Multiinstitutional Validation Study of Cyst Fluid Protein Biomarkers in Patients With Cystic Lesions of the Pancreas

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Objective: Prospective evaluation of 2 clinical-molecular models in patients with unknown pathology who underwent endoscopic ultrasound with fine-needle aspiration (EUS-FNA) for a cystic lesion of the pancreas.

Summary of Background Data: Preoperative prediction of histologic subtype (mucinous vs nonmucinous) and grade of dysplasia in patients with pancreatic cystic neoplasms is challenging. Our group has previously published 2 clinical-molecular nomograms for intraductal papillary mucinous neoplasms (IPMN) that incorporated both clinical/radiographic features and cyst fluid protein markers (sFASL, CA72-4, MMP9, IL-4).

Methods: This multiinstitutional study enrolled patients who underwent EUS-FNA for a cystic lesion of the pancreas. Treatment recommendations regarding resection were based on standard clinical, radiographic, and endoscopic features. Predicted

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probabilities of high-risk IPMN (high-grade dysplasia/invasive cancer) were calculated using the previously developed clinical-molecular nomograms.

Results: Cyst fluid was obtained from 100 patients who underwent diagnostic EUS-FNA. Within this group there were 35 patients who underwent resection, and 65 were monitored radiographically. Within the group that underwent resection, 26 had low-risk IPMN or benign non-IPMN lesions, and 9 had high-risk IPMN. Within the surveillance group, no patient progressed to resection or developed cancer after a median follow-up of 12months (range: 0.5–38). Using the clinical/radiographic nomogram alone, 2 out of 9 patients with high-risk IPMN had a predicted probability >0.5. In the clinical-molecular models, 6 of 9 patients in model 1, and 6 of 9 in model 2, had scores >0.5.

Conclusions: This prospective study of patients with unknown cyst pathology further demonstrates the importance of cyst fluid protein analysis in the preoperative identification of patients with high-risk IPMN. Longer follow-up is necessary to determine if this model will be useful in clinical practice.

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The management of patients with cystic neoplasms of the pancreas is challenging. Guidelines for resection are based on clinical, radiographic, and endoscopic characteristics, yet diagnostic accuracy is limited.[1–3] Our group has previously demonstrated that cyst fluid protein biomarkers can discriminate between cyst types[4] and predict degree of dysplasia in resected pancreatic cysts.[5–8] We have proposed 2 clinical-molecular models that combine 4 cyst fluid protein markers (Model 1: MMP9, CA72-4; Model 2: sFASL, IL-4)[8] with a previously published clinical/radiographic nomogram,[9] which discriminated between low-risk and high-risk intraductal papillary mucinous neoplasms (IPMN) with c-indices of 0.8.[7]

Prior models were developed using patients with pathologically confirmed IPMN after resection. This fails to take into consideration whether a given biomarker can differentiate dysplasia in patients with IPMN who do not undergo resection or in pancreatic cysts other than IPMN. In the current study, we aim to prospectively evaluate whether these models show similar performance in patients with pancreatic cystic lesions undergoing an initial evaluation with endoscopic ultrasound with fine needle aspiration (EUS-FNA).

METHODS

Patients with pancreatic cysts were enrolled to undergo EUS-FNA at 1 of 3 institutions (Memorial Sloan Kettering, Massachusetts General Hospital, Johns Hopkins Hospital) between 2008 and 2019. Cyst fluid was obtained during EUS-FNA and stored at −80 degrees. Patients were either resected or followed radiographically per institutional protocol, usually at 6-month to 1-year intervals. Clinical decision making was made at the discretion of the surgeon. High-risk lesions were defined as those with high-grade dysplasia/invasive carcinoma, or pathologically confirmed, unresectable pancreatic adenocarcinoma. Low-risk lesions included IPMN that harbored low-grade or intermediate-grade dysplasia, and benign histopathologies.

