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CLINICAL—PANCREAS

Cross Validation of the Monoclonal Antibody Das-1 in Identification of High-Risk Mucinous Pancreatic Cystic Lesions

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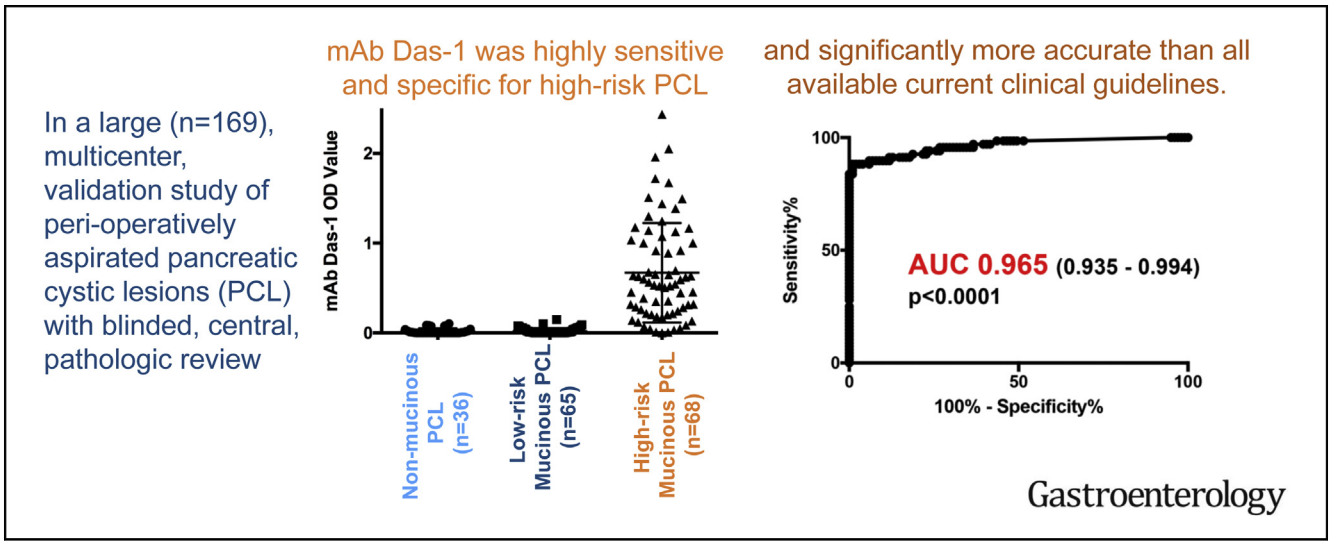

BACKGROUND & AIMS: Although pancreatic cystic lesions (PCLs) are frequently and incidentally detected, it is a challenge to determine their risk of malignancy. In immunohistochemical and enzyme-linked immunosorbent assay (ELISA) analyses of tissue and cyst fluid from pancreatic intraductal papillary mucinous neoplasms, the monoclonal antibody Das-1 identifies those at risk for malignancy with high levels of specificity and sensitivity. We aimed to validate the ability of Das-1 to identify high-risk PCLs in comparison to clinical guidelines and clinical features, using samples from a multicenter cohort. **METHODS:** We obtained cyst fluid samples of 169 PCLs (90 intraductal papillary mucinous neoplasms, 43 mucinous cystic neoplasms, and 36 non-mucinous cysts) from patients undergoing surgery at 4 tertiary referral centers (January 2010 through June 2017). Histology findings from surgical samples, analyzed independently and centrally re-reviewed in a blinded manner, were used as the reference standard. High-risk PCLs were those with invasive carcinomas, high-grade dysplasia, or intestinal-type intraductal papillary mucinous neoplasms with intermediate-grade dysplasia. An ELISA with Das-1 was performed in parallel using banked cyst fluid samples. We evaluated the biomarker's performance, generated area under the curve values, and conducted multivariate logistic regression using clinical and pathology features. **RESULTS:** The ELISA for Das-1 identified high-risk PCLs with 88% sensitivity, 99% specificity, and 95% accuracy, at a cutoff optical density value of 0.104. In 10-fold cross-validation analysis with 100 replications, Das-1 identified high-risk PCLs with 88% sensitivity and 98% specificity. The Sendai, Fukuoka, and American Gastroenterological Association guideline criteria identified high-risk PCLs with 46%, 52%, and 74% accuracy (P for comparison to Das-1 ELISA $<.001$). When we controlled for Das-1 in multivariate regression, main pancreatic duct dilation >5 mm (odds ratio, 14.98; 95% confidence interval, 2.63–108; $P < .0012$), main pancreatic duct dilation ≥ 1 cm (odds ratio, 47.9; 95% confidence interval, 6.39–490; $P < .0001$), and jaundice (odds ratio, 6.16; 95% confidence interval, 1.08–36.7; $P = .0397$) were significantly associated with high-risk PCLs. **CONCLUSIONS:** We validated the ability of an

ELISA with the monoclonal antibody Das-1 to detect PCLs at risk for malignancy with high levels of sensitivity and specificity. This biomarker might be used in conjunction with clinical guidelines to identify patients at risk for malignancy.

Keywords: Intraductal Papillary Mucinous Neoplasm (IPMN); Mucinous Cystic Neoplasm (MCN); Pancreatic Cancer; mAb Das-1.

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

Pancreatic cystic lesions (PCLs) are frequently found but there are a dearth of biomarkers to confidently identify those at high risk for malignancy.

NEW FINDINGS

In a large (n=169), multicenter, validation study of perioperatively aspirated PCL, mAb Das-1 was 88% sensitive and 99% specific for identifying high-risk PCL. mAb Das-1 was significantly more accurate than all available clinical guidelines ($P<.001$) in identifying high-risk lesions.

LIMITATIONS

This was a retrospectively collected, prospectively banked, surgical cohort, from large, tertiary care centers.

IMPACT

An ELISA with Das-1 might be used to analyze fluid from pancreatic cysts and determine their risk for malignancy.

Pancreatic cystic lesions (PCLs) have been increasingly recognized to have malignant potential and are readily detectable on cross-sectional imaging.[1–3] The overall prevalence of pancreatic cysts is estimated to be 2.6%–9.3% of asymptomatic patients undergoing abdominal computed tomography[4] and magnetic resonance imaging[5] scans, with their resections accounting for up to 20% of pancreatic resections in referral centers.[6] However, while a small proportion has malignant potential, the vast majority of these lesions are either benign or indolent.[7,8] Several clinical guidelines have been adopted to assist clinicians in determining when a lesion should be surgically resected.[7,9–11] However, validation studies have demonstrated that these guidelines have either inadequate sensitivity (7.3%–35.2%)[9,10] or inadequate specificity (23%–30%).[11] Given the prevalence of asymptomatic cysts and possible morbidity associated with surgical interventions, there is an unmet need for molecular tools to risk-stratify lesions.

