Changes to management influenced by next-generation sequencing of pancreatic duct aspirates among patients with main pancreatic duct dilatation of unclear etiology: a multicenter cohort study

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Background and Aims: Main pancreatic duct (MPD) dilatation of unclear etiology presents a diagnostic challenge because the management of neoplastic and non-neoplastic causes differs significantly. We aimed to assess the clinical utility of next-generation sequencing (NGS) in the management of MPD dilatation.

Methods: We retrospectively identified patients with dilated MPD (≥6 mm) of unclear etiology who underwent NGS at 2 academic institutions. The primary outcome was any change to management influenced by NGS compared with previous standard of practice. Adverse event rates of EUS fine-needle aspiration and ERCP were compared.

Results: A total of 62 patients with dilated MPD were identified; they had a mean age of 71.5 ± 12.1 years, and 53.2% were men. The majority had chronic pancreatitis (54.8%). The average MPD diameter was 8.6 ± 2.3 mm. MPD aspiration was performed by EUS fine-needle aspiration in 33 patients (53.2%) and ERCP in 29 patients (46.8%). The adverse event rate was 8.1%, with no difference between aspiration techniques observed. NGS mutations were present in 40 (64.5%) patients. The identification of mutations suggesting a neoplastic cause of MPD dilatation resulted in surgical management for 11 patients with no previous indication. In 6 cases with MPD ≥10 mm, dilatation was attributed to chronic pancreatitis and surgery was deferred despite previous practice of recommending surgery. Overall, management was changed in 17 (27.4%) patients.

Conclusions: In a multicenter cohort of patients with MPD dilatation, NGS of MPD aspirates was safe and changed surgical management for more than one-quarter of patients. Providers should consider MPD sampling with NGS as an ancillary diagnostic modality for patients presenting with MPD dilatation of unclear etiology. (Gastrointest Endosc 2026;104:59-68.)

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INTRODUCTION

Main pancreatic duct (MPD) dilatation can result from both benign and malignant conditions, including chronic pancreatitis (CP), pancreatic ductal adenocarcinoma (PDAC), and mucinous cystic lesions of the pancreas.[1] The importance of establishing the etiology of MPD dilatation is further magnified by the high risk of advanced neoplasia associated with main duct-intraductal papillary mucinous neoplasm (IPMN)[2] and the poor prognosis of PDAC.[3] Although obstructive causes of MPD dilatation may be radiographically apparent, cases of diagnostic uncertainty present a significant clinical challenge. Indeed, a premalignant or malignant pathology may be present in a substantial proportion of patients with MPD dilatation of unclear etiology,[1] therefore, highlighting the need for effective diagnostic algorithms.[4]

IPMNs are broadly categorized as main duct, mixed, or branch duct on the basis of the degree of MPD dilatation ($\geq$10 mm, $\geq$5 mm, and <5 mm, respectively).[5] However, this classification does not always correlate with histopathologic findings, because mucin production from branch duct IPMN or concomitant pathology such as CP may result in MPD dilatation without neoplastic involvement of the main duct epithelium. Nevertheless, surgical resection is generally recommended in cases of suspected main duct-IPMN, and MPD diameter $\geq$5 mm is considered a worrisome feature for pancreatic cysts.[5] In the absence of a solid or cystic lesion, the management of MPD dilatation is complex and depends on several factors, including the patient's fitness for surgery, MPD diameter, and the presence or absence of CP.[4] Moreover, although CP is a common benign cause of MPD dilatation, clinical and radiographic findings of CP do not exclude the possibility of a concomitant neoplastic pathology such as main duct-IPMN.[6] Diagnostic modalities capable of accurately characterizing the etiology of MPD dilatation are therefore of critical importance, particularly in cases with confounding factors such as the presence of CP.

In cases of isolated MPD dilatation, imaging alone cannot always differentiate between benign and malignant causes.[7] EUS and ERCP may provide useful ancillary information in cases of diagnostic uncertainty. Although EUS has high sensitivity for detecting occult pancreatic malignancies, it is limited by a lack of specificity,[8] and the diagnostic performance is further limited in the setting of CP.[9] Accurate diagnostic modalities for determining the etiology of MPD dilatation are therefore needed. Next-generation sequencing (NGS) has emerged as a promising ancillary test with superior diagnostic performance in determining cyst type and predicting progression to advanced neoplasia.[10] Incorporating NGS into management algorithms for pancreatic lesions also has demonstrated utility in several clinical scenarios, including in the evaluation of MPD dilatation of unclear etiology.[11] Herein, we aimed to further characterize the clinical utility of NGS performed on MPD aspirates in a multicenter cohort of patients with MPD dilatation of unclear etiology and compare the procedural safety and yield of EUS fine-needle aspiration (FNA) and ERCP for sample collection.

METHODS

Study design

A multicenter retrospective study design was used to assess patients who underwent NGS of MPD aspirates for evaluation of MPD dilatation of unclear etiology at 2 academic medical centers in the United States. The study was approved by institutional review boards at the University of Texas Southwestern Medical Center (no. STU-2019 to 1650) and was exempt from full review at the University of Wisconsin School of Medicine and Public Health.

