67.1	F	3.0	N	Y§	N	Y	1.2
Surgery	Benign	76.6	M	N	2.1	N	N	Y ¶	0.5
Surgery	Benign	70.6	M	N	1.0	N	N	Y ¶	3.5
Surgery	Benign	64.1	M	2.0	N	N	N	Y ¶	3.5
Surgery	Benign	77.5	M	N	1.7	N	N	Y ¶	1.0

*Patient outcome determined < 2 weeks post-IMP testing.

†Obstructive jaundice in a patient with a cystic lesion in the neck of the pancreas.

‡Complex multilocular cyst.

§Includes pancreatitis, steatorrhea, nausea, bloating, and/or upper abdominal discomfort.

||Main duct-associated lesion.

¶Duct dilation > 1 cm.

F indicates female; FNA, fine-needle aspiration; IMP, integrated molecular pathology; M, male; N, none indicated in clinical records of the patient; SHR, statistically higher risk; SI, statistically indolent; Y, present.

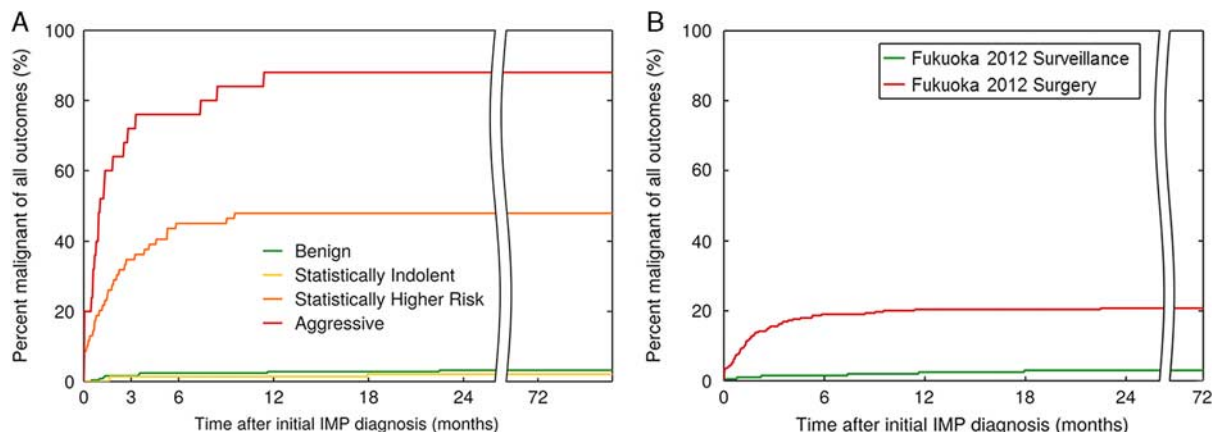


FIGURE 1. Proportion of malignant outcomes as a percentage of all outcomes by time by IMP diagnostic category: at 7.7 years from initial IMP test, 88% (22/25) of AGG diagnoses and 47% (33/70) of SHR diagnoses had malignant outcomes (A); by Fukuoka 2012 model diagnostic category: at 7.7 years from initial IMP test, only 21% (60/289) patients in the surgery category had malignant outcomes (B). IMP indicates integrated molecular pathology.

outcomes, supporting decisions for very close surveillance (~3 mo) or surgery. In this cohort, 55% of cases (269/492) were noted to have possible sampling limitations and 37% (178/475) had high-risk clinical circumstances. Overall, 68% of cases (334/492) had possible FNA sampling limitations and/or high-risk clinical circumstances (50% in the BEN, 25% in the SI, 18% in the SHR, and 7% in the AGG groups).

DISCUSSION

The major challenge in managing pancreatic cysts remains the early identification and treatment of patients at high risk of malignancy and the avoidance of unnecessary treatment or overly cautious surveillance in patients at low risk. We have demonstrated previously that IMP testing provides clinically valid information related to the presence, absence, or increased risk of malignancy.¹³ Here we report additional insights into the characteristics of patients examined in our previous report to better understand the

clinical utility of IMP in managing patients with pancreatic cysts.