PCLs can be broadly divided into non-mucinous and mucinous lesions. Non-mucinous PCLs include pseudocysts and serous cystadenoma that have no malignant potential, and cystic neuroendocrine tumors and solid pseudopapillary neoplasm, both of which have low-grade malignant potential. Mucinous PCLs consist of intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCNs) that are of varying malignant potential.[12] IPMNs are divided into 3 broad anatomic subtypes (main duct, branch duct, and mixed) based on the involvement of the pancreatic duct, and 4 epithelial subtypes (gastric [IPMN-G], intestinal [IPMN-I], pancreatobiliary, and oncocytic) with varying degrees of dysplasia (low [LGD], intermediate [IGD], and high [HGD] grade).[1–3,13] While main-duct and mixed-type IPMNs have a 48% and 42% likelihood of harboring invasive carcinoma, respectively, in branch duct lesions it is only 11%.[1,2] IPMN-Gs comprise the majority of branch-duct IPMNs, and rarely exhibit HGD. Conversely, IPMN-Is make up the majority of main-duct IPMNs and frequently exhibit HGD/invasive carcinoma.[13] Pancreatobiliary and oncocytic subtypes are rare, high-grade lesions, and typically present with large cystic tumors involving the main duct. The majority of the latter 2 subtypes contain invasive or minimally invasive components, respectively.[2]

We have previously developed a novel murine monoclonal antibody (mAb), Das-1, which reacts specifically with normal non-goblet and goblet colonic epithelium, but not with normal small intestinal enterocytes.[14–17] While absent in normal esophageal, gastric, and pancreatic epithelium, we have demonstrated that it is present in precancerous and cancerous conditions of these same tissues.[14,15,18–20] In a preliminary single-center study, we reported on the specific immunoreactivity of mAb Das-1 against resected tissue and cyst fluid from patients with high-risk IPMNs and associated invasive carcinomas.[15] Indeed, in a small cohort of patients with resected PCLs, evaluation of mAb Das-1 in perioperatively aspirated cyst fluid samples by ELISA assay demonstrated a sensitivity of 89% and specificity of 100% in detecting high-risk lesions.[15]

Here, we explore the ability of mAb Das-1 to segregate PCLs with high-risk for malignant behavior in a large, multicenter cohort of cyst fluid samples aspirated at the time of surgical resection. With blinded, centrally verified pathologic review of all cases, we ensured a comparison to a uniform gold standard. Finally, we evaluated the performance of mAb Das-1 against current available clinical guidelines and their constituent components.

Materials and Methods

Study Design and Subjects

The Institutional Review Boards of all centers approved this study and it is reported in accordance with STARD (Standards for Reporting of Diagnostic Accuracy Studies) and REMARK (Reporting Recommendations for Tumor Marker Prognostic

Abbreviations used in this paper: **AGA, American Gastroenterological Association; AUC, area under the curve; CEA, carcinoembryonic antigen; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; HGD, high-grade dysplasia; IGD, intermediate-grade dysplasia; IPMN, intraductal papillary mucinous neoplasm; IPMN-G, gastric-type intraductal papillary mucinous neoplasm; IPMN-I, intestinal-type intraductal papillary mucinous neoplasm; LGD, low-grade dysplasia; mAb, monoclonal antibody; MCN, mucinous cystic neoplasm; NGS, next-generation sequencing; OD, optical density; PCL, pancreatic cystic lesion.**

Most current article

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Studies) guidelines (Supplementary Statistical Checklists). Patients underwent surgical resection for PCLs with perioperative cyst fluid collection between January 2010 and June 2017 at 4 tertiary referral centers (Massachusetts General Hospital [n = 94], Johns Hopkins University [n = 46], Memorial Sloan Kettering Cancer Center [n = 37], and Washington University [n = 4]) with multidisciplinary PCL programs. The decision to resect a pancreatic cyst is multifactorial, and includes not only an assessment of the risk of the presence of HGD or invasive cancer within a cyst, but also the presence of other features, including symptoms secondary to the cyst, patient age, and/or comorbidities. All pancreatic cyst fluid was aspirated, aliquoted, and flash frozen (−80°C) at the time of surgical resection and stored in the respective institutions' biobanks. Retrospective inclusion of patients into the current study was on the basis of the availability of frozen, banked cyst fluid. Of the 181 patients with available cyst fluid for analysis, 12 patients did not have a sufficient quantity to analyze (Figure 1). Each institution provided clinical data on review of the enrolled patients' records.

Pathologic Evaluation of Tissue Specimens

De-identified, coded slides of all available patients were reviewed by one of the authors specialized in the field (MM-K). Analysis was performed blinded to the original pathologic diagnosis and immunoreactivity to mAb Das-1. Of those, all cases of IPMNs were histopathologically classified by main/branch duct involvement and by dysplastic grade. We used a 3-tiered grading system (LGD, IGD, and HGD) for the purpose of this study, and the LGD and IGD correspond to LGD in the recently recommended 2-tiered grading system.[21] Epithelial subtypes were determined on the basis of their epithelial morphology on routine H&E staining and, when available, immunoreactivity against mucin glycoproteins and/or CDX2, according to previously established criteria.[22,23] IPMN lesions were classified on a per-patient basis, based on the most predominant epithelial subtype, and the highest-grade lesion demonstrated. All cases of MCN were also classified by dysplastic grade.

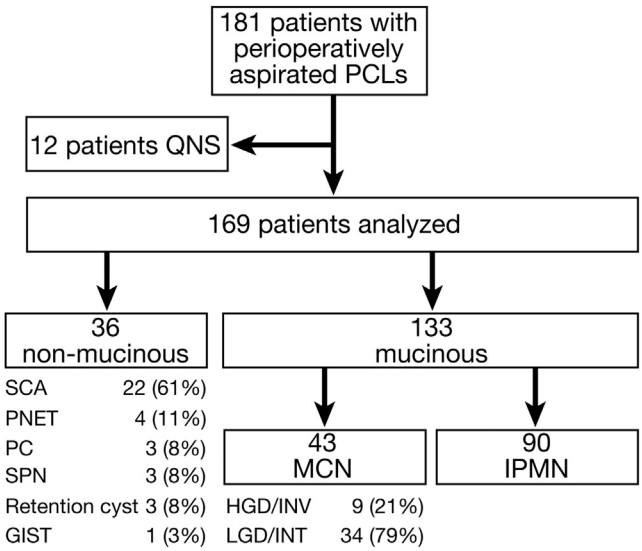

Figure 1. Flow diagram of patients evaluated. GIST, gastrointestinal stromal tumor; HGD/INV, high-grade dysplasia/invasive carcinoma; LGD/INT, low-grade/intermediate-grade dysplasia; MCN, mucinous cystic neoplasm; PC, pseudocyst; PNET, pancreatic neuroendocrine tumor; QNS, quantity not sufficient; SCA, serous cyst adenoma; SPN, solid pseudopapillary neoplasm.

For the purposes of this study, high-risk lesions (ie, those warranting definitive surgical management) were those pathologically verified to have invasive carcinoma in association with a PCL, HGD arising in an MCN or IPMN, or IPMN-I with IGD. IPMN-I with IGD were included in this high-risk category as we[2] and others[23] have found these cases frequently harbor multiple small foci of HGD and present in patients with (recurrent) pancreatitis,[24] both warranting surgical management. Low-risk lesions were defined as all other PCLs, including non-mucinous PCLs and IPMN-G and MCN with LGD or IGD. A separate analysis, defining high-risk lesions as "advanced neoplasia," meaning those lesions with HGD or invasive carcinoma (not including IPMN-I with IGD), was also performed in parallel.

Analyses of Cyst Fluid Aspirates for Monoclonal Antibody Das-1

De-identified frozen samples were processed blinded to their pathologic diagnosis. Fluid was assayed for total protein concentration and all samples were normalized to equal protein amount. Sandwich enzyme-linked immunosorbent assay (ELISA) was performed with mAb Das-1 IgM and mAb Das-1 IgG isotypes as described previously.[15] All experiments were conducted at least in duplicate and normalized with respect to reactivity of the positive control.

Statistical Analysis

Based on our preliminary evaluation of PCL cyst fluid,[15,25] we expected that both the sensitivity and specificity of the assay would be approximately 92%. We performed sample size calculations demonstrating that for a sample of 50 patients with high-risk lesions and 50 with low-risk lesions, the 95% confidence interval (CI) for an observed sensitivity or specificity of 92% would be 81%–98%. Our cohort includes 101 low-risk and 68 high-risk patients.