Participants

A total of 6369 patients seen in pancreatic cancer prevention clinics at 2 large academic hospitals in the United States were considered for this analysis (Fig. 1). Patients with pancreatic abnormalities identified on imaging presented to the clinic after either automatic referral or clinician-prompted referral (ie, recommendation for referral in radiology report). A multidisciplinary panel including gastroenterologists, surgeons, and radiologists reviewed available data and determined the need for diagnostic and therapeutic interventions including MPD sampling. Recommendations were then discussed with patients to determine the final treatment plan. Consecutive patients seen in these clinics were included if they established care in between 2016 and 2024, were age $\geq$18 years at the time of initial visit, had MPD diameter $\geq$6 mm on cross-sectional imaging with unclear etiology of dilatation at initial visit, and underwent MPD aspiration with NGS for evaluation of MPD dilatation. All patients underwent EUS as part of evaluation either before or at the time of procedure for NGS collection. Exclusion criteria included age <18 years, unavailability of NGS results, the presence of a pancreatic mass on cross-sectional imaging, and <12 months follow-up for patients enrolled in a lesion surveillance protocol. Demographic and clinical variables were abstracted by chart review.

Next-generation sequencing

NGS was performed at the University of Pittsburgh Medical Center (UPMC) using the PancreaSeq assay as previously described.[10] In brief, an amplification-based DNA/RNA-based assay was used to evaluate for a variety of genomic alterations including single-nucleotide variants, loss of heterozygosity, insertions, and deletions in genes of interest. Massive parallel sequencing with AmpliSeq primers was used on an Ion GeneStudio S5 Prime System (Thermo Fisher Scientific, Waltham, Mass, USA) and data were analyzed using Variant Explorer bioinformatics program (UPMC). The detected mutations were highlighted in a report that was subsequently uploaded to the medical record at each respective institution,

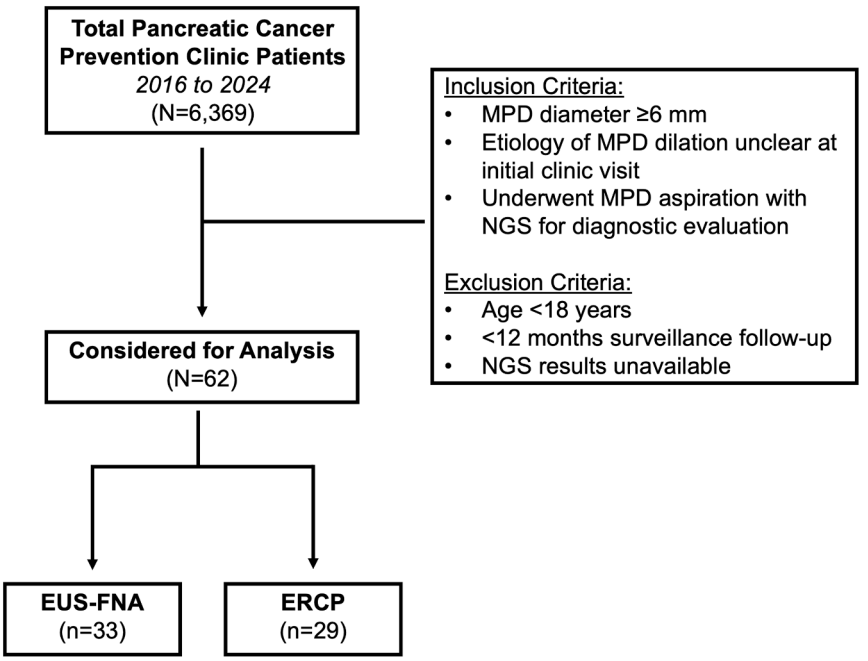

Figure 1. Patient population considered for analysis after application of inclusion and exclusion criteria stratified by technique used for aspiration of main pancreatic duct. *FNA*, Fine-needle aspiration; *MPD*, main pancreatic duct; *NGS*, next-generation sequencing.

and data were abstracted by chart review. *TP53*, *PTEN*, *SMAD4, CDKN2A*, and *PIK3CA* were considered high-risk mutations. In this analysis, positive NGS was defined as detection of any high-risk mutation or mutation suggestive of mucinous neoplasm (eg, *KRAS, GNAS*). The diagnostic performance of MPD aspirate NGS at identifying IPMN as the etiology of MPD dilatation was determined using the criterion standard of surgical histopathology.

Sample collection

MPD aspiration was conducted through either EUS-FNA or ERCP at the discretion of the endoscopist after multidisciplinary discussion yielded a recommendation for MPD sampling. EUS-FNA was performed using a linear EUS probe. The MPD and surrounding parenchyma was evaluated, and FNA was performed using a 22- or 25-gauge needle either through a transgastric or transduodenal approach. ERCP was performed using a duodenoscope to identify the major duodenal papillae. Cannulation of the major duodenal papillae was performed through insertion of a guidewire. The MPD was accessed under fluoroscopic guidance and a catheter was subsequently inserted to perform MPD aspiration before any contrast injection.

Regardless of collection technique, the decision to perform MPD aspiration was made after initial endosonographic evaluation failed to identify a pancreatic mass. Samples for NGS were stored in prespecified collection vials in stabilization media and sent to UPMC for analysis. Additional fluid was sent for biochemical testing and cytology if sample volume was adequate. When sample fluid was limited, NGS was prioritized. Volume of MPD fluid aspirated and postprocedural adverse events were abstracted by chart review for each collection technique.