Our previous comparison of IMP performance characteristics with those of the Fukuoka 2012 model in this patient cohort indicated similar sensitivity (83% and 91%, respectively), negative predictive value (NPV; both 97%), and probability of benign outcome at follow-up (both 97%), but significantly better specificity (91% vs. 46%; *P* < 0.0001) and positive predictive value (PPV; 58% vs. 21%; *P* < 0.0001) for IMP.¹³ Only 13% (66/492) of patients with outcomes data meeting study criteria had malignant outcomes at a median of 3 years' follow-up (minimum 2 years, maximum 7.7 years), supporting the need for surveillance rather than surgery in the majority (87%) of patients. Other retrospective record review studies also report low PPV for the Fukuoka 2012 guideline criteria in determining presence or risk of malignancy based on patient outcomes.³⁵⁻³⁷ The low PPV and specificity for malignancy of the Fukuoka 2012 criteria in all studies highlight a key problem with current patient management: too many patients undergo surgery

TABLE 2. IMP Diagnoses Can Guide Longer, More Relaxed Surveillance Intervals for Patients Who Would Otherwise Undergo Close Surveillance or Surgery

Fukuoka 2012 Criteria Recommendations	IMP Diagnosis and Management Recommendations			Total
	BEN or SI: 2-3 y Surveillance*†	SHR: 3 mo Surveillance or Surgery‡	AGG: Surgery	
2-3 y (size 0-1 cm)	45	2	2	49
1 y (size 1-2 cm)	103	3	0	106
3-6 mo (size 2-3 cm)	47	1	0	48
Consider surgery (> 3 cm or other Fukuoka criteria for surgery)	202	64	23	289
Total	397	70	25	492

*Patients who have possible FNA sampling limitations and/or clinical high-risk circumstances should undergo close surveillance (3 mo to 1 y).

†In this study cohort, 68% (334/492) of cases were noted to have possible FNA sampling limitations and/or high-risk clinical circumstances to consider when managing patients (50% in the BEN, 25% in the SI, 18% in the SHR, and 7% in the AGG groups).

‡Patients who have possible FNA sampling limitations and/or clinical high-risk circumstances should undergo surgical consult.

AGG indicates aggressive; BEN, benign; FNA, fine-needle aspiration; IMP, integrated molecular pathology; SHR, statistically higher risk; SI, statistically indolent.

for benign pancreatic cysts because of the high false-positive rate of most currently used diagnostic criteria. Furthermore, the PPV for the Fukuoka 2012 criteria is expected to be even lower in clinical practice, as a greater number of patients with benign disease are projected compared with study populations, owing to the low prevalence of malignancy in pancreatic cysts, the requirement for a reasonable clinical follow-up period to confirm benign disease in clinical studies, and/or the inclusion of only patients with surgical outcomes in study populations. A key advantage in using IMP as an adjunct to current guideline-recommended criteria is the improved risk stratification for malignancy due to the substantial reduction in false-positive cases without an increase in missed malignancies.

As expected given the low prevalence of malignancy, the NPV and sensitivity for malignancy of IMP testing and Fukuoka 2012 criteria have been consistently high and statistically similar in all studies.^{13,35–37} However, although infrequent, late detection of malignancy continues to occur when guideline-recommended criteria are used to manage patients.^{35–37} In our study, IMP testing identified malignancies that were missed by Fukuoka criteria alone, and

vice versa. Adjunct use of IMP alongside current management guidance could therefore reduce the number of missed malignancies, improving early detection of pancreatic cancer where it begins—cumulative DNA damage.