Optical density (OD) values are displayed with SDs and compared across patient groups using the Mann-Whitney test. The performance of the continuous mAb Das-1 OD values in predicting high-risk PCLs was described through receiver operating curves and the area under the receiver operating curves (AUC). The optimal cut point for predicting high-risk PCLs with mAb Das-1 was determined from the receiver operating curves utilizing Youden's statistic. The performance of the dichotomized mAb Das-1 and other clinical criteria for high-risk PCLs (Sendai guidelines, Fukuoka guidelines, and American Gastroenterological Association [AGA] guidelines) is described through sensitivity, specificity, and accuracy (the percent correctly identified by the screen, or the sum of the true positives and true negatives). Exact 95% CIs are given, and performance of these guidelines and mAb Das-1 are compared through exact paired-sample McNemar's tests for proportions. All of the tests were 2-sided and the significance level was set at .05. The analyses were performed with STATA, version 14 (StataCorp, College Station, TX) and SAS, version 9.2 (SAS Institute, Cary, NC).

Examination of Clinical Risk Factors Controlling for Monoclonal Antibody Das-1

We used logistic regression to examine associations between clinical risk factors and high-risk PCL, first examining unadjusted associations (odds ratios and 95% CIs) and then examining associations between clinical risk factors and high-risk PCL after controlling for the dichotomized mAb Das-1. Clinical factors examined were cyst size, MPD dilation (>5 mm, ≥1 cm), enhancing mural nodule, solid component, multifocality, any symptoms, weight loss, abdominal pain, jaundice, pancreatitis, as well as a previously validated composite clinical marker (jaundice or MPD dilation or cyst size ≥4cm).[26,27] Because of the high sensitivity and specificity of mAb Das-1, which led to a small number of false positives (n = 1) and negatives (n = 8), we used exact logistic regression to estimate the adjusted associations.

k-fold Validation

We used repeated k-fold cross-validation to estimate the sensitivity, specificity, and accuracy of the mAb Das-1 screen when applied to an independent sample of subjects. First, we randomly divided the sample into 10 equal-sized subsamples. Then, for each subsample, we took the subsample as a holdout validation data set; took the remaining subsamples as a training data set; determined the Das-1 cutoff in the training data set using Youden's index; and, using the cutoff from the training data set, determined the performance of the cutoff in the held-out validation data set. Performance results in the 10 validation data sets are then pooled to calculate sensitivity, specificity, accuracy, and their 95% CIs. Because these performance estimates depend on the original random sample division, we repeated this process 100 times and present the mean sensitivity, specificity, and accuracy across the 100 replications.

Results

Study Cohort

Demographic and clinical information on the examined study cohort are displayed in Table 1. Of the 181 patients with PCLs in the study, 169 patients had sufficient cyst fluid for analysis. Of these PCLs, 36 were non-mucinous and 133 were mucinous (43 MCNs and 90 IPMNs) (Figure 1). As expected, patients with MCN tended to be younger and have a female predominance.

Preoperative cyst fluid carcinoembryonic antigen (CEA) was available in 49 patients. Utilizing the previously established threshold of 192 ng/mL to discriminate a potential mucinous PCL,[28] there was a 50% sensitivity (95% CI, 0.329–0.671) and 92.3% specificity (95% CI, 0.640–0.998) for CEA accurately identifying a mucinous lesion (Supplementary Table 1). Cyst fluid cytology (n = 57) was not readily available in a large enough subgroup of the patients included in this cohort to provide a meaningful evaluation of its performance in parallel.

Among the 90 patients with IPMNs examined, 44 (49%) involved the main duct and 46 (51%) were exclusively branch duct lesions, with all epithelial subtypes represented (Supplementary Table 2). Preoperative assessment of main duct involvement was observed in 42 (46%) on cross-sectional imaging. Of the IPMN lesions, 19 harbored LGD, 21 IGD, 32 HGD, and 18 showed an invasive component. Only 9 of the MCN harbored HGD or invasive features.

Cyst Fluid Protein Analysis

Median cyst fluid protein concentration was 4.1 μg/μL (interquartile range, 1.61–9.56 μg/μL) and reflected the pathology of the resected specimens with low-grade IPMN-G/serous cyst adenoma and high-grade IPMN-I/colloid carcinoma at the extremes. Considering the lower end of the interquartile range, the vast majority of samples could be processed in duplicate (requiring 200 μg protein) with <150 μL cyst fluid.

Monoclonal Antibody Das-1 Identifies High-Risk Pancreatic Cystic Lesions

Cyst fluid from non-mucinous PCLs (n = 36) demonstrated very little reactivity with mAb Das-1 by sandwich ELISA assay (OD, 0.019 ± 0.032). Similarly, low-risk IPMNs and MCNs (n = 65) had minimal reactivity (OD, 0.019 ± 0.034). Conversely, high-risk IPMN and MCN lesions (n = 68) expressed a significantly higher amount of reactivity (OD, 0.670 ± 0.555) when compared with low-risk IPMNs and MCNs ($P < .0001$) and non-mucinous PCLs ($P < .0001$) (Figure 2*A*). Plotting the overall sensitivity and specificity of mAb Das-1 for high-risk PCLs as a continuous variable, AUC was 0.965 (95% CI, 0.935–0.994) (Figure 2*B*).

Table 1. Patient and Cyst Characteristics

Characteristic	All samples (n = 169)	IPMNs (n = 90)	MCNs (n = 43)	Non-mucinous cystic lesions (n = 36)
Female sex, n (%)	112 (66)	43 (48)	41 (95)	28 (78)
Age at surgery, *y*, mean (SD)	58.9 (15.2)	66.6 (12.1)	48.4 (14.2)	52.3 (12.5)
Symptoms, n (%)	76 (45)	44 (49)	22 (51)	10 (28)
Weight loss, n (%)	9 (5)	7 (8)	1 (2)	1 (3)
Abdominal pain, n (%)	50 (30)	24 (27)	18 (42)	8 (22)
Pancreatitis, n (%)	33 (20)	21 (23)	7 (16)	5 (14)
Jaundice, n (%)	7 (4)	7 (8)	0 (0)	0 (0)
Cyst size, *cm*, mean (SD)	5.04 (3.59)	4.07 (3.23)	6.30 (4.32)	5.88 (2.72)
Mural nodule n (%)	26 (15)	18 (20)	6 (14)	2 (6)