Clinical outcomes and changes to management

Clinical outcomes were determined for each patient after the index NGS encounter, including discharge from clinic, enrollment in a surveillance protocol, surgical resection, and development of advanced neoplasia. In cases in which patients underwent surgical resection, final pathologic diagnosis on surgical pathology was noted. Among patients in whom the decision was made to defer surgery, attention was paid to radiographic or pathologic progression during follow-up.

Changes to clinical management after undergoing NGS were considered in reference to previous institutional practices. Both institutions included in this analysis reference the International Association of Pancreatology guidelines for pancreatic cyst management.[5,12] The recommendations for management were made after multidisciplinary discussion considering these guidelines. In cases of MPD dilatation, surgical management was generally recommended for any patient with MPD diameter $\geq$10 mm. In cases in which MPD diameter was between 6 and 10 mm, surgery was recommended in cases in which at least 1 additional worrisome feature was present. If nonsurgical management was recommended, surveillance was dictated by clinical risk factors, MPD diameter, stability over time, and patient preference. After incorporation of NGS into the protocols of the institutions included in the current study, cases in which the etiology of MPD dilatation was unclear after initial

consultation and multidisciplinary discussion were referred for NGS. In this study, cases in which NGS influenced clinical management (ie, changed the recommendation for or against surgery) were noted.

Statistical analysis

Demographic and clinical characteristics were reported using means with SDs and frequencies with percentages, as appropriate. We then compared patients with and without high-risk mutations using either the χ^2 or Fisher exact test for categorical variables and *t* test for continuous variable. The Bonferroni correction was used to control for the family-wise error rate when applicable. A 2-sided *P* value <.05 was considered statistically significant. Standard 2 × 2 contingency tables were used to calculate the sensitivity and specificity of NGS at identifying IPMN with main duct involvement using only cases with surgical pathology available. Statistical analysis was performed using STATA software 14.2 (College Station, Tex, USA).

RESULTS

Patient and cyst characteristics

A total of 62 patients met inclusion criteria. The mean age of patients was 71.5 ± 12.1 years and those with positive NGS were significantly older than those with negative NGS (75.0 ± 7.0 years vs 65.1 ± 16.1 years; $P = .002$) (Table 1). The majority of patients (53.2%) were male. The most common race/ethnicity was non-Hispanic white (79.0%), followed by non-Hispanic black (11.3%) and Hispanic/Latino (4.8%). Diabetes was present in 54.8%, a personal history of nonpancreatic cancer was present in 30.6% of patients, and 32.3% had a family history of PDAC. Germline mutations were identified in 2 patients (3.2%; *PTEN* and *MUTYH*). In addition, 33 of 62 patients (53.2%) reported a history of smoking, 45.2% reported clinically significant alcohol consumption, and 14.5% received chronic opioids for pain management. The mean total follow-up period was 33.3 ± 26.3 months (Table 1).

Radiographic or clinical (clinician documentation of diagnosis) evidence of CP was evident in 34 of 62 patients (54.8%; Table 1). Patients with negative NGS were more likely than those with positive NGS to have CP (77.3% vs 42.5%; $P = .01$). Among 29 patients with CP, 17 (58.6%) had radiographic findings of CP, 9 (31.0%) had pancreatic calcifications, and 11 (37.9%) had a MPD stricture. EUS findings were suggestive of CP in 10 of 29 (34.5%) patients with a diagnosis of CP.

The pancreatic lesion prompting referral to pancreas cancer prevention clinic was identified incidentally in 59.7% of patients. Initial imaging modality was computed tomography in 53.2% of patients, magnetic resonance imaging in 45.2%, and EUS in 1.6%. A pancreatic cyst (suspected mixed type or main duct IPMN) was present at the index encounter in 37.1% of patients, and those with positive NGS were more likely to have a pancreatic cyst than those with negative NGS (47.5% vs 18.2%; $P = .02$). In these cases, the mean cyst size was 1.58 ± 1.02 cm with no difference between groups. The mean MPD diameter was 8.6 ± 2.3 mm; 8.5 ± 2.2 mm among those with positive NGS and 8.8 ± 2.6 mm among those with negative NGS ($P = .56$). Cytology was positive in 1 patient (1.6%) who underwent ERCP and was found to have *KRAS* and *GNAS* mutations on NGS with no high-risk mutations but declined surgery.

Before undergoing NGS, 85.5% of patients had at least 1 magnetic resonance imaging study, 64.5% had at least 1 computed tomography study, and 51.6% had at least 1 EUS to evaluate the pancreatic lesion. Considering patients with positive NGS findings ($n = 40$), 97.5% had mutations in either *KRAS* or *GNAS* suggesting a mucinous neoplasm. High-risk mutations were present in 10.0%; 2 with *TP53* (variant allele frequencies 7% and 1%), 1 with *TP53*/*SMAD4* (variant allele frequencies not available), and 1 with *PIK3CA* (variant allele frequency 10%). A total of 20 (32.3%) patients underwent surgery (16 pancreaticoduodenectomy and 4 distal pancreatectomy) in this cohort and 9 (14.5%) were diagnosed with advanced neoplasia (high-grade dysplasia or invasive adenocarcinoma). Patients with positive NGS were more likely to undergo surgical management of MPD dilatation than those with negative NGS (42.5% vs 13.6%; $P = .02$). An adverse event occurred in 6 of 17 (35.3%) patients undergoing surgery (intraabdominal infection in 3 patients, bleeding in 2 patients, delayed gastric emptying in 1 patient). The clinical risk factors, radiographic findings, and NGS results for each patient are summarized in Figure 2.