As with the Fukuoka 2012 model, a small number of FNs occurred with IMP testing. Given the nature of the study (patient medical record review), imaging and cytology specimens could not be rereviewed to determine whether interpretive errors had occurred. However, after careful review of the medical records by 4 experienced EUS-FNA specialists, these FNs were likely a result of EUS-FNA sampling limitations inherent to the particular characteristics of those cysts. EUS-FNA sampling limitations are well recognized and considered in diagnostic testing of other cancer types.^{27,28} Most IMP FN cases occurred in cysts associated with the main duct, possibly due to fluid flow, which can clear the lesion of representative material for testing. We also consider any significant pancreatic duct dilation (eg, >1 cm) as an indicator for the presence of a possible EUS-FNA sampling limitations in ductal lesions, where the origin of malignancy may be difficult to discern due to the heterogenous nature of such lesions. Multilocular cysts and cysts with solid components should also

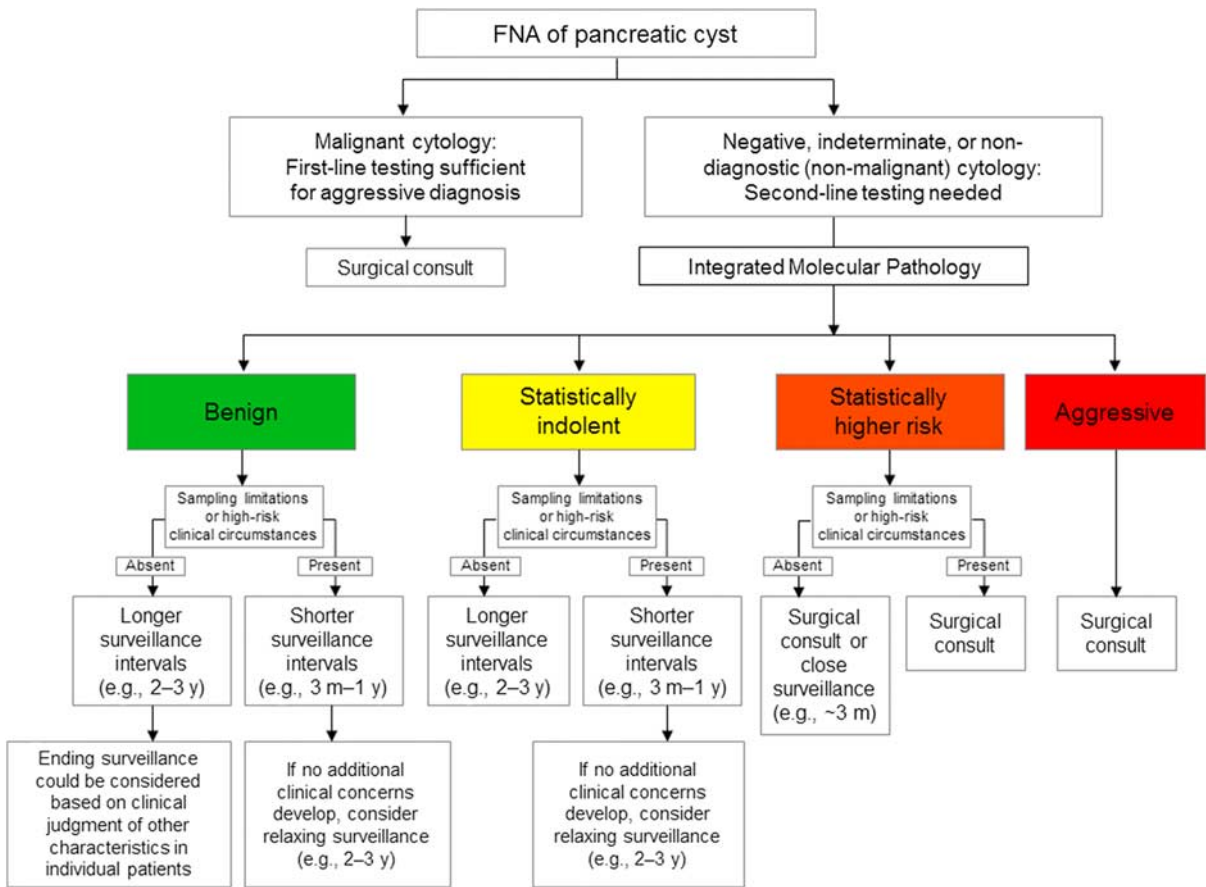


FIGURE 2. Management guidance for using second-line integrated molecular pathology diagnoses of endoscopic ultrasound-FNA samples in patients who have nonmalignant or nondiagnostic cytology. High-risk clinical circumstances include factors such as presence of symptoms, family history of pancreatic cancer, or a genetic disorder associated with an increased risk for pancreatic cancer, patient’s clinical history, and comorbidities (see Materials and methods section).^{11,12} FNA sampling limitations may be present for some cyst types such as main duct-associated neoplasms and complex cysts, as described in the Materials and methods section. FNA indicates fine-needle aspiration.

be considered limitations for diagnostic EUS-FNA sampling. Such lesions are often heterogenous and may contain multiple fluid-filled lobules and/or potentially solid components, which all may carry variable malignant potential, thus complicating the acquisition of a representative sample.³⁸ In this cohort, approximately 55% of cases were noted to have possible FNA sampling limitations based on medical record review. However, physician awareness of the potential for FNA sampling limitations may reduce the risk of missing malignancies if steps can be taken to ensure that representative samples are obtained for testing. For example, in a cyst with both a solid and a cystic component, samples should be obtained from both components for testing, thus mitigating the limitation, although we recognize that this might not be possible in all cysts. Clinical judgment is therefore vital in determining whether potential sampling limitations have been addressed on a case-by-case basis, with more or less aggressive patient management prescribed based on confidence of obtaining a representative sample(s).