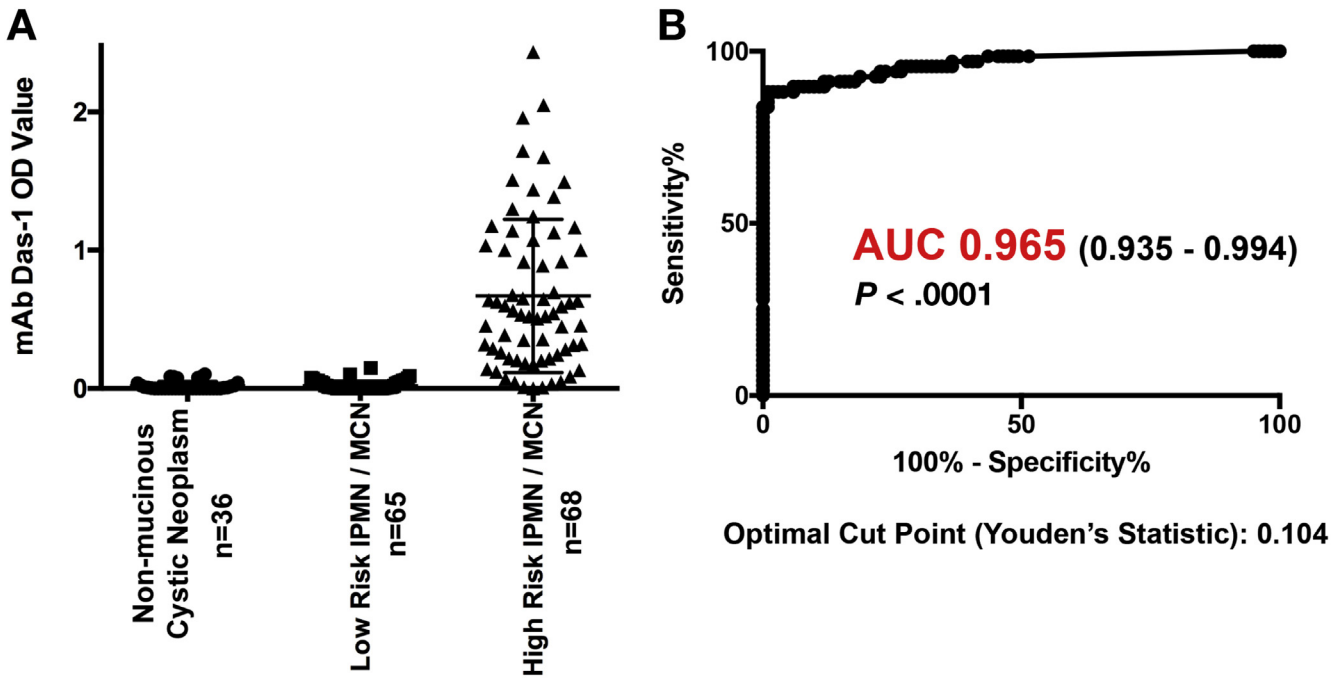

Figure 2. mAb Das-1 is highly sensitive and specific for high-risk pancreatic cystic lesions. (*A*) Cyst fluid immunoreactivity against mAb Das-1 by ELISA. OD values as determined by ELISA in high-risk IPMNs (invasive IPMN, HGD of any epithelial subtype, and IGD of intestinal subtype) and MCNs with HGD (n = 68), low-risk IPMNs and MCNs (n = 65), non-mucinous cystic neoplasms (serous cyst adenoma, pancreatic neuroendocrine tumors, pseudocysts, solid pseudopapillary neoplasm, retention cysts, and gastrointestinal stromal tumor) (n = 36). *Bars* indicate the mean and SD in each column. Reactivity of mAb Das-1 from fluid from high-risk IPMNs and MCNs was significantly higher than that from low-risk IPMNs/MCNs ($P < .0001$) and non-mucinous cystic lesions ($P < .0001$). (*B*) Receiver operating curve analysis of mAb Das-1 for the identification of high-risk pancreatic cystic lesions. The AUC was 0.965, which was highly significant ($P < .0001$). Utilizing Youden's statistic, an optimal binary cut point of 0.104 was selected and the sensitivity and specificity of Das-1 for segregating high-risk PCLs from low-risk PCLs were 88.2% and 99%, respectively.

While low-risk IPMNs (n = 31) had an OD of 0.025 ± 0.042, non-invasive high-risk IPMN (n = 41) had an OD of 0.610 ± 0.493 ($P < .0001$), and IPMN with an invasive component (n = 18) had an OD of 0.680 ± 0.555 ($P < .0001$), demonstrating a progressive increase in reactivity to mAb Das-1. The mAb Das-1 had a strong ability to segregate IPMN-G with LGD/IGD (n = 31; OD, 0.025 ± 0.042), which represent the majority of indolent branch duct IPMNs, from IPMN-G with HGD or invasive tubular carcinoma arising from IPMN-G (n = 14; OD, 0.636 ± 0.486) ($P < .0001$) or IPMNs of any type with HGD or with associated invasive carcinoma (n = 50; OD, 0.658 ± 0.473) ($P < .0001$). The reactivity of the biomarker with the various histologic subtypes and dysplastic grades of IPMNs are displayed in Supplementary Table 3. Among MCNs, lesions with LGD/IGD (n = 34) were non-reactive (OD, 0.014 ± 0.023) in comparison to MCNs with HGD (n = 9; OD, 0.924 ± 0.825) ($P < .0001$).

Evaluation of a Cutoff for Identification of High-Risk Pancreatic Cystic Lesions by Monoclonal Antibody Das-1

We have previously reported proposed preliminary OD cutoffs in our initial descriptions of an ELISA for mAb Das-1 in IPMNs and in a subsequent pilot abstract.[15,25] Our initial report of a cutoff[15] was based on the mean of only 9 samples of low-risk gastric-type IPMNs and twice their SD. Subsequently, improving our assay and utilizing a small, preliminary, previously reported cohort, we had initially estimated an optimal cutoff OD for high-risk lesions to be 0.120, based on maximization of sensitivity and specificity (data presented at Digestive Disease Week 2014).[25] With this cutoff, we reported a sensitivity of 84% and specificity of 100% for identifying high-risk IPMNs. However, this cohort did not include any non-mucinous cystic lesions, MCNs, or other PCLs, and represented a single-center experience only. Regardless, utilizing this cutoff, and the unique cases in the current study cohort (n = 126) as a validation set, this previously suggested cutoff (0.120) had a sensitivity and specificity of detecting high-risk lesions very similar to the prior report: 83.0% (95% CI, 0.679–0.928) and 100% (95% CI, 0.958–1.00), respectively.

Given our current much larger data set (n = 169), which encompasses all PCL subtypes, a statistically valid, optimal cutoff was calculated by Youden's index of ≥0.104. Utilizing this cutoff, the sensitivity and specificity for segregating high-risk PCLs from low-risk PCLs was 88.2% (95% CI, 0.781–0.948) and 99.0% (95% CI, 0.946–1.00), respectively. In our cohort of 169 patients there was only 1 case with an OD value between the 0.120 and 0.104 cutoffs—a patient with a high-risk MCNs that had an OD value of 0.119. The

Table 2. Performance of Clinical Guidelines and Monoclonal Antibody Das-1 in Segregating High-Risk Pancreatic Cystic Lesion

	Mucinous PCLs (n = 133)						All PCLs (n = 169)					
	Sensitivity		Specificity		Accuracy		Sensitivity		Specificity		Accuracy	
Variable	% (95% CI)	*P* value	% (95% CI	*P* value	% (95% CI	*P* value	% (95% CI	*P* value	% (95% CI	*P* value	% (95% CI	*P* value
mAb Das-1	88.2 (78.1–94.8)	ref	98.5 (91.7–100)	ref	93.2 (87.5–96.9)	ref	88.2 (78.1–94.8)	ref	99.0 (94.6–100)	ref	94.7 (90.1–97.5)	ref
Sendai guidelines	94.1 (85.6–98.4)	.3877	15.4 (7.63–26.5)	<.0001	55.6 (46.8–64.3)	<.001	94.1 (85.6–98.4)	.3877	13.9 (7.79–22.2)	<.0001	46.2 (38.5–54.0)	<.001
Fukuoka guidelines	97.1(89.8–99.6)	.1094	23.1 (13.5–35.2)	<.0001	60.9 (52.1–69.2)	<.001	97.1 (89.8–99.6)	.1094	20.8 (13.4–30.0)	<.0001	51.5 (43.7–59.2)	<.001
AGA guidelines	50.0 (37.6–62.4)	<.0001	93.8 (85–98.3)	.3750	71.4 (62.9–78.2)	<.001	50.0 (37.6–62.4)	<.0001	89.1 (81.4–94.4)	.0063	73.7 (66.0–79.9)	<.001

NOTE. *P* values reported in comparison to various guideline performance as compared to mAb Das-1. *P* values calculated using exact paired-sample McNemar's test of proportions.

small differences in sensitivity and specificity between the cutoffs are due to this single case.

Utilizing a cutoff of 0.104, it should be noted that there is only 1 patient who had a positive ELISA for mAb Das-1 (OD, 0.149) with a low-risk lesion. This patient had a 3-cm PCL with a mural nodule with a gastric-type IPMN with IGD on final surgical pathology. Similarly, there were 8 high-risk cases non-reactive to mAb Das-1. Of these lesions, 2 had malignant cytology identified on preoperative endoscopic ultrasound and 5 presented with an MPD dilated >1 cm or a focal mass on imaging.