Diagnostic performance of NGS

The diagnostic performance of NGS was evaluated among the subgroup of patients undergoing surgical resection for management of pancreatic lesions ($n = 20$). Specifically, the performance of identifying IPMN as the etiology of MPD dilatation was determined using a reference criterion standard of final surgical histopathology. The sensitivity of NGS (*KRAS* and/or *GNAS* mutation) for the diagnosis of IPMN with main duct involvement was 88.9% (95% CI, 66.6%-97.6%) and the specificity was 100.0% (95% CI, 34.2%-100.0%). The area under the receiver operating curve was 0.94 (95% CI, 0.83-1.00; Fig. 3).

Comparison of sample collection techniques

MPD aspiration technique varied depending on patient and provider factors, with 29 (46.8%) patients undergoing ERCP and 33 (53.2%) patients undergoing EUS-FNA. Patients undergoing EUS-FNA were older than those undergoing ERCP (74.4 ± 7.7 years vs 68.1 ± 15.2 years; $P = .04$). Otherwise, demographic variables and

TABLE 1. Demographic and clinical variables of study population stratified by the presence ($n = 40$) or absence ($n = 22$) of mutations on NGS of MPD aspirates

Variable	Overall ($N = 62$)	Positive NGS* ($N = 40$)	Negative NGS ($N = 22$)	*P* value
Age, y, mean (SD)	71.5 (12.1)	75.0 (7.0)	65.1 (16.1)	**.002**
Female sex, *n* (%)	29 (46.8%)	17 (40.5%)	12 (54.5%)	.36
Race/ethnicity				.63
Non-Hispanic white	49 (79.0%)	33 (82.5%)	16 (72.7%)	
Non-Hispanic black	7 (11.3%)	4 (10.0%)	3 (13.6%)	
Hispanic/Latino	3 (4.8%)	2 (5.0%)	1 (4.5%)	
Other	3 (4.8%)	1 (2.5%)	2 (9.1%)	
Diabetes, *n* (%)	34 (54.8%)	21 (52.5%)	13 (59.1%)	.62
Tobacco use, *n* (%)	33 (53.2%)	21 (52.5%)	12 (54.5%)	.88
Alcohol use, *n* (%)	28 (45.2%)	18 (45.0%)	10 (45.4%)	.97
Chronic opioids, *n* (%)	9 (14.5%)	3 (7.5%)	6 (27.3%)	.06
Chronic pancreatitis, *n* (%)†	34 (54.8%)	17 (42.5%)	17 (77.3%)	**.01**
Personal cancer history, *n* (%)	19 (30.6%)	11 (27.5%)	8 (36.4%)	.12
Family history of PDAC, *n* (%)	20 (32.3%)	12 (30.0%)	8 (36.4%)	.61
Germline mutation, *n* (%)	2 (3.2%)	2 (5.0%)	0 (0%)	.53
CA 19-9, U/mL, mean (SD)	119.0 (426.6)	32.3 (50.7)	316.14 (754.4)	.07
Lesion characteristics				
Cyst present, *n* (%)	23 (37.1%)	19 (47.5%)	4 (18.2%)	**.02**
Cyst size, cm, mean (SD)	1.58 (1.02)	1.58 (1.12)	1.60 (0.41)	.97
MPD diameter, mm, mean (SD)	8.6 (2.3)	8.5 (2.2)	8.8 (2.6)	.56
MPD aspirate analysis				
CEA, ng/mL, mean (SD)	472.0 (1255.3)	459.2 (1233.7)	496.6 (1346.8)	.93
Amylase, U/L, mean (SD)	95,008.4 (151,292.4)	94,372.7 (171,382.5)	96,491.5 (102,999.1)	.98
Cytology positive, *n* (%)‡	1 (1.6%)	1 (2.5%)	0 (0%)	.99
KRAS/GNAS positive, *n* (%)	39 (62.9%)	39 (97.5%)	0 (0%)	**<.001**
High-risk NGS mutation, *n* (%)	4 (6.5%)	4 (10.0%)	0 (0%)	.29
Follow-up, mo, mean (SD)	33.3 (26.3)	33.3 (24.7)	33.2 (29.7)	.99
Surgery, *n* (%)	20 (32.3%)	17 (42.5%)	3 (13.6%)	**.02**
Advanced neoplasia, *n* (%)#	9 (14.5%)	7 (17.5%)	2 (9.1%)	.47

Bold *P* values are statistically significant.
CA 19-9, Cancer antigen 19-9; *CEA*, carcinoembryonic antigen; *MPD*, main pancreatic duct; *NGS*, next-generation sequencing; *PDAC*, pancreatic ductal adenocarcinoma.
*Defined as any mutation on PancreaSeq assay.
†Identified through either clinical documentation or radiographic evidence.
‡Defined as positive or suspicious for malignancy.
#Defined as either high-grade dysplasia or invasive adenocarcinoma.

pancreatic lesion characteristics did not differ between those who underwent EUS-FNA and ERCP (Table 2). At the time of EUS-FNA or ERCP, 41.9% received indomethacin, 100.0% received intravenous fluids, and 21.0% underwent MPD stenting. Considering just patients who underwent ERCP for MPD sampling ($n = 29$), 89.7% received indomethacin, 100.0% received intravenous fluids, and 44.8% underwent prophylactic MPD stenting.