Cyst fluid samples were analyzed for 4 proteins (sFASL, IL-4, MMP9, CA72-4) using Luminex Multiplex Bead Immunoassays per standard protocol at the University of Pittsburgh Cancer Institute Luminex facility.[4,7,8,10] Out-of- range values indicated concentrations below the lowest extrapolated value. Half of the lowest possible value was used for patients with out-of-range values in model 1, and patients were included in the $<84^{th}$ percentile for IL-4 and $<90^{th}$ percentile for sFASL for model 2.

Two clinical-molecular models were created by combining the results from the cyst fluid analysis and the clinical/radiographic nomogram.[7–9] Predicted probabilities were calculated using the following formulas: Model 1 = clinical nomogram + log-CA72-4 + log-MMP9, and Model 2 = clinical nomogram + branch versus main/mixed duct and (IL-4 $\leq$ 84th percentile) and (sFASL $\leq$ 90th percentile) + main/mixed duct and (IL-4 $\leq$ 84th percentile) and (sFASL $\leq$ 90th percentile) + (IL-4 $>$ 84th percentile or sFASL $>$ 90th percentile). Comparisons between groups were performed with Fisher exact and Wilcoxon rank sum tests. All tests were two-sided and $P < 0.05$ was considered significant. SAS version 9.4 (SAS institute Inc., Cary, NC) was used for analysis.

RESULTS

There were 100 patients included in the study, 35 patients had known final pathology (9 high-risk disease and 26 low-risk disease) and 65 patients did not undergo resection and were followed with radiographic surveillance (Table 1). Median cyst size was 3.2 cm (interquartile range 2.6–4.2cm), and 7 patients had main pancreatic duct dilatation. Median follow-up of patients who underwent surveillance was 12 months (range 0.5–39). All resected patients had operations within 12 months of EUS-FNA, with a median of 2 months before operation.

Three of the 4 cyst fluid markers demonstrated a significant difference in the log concentrations between patients with high-risk lesions, low-risk lesions, and those who underwent surveillance (sFASL, 5.8 vs 3.5 vs 2.9, $P = 0.006$; MMP-9, 10.9 vs 8.7 vs 7.8, $P = 0.047$; CA72-4, 2.3 vs −0.7 vs −0.5, $P = 0.019$). There was no significant difference in log concentration of IL-4 between groups (1.64 vs 0.32 vs 0.32, $P = 0.22$).

Predicted probabilities using the clinical/radiographic nomogram alone ranged from 0.09 to 0.69 (Fig. 1A), and 2 of 9 patients with high-risk disease had predicted probabilities greater than 0.5.

In clinical-molecular model 1 (Fig. 1B), all patients with high-risk disease were in the top third of the cohort with predicted probabilities between 0.23 and 0.96 (range 0.005–0.96). In this model, 6 of 9 high-risk patients had a predicted probability greater than 0.5. Predicted probabilities using clinical-molecular model 2 (Fig. 1C) ranged between 0.18 and 0.77. There were 8 patients with predicted probabilities greater than 0.5, 6 of whom had high-risk disease.

When the 2 models were combined, 8 of 9 cases (89%) with high-risk disease had a score greater than 0.5 by at least 1 of the 2 models (Fig. 1D). A score greater than 0.5 in at least one of the models was present in 3 of 23 patients (9%) with low-risk disease, and in 4 of 65 patients (6%) who underwent radiographic surveillance.

DISCUSSION

The challenge with biomarker validation in patients with pancreatic cysts is that the majority of patients are followed radiographically, and in this group the exact histopathology cannot be determined. In this study, we sought to

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TABLE 1. Baseline Clinical and Pathologic Characteristics of the Cohort