When defining high-risk lesions strictly as those with HGD/invasive component, mAb Das-1 continued to have strong diagnostic performance with a sensitivity and specificity of 88.3% (95% CI, 77.4%–95.2%) and 92.7% (95% CI, 86.0%–96.8%), respectively (Supplementary Table 4).

k-Fold Validation of Sensitivity and Specificity

To estimate the performance of mAb Das-1 on an independent sample, we performed 10-fold cross validation with 100 replications. Cross-validated sensitivity and specificity were 88.0% (95% CI, 77.9%–94.5%) and 97.6% (95% CI, 92.5%–99.4%), respectively.

Monoclonal Antibody Das-1 Is Superior to Current Clinical Guidelines for Identifying High-Risk Pancreatic Cystic Lesions

We next compared the performance of mAb Das-1 to the available clinical guidelines[7,29,30] (Table 2). The Sendai guidelines had an overall sensitivity, specificity, and accuracy of 94.1% (95% CI, 85.6%–98.4%), 13.9% (95% CI, 7.79%–22.2%), and 46.2% (95% CI, 38.5%–54.0%), respectively, for identifying high-risk lesions. In comparison, the Fukuoka guidelines were significantly more accurate ($P < .012$) with a sensitivity, specificity, and accuracy of 97.1% (95% CI, 89.8%–99.6%), 20.8% (95% CI, 13.4%–30.0%), and 51.5% (95% CI, 43.7%–59.2%), respectively. The revised International Association of the Pancreas consensus guidelines from 2017 have few changes for the indications for surgery from the Fukuoka guidelines, and thus the performance of these updated guidelines were identical to the Fukuoka guidelines.[31] The AGA guidelines were significantly more accurate than the Sendai guidelines ($P < .001$), as well as the Fukuoka guidelines ($P < .001$), with a sensitivity, specificity, and accuracy of 50.0% (95% CI, 37.6%–62.4%), 89.1% (95% CI, 81.4%–94.4%), and 73.7% (95% CI, 66.0%–79.9%), respectively. A validated composite clinical risk indicator[27] was also significantly more accurate than the Sendai guidelines ($P < .003$) (Supplementary Table 5). However, with a sensitivity, specificity, and accuracy of 88.2%, 99.0%, and 94.7%, respectively, the performance of mAb Das-1 was significantly ($P < .001$) more accurate than that of the Sendai, Fukuoka, or AGA guidelines, or the composite risk indicator (Table 2). The same was true when defining high-risk lesions strictly as those with a HGD/invasive component (Supplementary Table 4).

Evaluation of Clinical Risk Factors Associated With High-Risk Pancreatic Cystic Lesions in Association With Monoclonal Antibody Das-1

In performing a univariate analysis to identify high-risk PCL in our cohort utilizing all of the component clinical indicators in the current guidelines, significant predictors included: mAb Das-1 (OR, 750; 95% CI, 91.5–6145.1; $P < .0001$), MPD dilation >5 mm (OR, 13.0; 95% CI, 5.77–29.28; $P < .0001$), and ≥1 cm (OR, 15.6; 95% CI, 4.45–54.9; $P < .0001$), solid component (OR, 4.07; 95 CI, 1.77–9.34; $P = .0009$), any symptoms (OR, 2.01; 95% CI, 1.08–3.76; $P = .0280$), jaundice (OR, 2.41; 95% CI, 1.11–5.23; $P = .0260$), and the composite risk indicator (OR, 3.81; 95% CI, 1.63–8.86; $P = .0013$) (Table 3).

We then examined the associations between clinical risk factors and high-risk PCL after controlling for mAb Das-1 reactivity. Using exact logistic regression with only 2 independent variables (the clinical variable of interest and mAb Das-1), MPD dilation >5 mm (OR, 14.98; 95% CI, 2.63–108; $P < .0012$) and ≥1 cm (OR, 47.9; 95% CI, 6.39–490; $P < .0001$), and jaundice (OR, 6.16; 95% CI, 1.08–36.7; $P = 0.0397$) were still significantly associated with high risk, even after controlling for mAb Das-1 reactivity. Given the highly sensitive and specific nature of mAb Das-1 in being able to detect high-risk lesions with only 9 misclassifications in our cohort of 169 patients, a statistically valid multivariate model or the creation of a risk-prediction model was not feasible.

Discussion

PCLs are very frequently, incidentally identified in patients,[4,5] without clinical guidelines or biomarkers that can reliably identify lesions that necessitate definitive management. While data from long-term cohorts of PCL have identified a small but ongoing risk to the development of carcinoma,[32,33] this must be balanced against the increasing data demonstrating low yield and high potential morbidity of surgical intervention in elderly patients with non-worrisome PCLs.[8,34] In our present study, we demonstrate that mAb Das-1 reliably identifies high-risk PCLs in a large, pathologically verified, multicenter cohort of patients. With a sensitivity of 88.2%, specificity of 99%, and overall accuracy of 94.7%, the biomarker was significantly more accurate than currently available guidelines ($P < .001$).

There are several limitations of our study. Our study cohort is retrospectively collected, prospectively banked surgical specimens from large, tertiary care centers, which introduces surgical selection, referral, and treatment access biases. While several validation and exploration studies in PCL biomarkers have reported larger non-pathologically verified cohorts, few have demonstrated a similarly sized and powered surgical resection cohort as the one utilized herein from 4 high-volume, geographically diverse centers.[35] Without a prospective cohort of patients followed, it is impossible to assess the malignant potential of a PCL within a patient's lifetime, however, evaluation against a gold standard of blinded, pathology review is ultimately superior to surrogate end points and assumptions of indolence based on clinical/radiographic follow-up. As such, this study does not address the optimal approach of integrating mAb Das-1 into PCL surveillance programs. We are currently studying the utilization of this marker on a prospective basis to validate its use in this fashion. With multiple promising biomarkers becoming available to risk stratify PCL, further studies are currently ongoing, to prospectively validate these various markers against one another, in the same cohorts.

Table 3. Univariate and Multivariate Analysis of Monoclonal Antibody Das-1 and Clinical Indicators for Predicting High-Risk Pancreatic Cystic Lesions

	Univariate analysis				Multivariate analysis controlling for Das-1	
Variable	Sensitivity, %	Specificity, %	OR (95% CI)	*P* value	Adjusted OR (95% CI)	*P* value
mAb Das-1	88.2 (78.1–94.8)	99.0 (94.6–100)	750 (91.5–6145.1)	<.0001	—	—
Cyst size ≥3 cm	65.7 (53.1–76.8)	28.7 (20.1–38.6)	0.738 (0.382–1.43)	.3665	0.597 (0.116–3.33)	.7001
Cyst size ≥4 cm	47.1 (34.8–59.6)	47.5 (37.5–57.7)	0.805 (0.435–1.49)	.4900	1.76 (0.358–11.4)	.6624
MPD >5 mm	52.9 (40.4–65.2)	90.1 (82.5–95.1)	13.0 (5.77–29.28)	<.0001	14.98 (2.63–108)	.0012
MPD ≥1 cm	32.4 (21.5–44.8)	97 (91.6–99.4)	15.6 (4.45–54.9)	<.0001	47.9 (6.39–490)	<.0001
Mural nodule	20.6 (11.7–32.1)	88.0 (80.0–93.6)	1.92 (0.829–4.46)	.1280	1.28 (0.084–9.76)	1.0000
Solid component	30.9 (20.2–43.3)	90.0 (82.4–95.1)	4.07 (1.77–9.34)	.0009	3.24 (0.384–20.3)	.3258
Multifocal	28.8 (17.8–42.1)	87.1 (79–93)	2.26 (1.01–5.02)	.0463	2.41 (0.290–14.6)	.5022
Symptoms	55.9 (43.3–67.9)	61.2 (50.8–70.9)	2.01 (1.08–3.76)	.0280	5.25 (0.946–53.9)	.0604
Weight loss	5.97 (1.65–14.6)	95.0 (88.8–98.4)	1.20 (0.310–4.64)	.7916	6.16 (0.51–49.3)	.1628
Abdominal pain	32.8 (21.8–45.4)	72.3 (62.5–80.7)	1.25 (0.639–2.44)	.5182	1.06 (0.154–5.62)	1.0000
Jaundice	27.9 (17.7–40.1)	85.9 (77.4–92.0)	2.41 (1.11–5.23)	.0260	6.16 (1.08–36.7)	.0397
Pancreatitis	10.3 (4.24–20.1)	100.0 (96.3–100.0)	—	.9752	—	—
Composite risk (jaundice or MPD dilation or cyst size ≥4 cm)	88.2 (78.1–94.8)	34.7 (25.5–44.8)	3.81(1.63–8.86)	.0019	—	—

MPD, main pancreatic duct.