Among 23 patients with pancreatic cyst present at the time of NGS, the cyst was aspirated in 6 (26.1%) cases. When the cyst was not aspirated, the cyst was either believed to be extrapancreatic, a pseudocyst, too small for sampling, or no window for aspiration was identified. Pancreatoscopy was performed in 2 patients undergoing ERCP, and in each case villous frond-like projections were identified in the MPD.

Volume of aspirated fluid was available in 32 (51.6%) patients. The mean collection volume was 3.4 ± 2.3 mL. There was insufficient collection volume data among patients undergoing ERCP ($n = 2$) to statistically compare with those undergoing EUS-FNA ($n = 30$). A procedural adverse event was present in 5 (8.1%) patients with no significant difference in adverse event rate between those undergoing ERCP and EUS-FNA (6.9% vs 9.1%; $P = .99$). All reported adverse events were pancreatitis and required hospital admission in each case (Table 2).

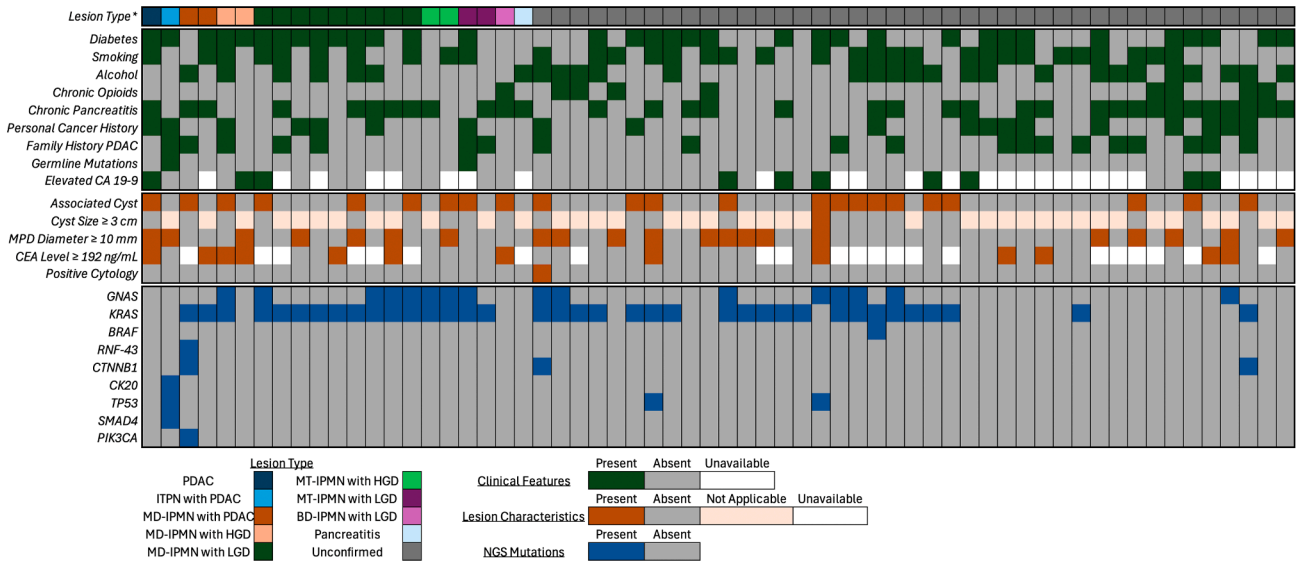

Figure 2. An oncoplot representing clinical characteristics, radiographic findings, laboratory values, and NGS findings among 62 patients undergoing pancreatic duct aspiration for evaluation of main pancreatic duct dilatation of unclear etiology. *Confirmed histologically either on biopsy specimen or surgical resection specimen. *BD-IPMN*, Branch duct-intraductal papillary mucinous neoplasm; *CEA*, carcinoembryonic antigen; *HGD*, high-grade dysplasia; *ITPN*, intraductal tubulopapillary neoplasm; *LGD*, low-grade dysplasia; *MD-IPMN*, main duct-intraductal papillary mucinous neoplasm; *MPD*, main pancreatic duct; *MT-IPMN*, mixed type-intraductal papillary mucinous neoplasm; *NGS*, next-generation sequencing; *PDAC*, pancreatic ductal adenocarcinoma.