	All Patients n = 100	High-Risk* n = 9	Low-Risk n = 26	Surveillance n = 65
Age, yrs	69 (61–76)	70 (65–77)	64 (51–72)	69 (64–76)
Sex				
Female	58 (58)	2 (22)	18 (69)	38 (58)
Male	42 (42)	7 (78)	8 (31)	27 (42)
BMI, kg/m^2	27 (24–32)	26 (23–27)	29 (23–33)	27 (24–32)
Symptoms				
Yes	8 (8)	5 (56)	2 (8)	1 (2)
No	92 (92)	4 (44)	24 (92)	64 (98)
Weight loss				
Yes	7 (7)	3 (33)	2 (8)	2 (3)
No	93 (93)	6 (67)	24 (92)	63 (97)
Pancreatitis				
Yes	6 (6)	2 (22)	1 (4)	3 (5)
No	94 (94)	7 (78)	25 (96)	62 (95)
Diabetes				
Yes	25 (25)	4 (44)	7 (27)	14 (22)
No	75 (75)	5 (56)	19 (73)	51 (78)
Smoking History†				
Yes	40 (40)	3 (33)	10 (38)	27 (42)
No	59 (59)	6 (67)	16 (62)	37 (57)
Radiographic duct type				
Main duct	7 (7)	4 (44)	0 (0)	3 (5)
Branch duct	93 (93)	5 (56)	26 (100)	62 (95)
Cyst diameter, cm	3.2 (2.6–4.2)	5.6 (4.6–6.7)	3.9 (2.8–4.5)	2.9 (2.5–3.8)
Cyst location				
Head/uncinate	44 (44)	2 (22)	8 (31)	34 (52)
Body/tail	53 (53)	5 (56)	18 (69)	30 (46)
Throughout	2(2)	2 (22)	0 (0)	0 (0)
Extrapancreatic	1 (1)	0(0)	0 (0)	1 (2)
Mural nodule/solid component				
Yes	27 (27)	9 (100)	5(19)	13 (20)
No	73 (73)	0 (0)	21 (81)	52 (80)
Cyst fluid CEA, ng/mL§	84.9 (7.2–439.1)	664.0 (96.1–9251)	126.6 (34.2–1028)	54.9 (2.4–247.5)
Operation				
Pancreatoduodenectomy	7 (7)	0 (0)	7 (27)	–
Distal pancreatectomy	16 (16)	1 (11)	15 (58)	–
Total pancreatectomy	2 (2)	1 (11)	1 (4)	–
Enucleation	3 (3)	0 (0)	3 (12)	–
Exploration ± biopsy	4 (4)	4 (45)	0 (0)	–
None	68 (68)	3 (33)	0 (0)	65 (100)

Displayed as n (%) or median (IQR).

BMI indicates Body Mass Index; CEA, carcinoembryonic antigen; IQR, interquartile range.*Includes patients with unresectable, biopsy-proven adenocarcinoma arising from IPMN.

†Unknown in 1 patient.

§Not obtained in 11 patients.

prospectively evaluate the utility of 2 clinical-molecular models that we had previously developed from the fluid of patients who had undergone resection. In our current study, a subset underwent resection (35%), whereas the majority of patients continued with radiographic surveillance. In each of the clinical-molecular models, 6 of 9 patients with high-risk lesions had predicted probabilities greater than 0.5. More than 90% of patients with low-risk or unknown pathology had scores below 0.5. Only 1 high-risk case had a low predicted probability according to both models whereas the remaining 8 cases had a high predicted probability using model 1 and/or 2.

These data suggest that the combination of clinical presentation, radiographic imaging, and cyst fluid protein markers increases diagnostic accuracy. The primary strength of this study is that the patients included had unknown pathology. This differs from the majority of other biomarker studies in this field, which have almost solely been conducted on samples with known pathology after resection. This approach excludes the majority of patients with pancreatic cysts.

Our ability to perform in-depth statistical analysis was limited as the patients on whom we have final pathology were few in number, and patients followed radiographically are unable to be classified as low-risk or high-risk. We were encouraged by the fact that the vast majority of unknown patients had low scores in both models, and that no patient in this group progressed during follow-up. Median follow-up is currently short (12 months), and thus with a longer duration of follow-up a more definitive conclusion will be possible.

In conclusion, the combination of clinical and radiographic features combined with cyst fluid protein markers demonstrate improved discrimination between high-risk and low-risk cystic lesions of the pancreas in this study. Longer term follow-up will strengthen this dataset and is necessary to confirm low-risk pathology in patients who are recommeded surveillance.