While the specific identity of the antigen that mAb Das-1 is reactive to currently remains under investigation, limited by its large molecular weight (>200 kDa) and heavy glycosylation, previous examination of the biomarker in fetal tissues has demonstrated expression of the Das-1 antigen in organs arising from the primitive gut (oropharynx, lung, esophagus, stomach, biliary tree, pancreas, liver, and intestine).[36] Several investigators have demonstrated that while Das-1 is expressed in the fetal esophagus, stomach, small bowel, and pancreas, it is lost in the respective adult organs and reappears in precancerous and cancerous conditions like Barrett's esophagus/esophageal adenocarcinoma,[14,37] incomplete-type gastric intestinal metaplasia/gastric adenocarcinoma,[18,38] small intestinal adenomas/adenocarcinoma,[19] and pancreatic intraepithelial neoplasia/pancreatic adenocarcinoma.[20] This pattern of expression, loss, and re-emergence appears to suggest its role as an oncofetal marker. Previous in vitro experiments have demonstrated that the Das-1 antigen is externalized and released from cells into the culture medium,[39] which may explain the intense staining of extracellular mucin we have observed in colloid carcinomas previously,[15] and the abundant presence in cyst fluid here. It is also promising that even in acellular cyst fluid aspirates, mAb Das-1 is still readily assayable. Ultimately, the presence of Das-1 in fetal pancreatic tissue, loss in normal pancreas and pancreatitis,[15] and re-emergence in dysplastic PCLs suggests that identification of this antigen may both help advance our knowledge of pathophysiologic mechanisms of malignant transformation and improve our diagnostic capacity for these lesions.

IPMNs progress from LGD and IGD to HGD (carcinoma in situ) and invasive carcinoma. We have previously demonstrated in a cohort of 94 patients with resected IPMNs that by immunohistochemistry, mAb Das-1 expression was preferentially expressed in higher-grade lesions.[15] We confirmed in our present study our initial observation that mAb Das-1 is minimally reactive to IPMN-G with LGD/IGD (3% [1 of 31]), which most frequently represent indolent, branch duct lesions. In addition, among the 43 MCNs in the current cohort, mAb Das-1 had a 100% sensitivity and specificity for identifying MCNs harboring HGD. While guidelines typically recommend the resection of all MCN lesions, the ability to reliably assess the development of dysplasia in MCNs may prospectively improve the timing of surgical intervention and avoid morbid interventions in those with significant comorbidities. Of the 8 high-risk lesions that were non-reactive with mAb Das-1, there was no clear bias to histologic subtype, and of these cases, 2 had preoperative endoscopic ultrasound/fine-needle aspiration with positive cytology and 5 of the lesions had an MPD >1 cm or a focal mass on imaging. Thus, in practice, 7 of 8 of these would have likely been referred to resection on clinical grounds alone, given the very high specificity of positive cytology and severe MPD dilation for high-risk lesions (cytology >90%,[40] MPD ≥1 cm 97%).

Classically, CEA has been utilized to discriminate mucinous from non-mucinous PCLs, traditionally with a cutoff of 192 ng/mL[28] though there has been considerable controversy regarding an optimal cutoff value, with only moderate reported accuracy.[41,42] In our available cohort, CEA was only 50% sensitive for identifying mucinous lesions. Other techniques, including cyst fluid glucose[43] and combinations of clinical indicators,[27] have been utilized to distinguish mucinous from non-mucinous lesions. While specific genetic alterations identified with next-generation sequencing (NGS) can subtype PCLs with moderate accuracy,[26,44,45] mAb Das-1 is completely non-reactive with the entire gamut of non-mucinous PCLs by ELISA (Figure 2).

Given the high prevalence of PCL in clinical practice, clinical guidelines have been adopted[7,29,30] to aid clinicians in attempting to risk stratify patients in the absence of adequate biomarkers. Validation cohorts have proven these to be inadequate in either their sensitivity or specificity, especially among branch duct lesions.[9–11] While these guidelines are not meant to be applied retroactively to surgical series, given the inherent biases associated with this, ideally a clinical risk model would have reasonable accuracy even in these cohorts. In our cohort of 169 resected PCLs, as expected, while the Sendai guideline had a high sensitivity (94.1%), it was non-specific (13.9%) for high-risk lesions, which were improved upon in the Fukuoka guidelines (specificity 20.8%) without sacrificing sensitivity. Given their similarity, the performance of the 2012 Fukuoka guidelines and the 2017 International Association of Pancreatology updated guidelines in identifying high-risk lesions was identical. The AGA guidelines (AUC 0.696) were considerably more specific (89.1%), but at the cost of reduced sensitivity (50.0%). Overall, the AGA and Fukukoa guidelines were significantly more accurate than the Sendai guidelines and the AGA guidelines were more accurate than the Fukuoka guidelines (Table 2). In comparison, mAb Das-1 reactivity was significantly more accurate than any of the available guidelines ($P < .001$). Utilizing univariate regression modeling, several of the same clinical factors that are constituent elements of the current clinical guidelines remained significant predictors of risk (MPD dilation, MPD involvement, solid component, symptoms, and jaundice). While several of these traditional high-risk clinical features like dilated MPD >1 cm, mural nodule, solid component, pancreatitis, jaundice, or weight loss were highly specific (97.0%, 88.0%, 90.0%, 100.0%, 85.9%, and 95.0%, respectively), they were highly insensitive (32.4%, 20.6%, 30.9%, 10.3%, 27.9%, and 5.97%, respectively). Therefore, their presence clinically should not be discounted, but they are insufficient to identify all high-risk lesions. Multivariate regression modeling controlling for mAb Das-1 and these clinical indicators demonstrated the presence of MPD dilation or jaundice to still be significantly associated with high-risk PCLs, independent of mAb Das-1 reactivity. Interestingly, in examining patients without any high-risk features (no dilated pancreatic duct >1 cm, weight loss, jaundice, pancreatitis, mural nodule, or solid component [n = 84]), the sensitivity, specificity, and AUC of mAb Das-1 are 94.7% (95% CI, 74.0–99.9), 100.0% (95% CI, 94.5–100.0), and 0.97 (95% CI, 0.92–1.00). Therefore, among these lesions without

obvious clinical high-risk features, mAb Das-1 may be of particular benefit.

There have been several studies attempting to identify new biomarkers for high-risk PCLs, including microRNAs, NGS, and telomerase activity.[45–48] Singhi et al[44] reported a panel of NGS targets with specific thresholds of mean allele frequency that demonstrate high accuracy for advanced neoplasia. Similarly, targeted mass spectrometry analysis of cyst fluid for mucin-5AC and mucin-2 has been reported as accurate in identifying advanced neoplasia.[49] While these are promising initial studies, their results need to be confirmed by multicenter validation cohorts. In this study, we conducted multicenter validation on mAb Das-1 as a biomarker for high-risk PCL. As mAb Das-1 is non-reactive to normal gastric mucosa and duodenal mucosa,[16] it is not susceptible to contamination that can affect cytology interpretation or even mean allele frequency in NGS. Also, in comparison to the technical expertise required for NGS or targeted mass spectrometry, ELISA is simple, highly reproducible, and inexpensive. Indeed, the non-commercialized cost of performing the assay for an entire plate (40 samples) is $50–$100. In addition, we found that the vast majority (81%) could be analyzed with as little as 125 μL cyst fluid, and 94% could be completed with 500 μL. As the ELISA assay is further refined, it is likely that smaller volumes of fluid will be required.