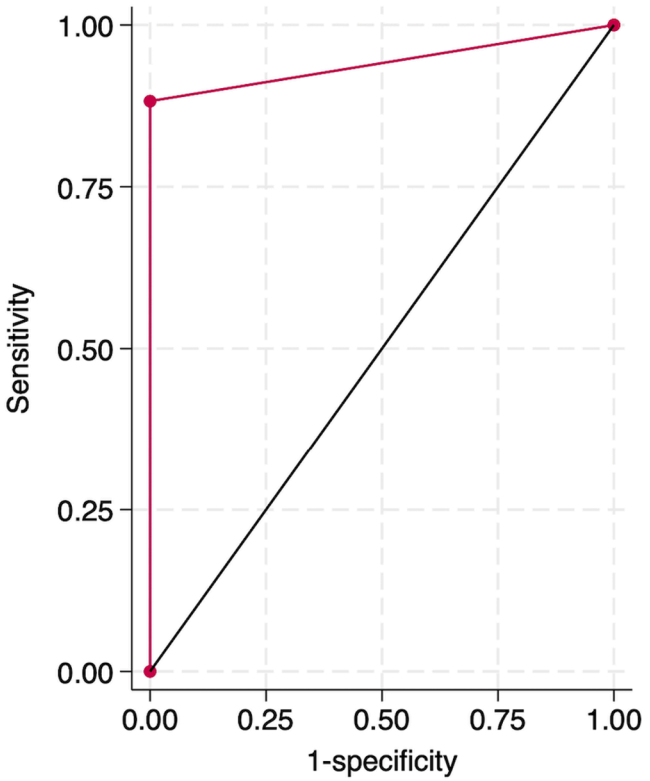

Figure 3. Receiver operating curve for the detection of next-generation sequencing mutations identified from main pancreatic duct aspirates at detecting intraductal papillary mucinous neoplasm with main duct involvement in the setting of ductal dilatation without associated pancreatic mass.

Clinical outcomes and changes to management

A total of 21 patients included in the current study had MPD diameter ≥10 mm at the time of referral, 10 of whom had CP. Before incorporating NGS into institutional practice, surgery would be recommended for these patients, including those with CP, given concern for MD-IPMN. However, in 6 patients with MPD diameter ≥10 mm and CP, MPD dilatation was attributed to CP after negative NGS and surgery was deferred. None of these patients have progressed on follow-up. The average follow-up interval among these 6 patients was 12.9 ± 13.0 months.

A total of 41 patients included in the current study had MPD diameter between 6 mm and 10 mm at the time of referral. Among these 41 patients, 32 had no other worrisome features, and surveillance would have been recommended before consideration of NGS results. In 11 cases in which NGS revealed mutations suggesting the presence of a mucinous neoplasm (*KRAS* and/or *GNAS*), the decision was made to pursue surgery, given concern for IPMN with main duct involvement. An IPMN was confirmed in all cases with involvement of the MPD (MD-IPMN or mixed type-IPMN) in 9 cases. Advanced neoplasia was present at the time of surgical resection in 4 patients.

High-risk mutations were present in 4 patients, 2 of whom underwent surgery. Two patients with *TP53* mutations elected to forgo surgery despite the recommendation to do so. One of these patients declined further surveillance, whereas the other has been followed with serial imaging

TABLE 2. Demographic and clinical variables of study population stratified by whether MPD dilatation was aspirated by ERCP ($n = 29$) or EUS-FNA ($n = 33$)

Variable	Overall ($N = 62$)	ERCP ($n = 29$)	EUS-FNA ($n = 33$)	*P* value
Age, y, mean (SD)	71.5 (12.1)	68.1 (15.2)	74.4 (7.7)	**.04**
Female sex, *n* (%)	29 (46.8%)	13 (44.8%)	16 (48.5%)	.77
Race/ethnicity				.33
Non-Hispanic white	49 (79.0%)	22 (72.4%)	28 (84.8%)	
Non-Hispanic black	7 (11.3%)	4 (13.8%)	3 (9.1%)	
Hispanic/Latino	3 (4.8%)	1 (3.4%)	2 (6.1%)	
Other	3 (4.8%)	3 (10.3%)	0 (0.0%)	
Lesion characteristics				
Cyst present, *n* (%)	23 (37.1%)	13 (44.8%)	10 (30.3%)	.23
Cyst size, mm, mean (SD)	1.58 (1.02)	1.28 (0.66)	1.98 (1.3)	.10
MPD diameter, mm, mean (SD)	8.6 (2.3)	8.1 (2.2)	9.0 (2.4)	.12
Aspirate volume, mL, mean (SD)	3.6 (2.6)	6.5 (4.9)	3.4 (2.3)	–*
Procedural adverse event	5 (8.1%)	2 (6.9%)	3 (9.1%)	.99

Bold *P* values are statistically significant.
FNA, Fine-needle aspiration; *MPD*, main pancreatic duct.
*Unable to perform statistical comparison because of the high degree of missing data among patients undergoing ERCP.

without progression after 11 months of follow-up. A patient with mutations in *TP53* and *SMAD4* underwent a pancreatoduodenectomy and was diagnosed with intraductal tubulopapillary neoplasm with associated PDAC. Finally, a patient with a mutation in *PIK3CA* underwent robotic pancreatoduodenectomy and was diagnosed with MD-IPMN with focal adenocarcinoma. Notably, this patient had no indication for surgery (ie, MPD <10 mm and no other worrisome features) before the identification of the high-risk mutation. Considering 6 patients for whom surgical management was deferred after reassuring NGS, and 11 patients for whom surgical management was pursued only after considering NGS findings, management was changed or influenced in 17 of 62 (27.4%) patients (Fig. 4).

DISCUSSION

Management of MPD dilatation is a diagnostic and therapeutic challenge, particularly in the presence of CP. Herein, we describe how NGS performed on MPD aspirates was used to guide management and change clinical practice at 2 large academic medical centers. Indeed, one-quarter of patients undergoing NGS had some change to clinical management. In particular, differentiating between neoplastic (eg, main duct-IPMN) and non-neoplastic (eg, CP) etiologies of MPD dilatation directed the surgical management of pancreatic lesions for these patients. Several patients with MPD dilatation and underlying CP avoided surgery after negative NGS suggested the absence of a concomitant neoplastic process, whereas others with no pre-existing surgical indication and NGS mutations underwent surgery on the basis of NGS. In refining the population of patients undergoing surgery for MPD dilatation of unclear etiology, NGS has the potential to reduce unnecessary surgery, improve cost-effectiveness, and expedite diagnosis of high-risk pathology. More than 50% of patients seen at pancreas cancer prevention clinics in the present study were referred for incidentally detected lesions, underscoring the importance of effective and accurate management algorithms as the number of patients with identified lesions increases. Although the clinical utility of NGS for managing MPD dilatation has previously been described,[11] our findings add to this literature by providing a large multicenter analysis with follow-up approaching 3 years and by evaluating the efficacy and safety of different modalities for MPD aspiration.