In addition, 1 of the 2 patients who were FN by both IMP and the Fukuoka 2012 model had a strong family history of pancreatic cancer (siblings and cousins), a known risk factor for malignancy.²⁹ This particular patient presented with a 1.2 cm cyst at the time of IMP testing and no worrisome features, and over the course of 11.6 months developed a malignant solid component to the cyst. Consistently, we believe that patients with known risk factors for pancreatic cancer (including family history of pancreatic cancer, Peutz-Jegher's syndrome, familial atypical mole malignant melanoma syndrome, Lynch Syndrome, and BRCA mutations)²⁹⁻³⁴ should be managed more cautiously with shorter surveillance intervals. Overall, 37% of patients had high-risk clinical circumstances in this cohort.

Practical guidance for using IMP testing to help manage patients in the absence of frank cytologic malignancy has been derived from the results of this study (Fig. 2). On the basis of our findings, any suspicion of EUS-FNA sampling limitations that cannot be confidently mitigated, and the presence of high-risk clinical circumstances (as described in the Materials and methods section), should be considered when making management decisions and are indications for more cautious management. For BEN or SI IMP diagnoses, a surveillance interval of 2 to 3 years is supported for patients without EUS-FNA sampling limitations and high-risk clinical circumstances. This is based on the high NPV, high specificity, and high probability of benign outcome at a median of 3 years follow-up for these diagnostic categories¹³ and the observation that no patient in these categories had a malignant outcome beyond a surveillance interval of 2 years from initial IMP diagnosis (Fig. 1A). Most of these patients had cysts > 1 cm in size and would therefore have been managed with close surveillance or even surgery based on Fukuoka 2012 criteria alone, resulting in unnecessary surgical risk. Most of the few instances of malignant outcome in the SI and BEN categories were detected shortly after IMP testing (< 6 mo) and were those in which FNA sampling limitations existed. Continued, more frequent surveillance (every 3 mo to 1 y) is advised for the minority of BEN/SI IMP diagnoses with suspected EUS-FNA sampling limitations and/or high-risk clinical circumstances. If no additional clinical concerns develop, then surveillance could be relaxed (to

every 2 to 3 y), based on clinical judgment. The American Gastroenterological Association 2015 guidelines suggest magnetic resonance imaging at 1 year then every 2 years for cysts < 3 cm without a solid component or dilated duct, for a total of 5 years if there is no change in size or characteristics¹⁵; our analysis did not address this new guideline.