In conclusion, mAb Das-1 is a sensitive and highly specific biomarker for the detection of high-risk and malignant PCLs. The inclusion of the Das-1 marker into the analysis of cyst fluid may aid in the preoperative diagnosis and risk stratification of patients with PCLs.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at https://doi.org/10.1053/j.gastro.2019.05.014.

References

1. Crippa S, del Castillo CF, Salvia R, et al. Mucin-producing neoplasms of the pancreas: an analysis of distinguishing clinical and epidemiologic characteristics. Clin Gastroenterol Hepatol 2010;8:213–219.e4.
2. Mino-Kenudson M, Fernandez-del Castillo C, Baba Y, et al. Prognosis of invasive intraductal papillary mucinous neoplasm depends on histological and precursor epithelial subtypes. Gut 2011;60:1712–1720.
3. Castillo CF, Adsay NV. Intraductal papillary mucinous neoplasms of the pancreas. Gastroenterology 2010;139:708–713.e2.
4. Laffan TA, Horton KM, Klein AP, et al. Prevalence of unsuspected pancreatic cysts on MDCT. Am J Roentgenol 2008;191:802–807.
5. de Oliveira PB, Puchnick A, Szejnfeld J, et al. Prevalence of incidental pancreatic cysts on 3 tesla magnetic resonance. PLoS One 2015;10:e0121317.
6. Farrell JJ, Brugge WR. Intraductal papillary mucinous tumor of the pancreas. Gastrointest. Endosc 2002;55:701–714.
7. Vege SS, Ziring B, Jain R, et al. American Gastroenterological Association Institute guideline on the diagnosis and management of asymptomatic neoplastic pancreatic cysts. Gastroenterology 2015;148:819–822.
8. Crippa S, Bassi C, Salvia R, et al. Low progression of intraductal papillary mucinous neoplasms with worrisome features and high-risk stigmata undergoing non-operative management: a mid-term follow-up analysis. Gut 2017;66:495–506.
9. Xu M-M, Yin S, Siddiqui AA, et al. Comparison of the diagnostic accuracy of three current guidelines for the evaluation of asymptomatic pancreatic cystic neoplasms. Medicine 2017;96:e7900.
10. Ma GK, Goldberg DS, Thiruvengadam N, et al. Comparing American Gastroenterological Association Pancreatic cyst management guidelines with Fukuoka Consensus guidelines as predictors of advanced neoplasia in patients with suspected pancreatic cystic neoplasms. J Am Coll Surg 2016;223:729–737.e1.
11. Tang R, Weinberg B, Dawson D, et al. Evaluation of the guidelines for management of pancreatic branch-duct intraductal papillary mucinous neoplasm. Clin Gastroenterol Hepatol 2008;6:815–819.
12. Brugge WR, Lauwers GY, Sahani D, et al. Cystic neoplasms of the pancreas. N Engl J Med 2004;351:1218–1226.
13. Adsay NV, Pierson C, Sarkar F, et al. Colloid (mucinous noncystic) carcinoma of the pancreas. Am J Surg Pathol 2001;25:26–42.
14. Das KM, Prasad I, Garla S, et al. Detection of a shared colon epithelial epitope on Barrett epithelium by a novel monoclonal antibody. Ann Intern Med 1994;120:753–756.
15. Das KK, Xiao H, Geng X, et al. mAb Das-1 is specific for high-risk and malignant intraductal papillary mucinous neoplasm (IPMN). Gut 2014;63:1626–1634.
16. Das KM, Sakamaki S, Vecchi M, et al. The production and characterization of monoclonal antibodies to a human colonic antigen associated with ulcerative colitis: cellular localization of the antigen by using the monoclonal antibody. J Immunol 1987;139:77–84.
17. Halstensen TS, Das KM, Brandtzaeg P. Epithelial deposits of immunoglobulin G1 and activated complement colocalise with the M(r) 40 kD putative autoantigen in ulcerative colitis. Gut 1993;34:650–657.
18. Mirza ZK, Das KK, Slate J, et al. Gastric intestinal metaplasia as detected by a monoclonal antibody is highly associated with gastric adenocarcinoma. Gut 2003;52:807–812.
19. Onuma EK, Amenta PS, Jukkola AF, et al. A phenotypic change of small intestinal epithelium to colonocytes in small intestinal adenomas and adenocarcinomas. Am J Gastroenterol 2001;96:2480–2485.
20. Das KK, Das KM, Mino Kenudson M. MAb DAS-1, a monoclonal antibody reactive against a colonic phenotype, identifies pancreatic adenocarcinoma and

high-grade pancreatic intraepithelial neoplasm (PanIN) with high specificity. Gastroenterology 2011;140(Suppl 1): S-341.
21. Basturk O, Hong S-M, Wood LD, et al. A revised classification system and recommendations from the Baltimore Consensus meeting for neoplastic precursor lesions in the pancreas. Am J Surg Pathol 2015;39:1730–1741.
22. Furukawa T, Klöppel G, Volkan Adsay N, et al. Classification of types of intraductal papillary-mucinous neoplasm of the pancreas: a consensus study. Virchows Arch 2005;447:794–799.
23. Adsay NV, Merati K, Basturk O, et al. Pathologically and biologically distinct types of epithelium in intraductal papillary mucinous neoplasms: delineation of an“ intestinal” pathway of carcinogenesis in the pancreas. Am J Surg Pathol 2004;28:839–848.
24. Morales-Oyarvide V, Mino Kenudson M, Ferrone CR, et al. Acute pancreatitis in intraductal papillary mucinous neoplasms: a common predictor of malignant intestinal subtype. Surgery 2015;158:1219–1225.
25. Das KK, Marchegiani G, Geng X, et al. Comparison of the International Consensus Guidelines for Management of Intraductal Papillary Mucinous Neoplasm (IPMN) with analysis of pancreatic cyst fluid aspirates for mAb-Das-1 reactivity in identifying high-risk and malignant IPMN. Gastroenterology 2014;146(Suppl 1): S-25.
26. Springer S, Wang Y, Dal Molin M, et al. A combination of molecular markers and clinical features improve the classification of pancreatic cysts. Gastroenterology 2015;149:1501–1510.
27. **Masica DL**, **Dal Molin M**, Wolfgang CL, et al. A novel approach for selecting combination clinical markers of pathology applied to a large retrospective cohort of surgically resected pancreatic cysts. J Am Med Inform Assoc 2016;24:145–152.
28. Brugge WR, Lewandrowski K, Lee-Lewandrowski E, et al. Diagnosis of pancreatic cystic neoplasms: a report of the cooperative pancreatic cyst study. Gastroenterology 2004;126:1330–1336.
29. Tanaka M, Chari S, Adsay V, et al. International Consensus Guidelines for Management of Intraductal Papillary Mucinous Neoplasms and Mucinous Cystic Neoplasms of the Pancreas. Pancreatology 2006; 6:17–32.
30. Tanaka M, Fernández Del Castillo C, Adsay V, et al. International consensus guidelines 2012 for the management of IPMN and MCN of the pancreas. Pancreatology 2012;12:183–197.
31. Tanaka M, Castillo CF-D, Kamisawa T, et al. Revisions of international consensus Fukuoka guidelines for the management of IPMN of the pancreas. Pancreatology 2017;7:738–753.
32. Tanno S, Nakano Y, Sugiyama Y, et al. Incidence of synchronous and metachronous pancreatic carcinoma in 168 patients with branch duct intraductal papillary mucinous neoplasm. Pancreatology 2010;10:173–178.
33. Lawrence SA, Attiyeh MA, Seier K, et al. Should patients with cystic lesions of the pancreas undergo long-term radiographic surveillance?: Results of 3024 patients evaluated at a single institution. Ann Surg 2017;266:536–544.
34. Kwok K, Chang J, Duan L, et al. Competing risks for mortality in patients with asymptomatic pancreatic cystic neoplasms: implications for clinical management. Am J Gastroenterol 2017;112:1330–1336.
35. Efishat Al MA, Attiyeh MF, Eaton AA, et al. Multi-institutional validation study of pancreatic cyst fluid protein analysis for prediction of high-risk intraductal papillary mucinous neoplasms of the pancreas. Ann Surg 2018; 268:340–347.
36. Badve S, Lôgdberg L, Sokhi R, et al. An antigen reacting with Das-1 monoclonal antibody is ontogenically regulated in diverse organs including liver and indicates sharing of developmental mechanisms among cell lineages. Pathobiology 2000;68:76–86.
37. Piazuelo MB, Haque S, Delgado A, et al. Phenotypic differences between esophageal and gastric intestinal metaplasia. Mod Pathol 2003;17:62–74.
38. Watari J, Das KK, Amenta PS, et al. Effect of eradication of *Helicobacter pylori* on the histology and cellular phenotype of gastric intestinal metaplasia. Clin Gastroenterol Hepatol 2008;6:409–417.
39. Kesari KV, Yoshizaki N, Geng X, et al. Externalization of tropomyosin isoform 5 in colon epithelial cells. Clin Exp Immunol 1999;118:219–227.
40. Genevay M, Mino Kenudson M, Yaeger K, et al. Cytology adds value to imaging studies for risk assessment of malignancy in pancreatic mucinous cysts. Ann Surg 2011;254:977–983.
41. Frossard J. Performance of endosonography-guided fine needle aspiration and biopsy in the diagnosis of pancreatic cystic lesions. Am J Gastroenterol 2003; 98:1516–1524.
42. Jin DX, Small AJ, Vollmer CM, et al. A lower cyst fluid CEA cut-off increases diagnostic accuracy in identifying mucinous pancreatic cystic lesions. JOP 2015;16:271–277.
43. Zikos T, Pham K, Bowen R, et al. Cyst fluid glucose is rapidly feasible and accurate in diagnosing mucinous pancreatic cysts. Am J Gastroenterol 2015;110:909–914.
44. Singhi AD, McGrath K, Brand RE, et al. Preoperative next-generation sequencing of pancreatic cyst fluid is highly accurate in cyst classification and detection of advanced neoplasia. Gut 2018;67:2131–2141.
45. Jones M, Zheng Z, Wang J, et al. Impact of next-generation sequencing on the clinical diagnosis of pancreatic cysts. Gastrointest Endosc 2016;83:140–148.
46. Farrell JJ, Toste P, Wu N, et al. Endoscopically acquired pancreatic cyst fluid microRNA 21 and 221 are associated with invasive cancer. Am J Gastroenterol 2013; 108:1352–1359.
47. Kanda M, Knight S, Topazian M, et al. Mutant GNAS detected in duodenal collections of secretin-stimulated pancreatic juice indicates the presence or emergence of pancreatic cysts. Gut 2013;62:1024–1033.
48. Hata T, Molin MD, Suenaga M, et al. Cyst fluid telomerase activity predicts the histologic grade of cystic neoplasms of the pancreas. Clin Cancer Res 2016;22:5141–5151.