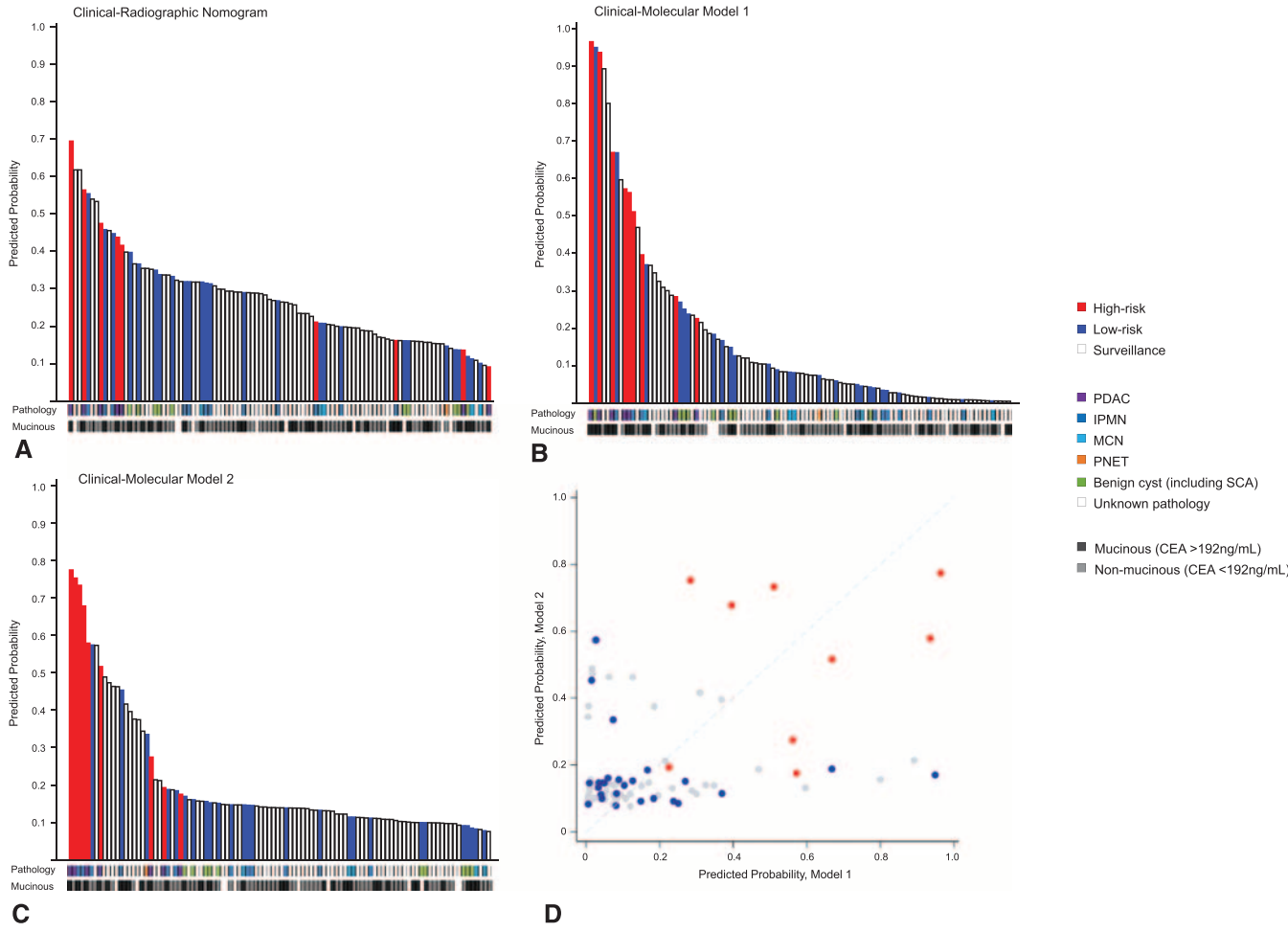

FIGURE 1. Waterfall plots demonstrating predicted probability of the clinical/radiographic nomogram alone (A), clinical-molecular model 1 (B), clinical-molecular model 2 (C), and a scatterplot comparing the predicted probabilities of models 1 and 2 (D).

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