In the IMP SHR category, either surgery or short surveillance interval (~3 mo) is suggested in patients without EUS-FNA sampling limitations and/or high-risk clinical circumstances due to the higher risk SHR patients have for malignant outcome.¹³ A short surveillance interval could be considered instead of surgery given that approximately only half of SHR patients had a malignant outcome, and that most malignant outcomes in SHR patients were confirmed within 3 months of IMP testing (Fig. 1A). Patients who have SHR diagnoses with possible EUS-FNA sampling limitations and/or high-risk clinical circumstances, and those who have AGG diagnoses, should be sent for surgical consultation. This is based on their significantly higher risk for malignant outcome due to the presence of confirmed malignant outcomes within 3 months of IMP testing in the majority of these patients in this study (Fig. 1A).

The primary limitation of this dataset is the retrospective nature of the outcomes data; as a result, information that would be used for the categorization of patients according to Fukuoka 2012 criteria, or for IMP diagnosis, was not specified in medical records in a significant proportion of patients.¹³ However, performance of the Fukuoka 2012 criteria could only be further reduced by having such information, because the comparatively poor performance was due to false-positive cases. An additional limitation is the follow-up period. Considering the natural history of pancreatic cysts, longer follow-up would be required to strengthen the findings regarding benign outcomes; such data may allow further extension of surveillance intervals recommended for BEN and SI IMP diagnoses. As our cohort comprised only patients with negative, nondiagnostic, indeterminate, or acellular cytology results (not all patients with a pancreatic cyst), this model of the Fukuoka 2012 criteria does not exactly correspond with the published guidance. As discussed previously,¹³ although we considered cyst size > 3 cm an indication for surgery, we acknowledge that this may differ slightly from the Fukuoka 2012 guidance. Complete exclusion of cyst size from the Fukuoka 2012 model resulted in 3 additional FN cases and 46 fewer false positives but overall had minimal impact on its performance in this cohort and no impact on the conclusions, as reported earlier.^{13,39,40} In addition, there was no evidence of abrupt duct caliber changes in any of the imaging records from patients in this cohort. Thus, in the Fukuoka 2012 model, the "worrisome feature" of abrupt duct caliber change did not contribute to any false-positive diagnoses and, more importantly, the absence of any recorded abrupt duct caliber changes (given that none were indicated in records) did not contribute to any FN diagnoses. Therefore, removal of this criterion would not affect the output of our Fukuoka model in this cohort.

In conclusion, the results described herein indicate that in the absence of frank cytologic malignancy following EUS-FNA, IMP can help guide physician management of pancreatic cysts by increasing confidence that observation is a safe and more appropriate management strategy in the

majority of patients, allowing longer surveillance intervals than suggested by current Fukuoka guidance. IMP also identifies patients at high risk for malignancy for cysts of all sizes that merit EUS-FNA on clinical or imaging criteria, thereby providing reliable evidence that surgical consultation or close surveillance is needed when cytology results are insufficient. IMP does so with high sensitivity for malignancy, which can be further enhanced by taking possible EUS-FNA sampling limitations and high-risk clinical circumstances into consideration. Adjunctive IMP testing could therefore help to limit false-positive diagnoses and reduce the risk of FN diagnoses determined by guideline criteria alone. When used in the clinic, IMP is a useful diagnostic tool that aids the management of pancreatic cysts by limiting overtreatment and surveillance of inconsequential disease while enabling early detection of malignancy.

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