49. Jabbar KS, Arike L, Verbeke CS, et al. Highly accurate identification of cystic precursor lesions of pancreatic cancer through targeted mass spectrometry: a phase IIc diagnostic study. J Clin Oncol 2018;36:367–375.

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Conflicts of interest
These authors disclose the following: Koushik K. Das, Mari Mino-Kenudson, and Kiron M. Das have been granted a patent for the use of monoclonal antibody Das-1 in the detection of cancerous lesions of pancreas. This patent has not been licensed and these authors hold no commercial interests at this time. The remaining authors disclose no conflicts.

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Supplementary Table 1. Performance of Carcinoembryonic Antigen (>192 ng/mL) in Assessing Mucinous vs Non-Mucinous Pancreatic Cystic Lesions (n = 49)

Variable	Data
Sensitivity for mucinous cystic lesions, % (95% CI)	50.0 (0.329–0.671)
Specificity for mucinous cystic lesions, % (95% CI)	92.3 (0.64–0.998)
AUC (95% CI)	0.712 (0.6–0.824)
Positive predictive value, % (95% CI)	94.7 (74–99.9)
Negative predictive value, % (95% CI)	40.0 (22.7–59.4)
Likelihood ratio (95% CI)	6.5 (0.961–43.9)

Supplementary Table 2. Intraductal Papillary Mucinous Neoplasm Cohort Characteristics

Characteristic	n (%)
IPMN location	
Head/uncinate	37 (41)
Neck	12 (13)
Body/tail	13 (15)
Multifocal	28 (31)
Anatomic subtype	
Main duct	14 (16)
Branch duct	46 (51)
Mixed type	30 (33)
Preoperative main duct involvement	42 (46)
Epithelial subtype	
Gastric	39 (43)
Intestinal	24 (27)
Pancreatobiliary	5 (6)
Oncocytic	4 (4)
Invasive	18 (20)
Invasive tubular	6 (33[a])
Invasive colloid	9 (50[a])
Invasive with concomitant adenocarcinoma	3 (17[a])
Dysplastic grade	
Low	19 (21)
Intermediate	21 (23)
High	32 (36)

[a]Percent of invasive.

Supplementary Table 3. Monoclonal Antibody Das-1 Reactivity by Epithelial Subtype and Dysplastic Grade of Intraductal Papillary Mucinous Neoplasm

Variable	n	mAb Das-1–positive, n (%)
Gastric type		
Low grade	19	0 (0)
Intermediate grade	12	1 (8.3)
High grade	8	7 (88)
Intestinal type		
Intermediate grade	9	6 (67)
High grade	15	14 (93)
Oncocytic type		
High grade	4	3 (75)
Pancreaticobiliary type		
High grade	5	4 (80)
Invasive IPMN		
Tubular carcinoma	6	6 (100)
Colloid carcinoma	9	8 (89)
Invasive IPMN with concomitant pancreatic adenocarcinoma	3	3 (100)

Supplementary Table 4. Performance of Monoclonal Antibody Das-1 and Clinical Guidelines in Assessing High-Risk Lesions, Defined as Those With Invasive Component or High-Grade Dysplasia of Any Epithelial Subtype

Variable	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
mAb Das-1	88.3 (77.4–95.2)	92.7 (86.0–96.8)	86.9 (75.8–94.2)	93.5 (87.1–97.4)
Sendai guidelines	95.0 (86.1–99.0)	13.8 (7.91–21.7)	37.7 (30.0–46.0)	83.3 (58.6–96.4)
Fukuoka guidelines	96.7 (88.5–99.6)	19.3 (12.3–27.9)	39.7 (31.7–48.1)	91.3 (72.0–98.9)
AGA guidelines	53.3 (40.0–66.3)	88.1 (80.5–93.5)	71.1 (55.7–83.6)	77.4 (69–84.4)

NPV, negative predictive value; PPV, positive predictive value.

Supplementary Table 5. Performance of Composite Clinical Parameters in Assessing High-Risk Pancreatic Cystic Lesions

Variable	Sensitivity, % (95% CI)	Specificity, % (95% CI)	LR (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
Composite high risk[a] (jaundice, MPD dilation, or cyst ≥4 cm)	88.2 (78.1–94.8)	34.7 (25.5–44.8)	1.35 (1.14–1.59)	47.6 (38.7–56.7)	81.4 (66.6–91.6)
Composite high risk[b] (jaundice, MPD dilation, or cyst ≥4 cm)	86.3 (73.7–94.3)	43.6 (27.8–60.4)	1.53 (1.14–2.06)	66.7 (54–77.8)	70.8 (48.9–87.4)

LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.
[a]Considering high risk as IPMN/MCN with HGD and/or invasion or IPMN-I with IGD.
[b]Considering high risk as only IPMN with HGD and/or invasion, excluding other PCLs.