Several studies have evaluated the use of molecular genetic testing from pancreatic fluid samples, generally endoscopically collected from the duodenum after secretin administration, for identifying patients at risk for PDAC. Mutations in *KRAS* isolated from pancreatic fluid have been described in high proportions of patients with PDAC.[13] High-risk NGS mutations (eg, *SMAD4*, *TP53*)[14,15] and methylated DNA[16] isolated from pancreatic fluid specimens have also been suggested to accurately identify patients with pancreatic malignancy. Indeed, a meta-analysis evaluating the diagnostic performance of pancreatic fluid DNA alterations at predicting the development of PDAC suggests near 100% specificity, but only modest sensitivity.[17] In contrast to these methods, we describe a method for detecting genomic alterations from MPD fluid collected from direct aspiration either through MPD cannulation during ERCP or EUS-FNA. Simpson et al[18] previously evaluated the use of direct MPD fluid collection for risk stratifying pancreatic lesions and found similar yield of genetic mutations when comparing ERCP and EUS-FNA with a greater adverse event rate among those undergoing ERCP (28.1% vs 9.0%). Although we describe similar methods for MPD

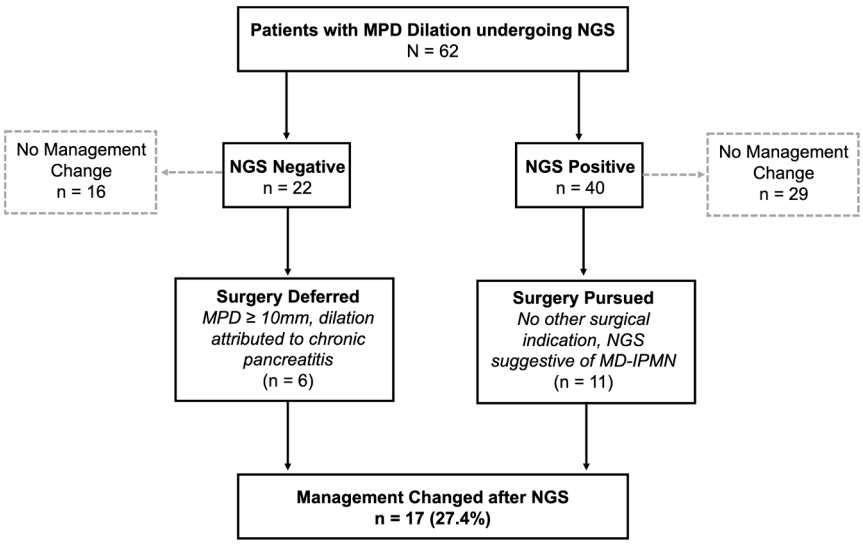

Figure 4. Summary of changes to clinical management after undergoing NGS of pancreatic duct aspirates for evaluation of MPD dilatation. *MD-IPMN*, Main duct-intraductal papillary mucinous neoplasm; *MPD*, main pancreatic duct; *NGS*, next-generation sequencing.

fluid collection to this study, we highlight our institutions use of a combined DNA/RNA-based NGS assay (PancreaSeq) to detect mutations of interest in these samples and subsequently guide management.

Although the absence of NGS mutations contributed to the decision to defer surgery in 6 patients with MPD diameter ≥10 mm, NGS results were not the only reassuring factor. Indeed, in each of these cases MPD diameter was the only concerning clinical feature and ductal dilatation was attributed to an established diagnosis of CP. None of these patients progressed during follow-up. Conversely, there were 3 patients who underwent surgical management of MPD dilatation despite negative NGS. In these cases, the decision to pursue surgery was supported by multiple worrisome features or high-risk stigmata (eg, elevated cancer antigen 19-9, acute pancreatitis). In 2 of these cases surgical pathology confirmed a mucinous cystic neoplasm, and in 1 case high-grade dysplasia was present. Despite these false negatives, MPD aspirate NGS was overall accurate at diagnosing IPMN as the etiology of MPD dilatation with sensitivity and specificity of 89% and 100%, respectively. Our reported test characteristics are similar to previously reported results for NGS performed on pancreatic cyst aspirates.[10] Nevertheless, the potential for false negatives observed herein highlights the role of NGS as an ancillary diagnostic test, the results of which should be weighed against other clinical and morphologic findings when determining whether nonsurgical management is appropriate.

More than one-third of patients with NGS mutations identified in our cohort underwent surgical management, and in more than one-half of these cases (11 of 17) no other definitive surgical indication was present before NGS. In all 11 cases, this decision was the result of positive mutations in *KRAS* and/or *GNAS* suggesting the presence of a mucinous cystic neoplasm. Surgical pathology confirmed main duct involvement of a mucinous cystic neoplasm in 9 of these patients, and advanced neoplasia was present in 4 cases. Considering a concomitant neoplastic process even when an alternative etiology of MPD dilatation is present is necessary, particularly in cases of CP, given the established association with the development of PDAC.[19] In our cohort, CP was present in more than one-third of patients with NGS mutations, suggesting both neoplastic and nonneoplastic contributions to MPD dilatation. In 5 of these cases, surgical management was pursued after NGS despite no previous surgical indication, and high-grade dysplasia was present in 1 case. It is also important to note that just more than one-half (21 of 40) of patients with NGS mutations did not undergo surgery and opted for surveillance. These cases highlight how other factors such as surgical candidacy, clinical features, radiographic findings, and patient preference also were considered in clinical decision-making.

High-risk NGS mutations were isolated from MPD aspirates from 4 patients, and in 3 such cases an existing surgical indication was present (MPD diameter ≥10 mm). Two patients declined surgery, and 2 proceeded with surgical resection and were diagnosed with invasive adenocarcinoma. Although high-risk genomic alterations have been suggested to predict the presence of or progression to advanced neoplasia in samples from pancreatic fluid[13-15,17] and cyst aspirates,[10] our sample size is insufficient to comment on diagnostic performance of high-risk NGS mutations isolated from direct MPD aspirates for predicting advanced neoplasia. Nevertheless, the clinical practice at both institutions in this study is to recommend surgical management for any patient with a high-risk NGS mutation identified.

The adverse event rate of MPD aspiration in our cohort was 8.1%, with each of these patients developing postprocedural pancreatitis requiring hospital admission. Among patients undergoing EUS-FNA, the adverse event rate was 9.1%, which is greater than reported rates of pancreatitis among patients undergoing EUS-FNA for evaluation of pancreatic cystic lesions.[20] This discrepancy may result from a combination of a smaller sample size in our study, selection bias, and increased risk resulting from direct aspiration of the MPD during EUS-FNA. The adverse event rate among those undergoing ERCP was 6.9% and similar to previously reported values.[21] We detected no statistical difference in adverse event rates between EUS-FNA and ERCP. This finding is in contrast to Simpson et al,[18] who reported a greater rate of adverse events for ERCP compared with EUS-FNA (28.1% vs 9.0%) when accessing the MPD directly for genomic analysis; however, a broader definition of adverse events was used in this study compared with the present analysis. The retrospective nature of our analysis also limited our ability to identify mild adverse events that did not result in an inpatient evaluation and may have contributed to this discrepancy. Finally, although we are unable to compare to the study conducted by Simpson et al,[18] we reported rates of preventative interventions for post-ERCP pancreatitis (89.7% indomethacin, 100.0% intravenous fluids, and 44.8% PD stenting) similar to a national survey of endoscopists.[22] The minimum volume to conduct the PancreaSeq assay is 200 μL. Although we are unable to compare the yield (volume of aspirated fluid) between techniques because of missing data, the mean aspirate volume was 3.6 mL among 32 patients with available data. NGS could therefore be conducted with sufficient volume to send for additional biochemical and cytologic studies as indicated in the majority of cases.

In addition to considering potential risks of MPD sampling, surgical adverse events must also be considered when assessing the utility of NGS. A surgical adverse events occurred in about one-third of patients undergoing surgery in the present study, including 5 patients who were not found to have advanced neoplasia at the time of surgery. These adverse events should be balanced against those that may have been avoided among patients for whom surgery was deferred after undergoing NGS (6 in the present study). Indeed, our objective for incorporating MPD aspirate NGS into management algorithms is to refine the population of patients undergoing surgery to those most likely to benefit. Nevertheless, the risks of both endoscopy and surgery must be carefully considered on a case-by-case basis.

Our study is limited by the retrospective design. Moreover, our outcomes of interest (eg, surgery, advanced neoplasia, adverse events) were rare among this cohort, limiting our ability to draw conclusions regarding the natural history of pancreatic lesions with varying mutation profiles. In particular, we identified just 4 patients with high-risk NGS mutations, and surgical management was declined in 2 of these cases. Therefore, although previous studies have documented the diagnostic performance of NGS at risk stratifying pancreatic cystic lesions,[10] we are limited in our ability to comment on how this applies to the management of MPD dilatation of unclear etiology. Although our mean follow-up approached 3 years, this may be insufficient to capture the progression of mucinous neoplasms to advanced neoplasia in all cases. This is of particular relevance among those undergoing active surveillance. The small sample size and relatively short follow-up interval among patients not undergoing surgery limits our ability to provide a rigorous characterization of NGS performance in identifying neoplastic etiologies of MPD dilatation. Finally, comparison of adverse events between techniques for MPD aspiration may have been influenced by variation in institutional practices. Indeed, 87.9% of patients undergoing EUS-FNA were from one institution and all patients undergoing ERCP were from the other institution.

In a multicenter cohort of patients evaluated for MPD dilatation of unclear etiology, we demonstrated that NGS from MPD aspirates resulted in clinically significant changes to management in more than one-quarter of patients. Namely, NGS assisted in differentiating between neoplastic and nonneoplastic etiologies of MPD dilatation through the identification of mutations associated with mucinous neoplasms with resultant changes in surgical management. Moreover, the identification of NGS mutations in patients with CP highlights the possibility of concomitant neoplastic and nonneoplastic contributions to MPD dilatation and suggests that even patients with known CP may benefit from duct sampling and NGS when MPD dilatation is present. We also reported a low rate of procedural adverse events with no significant difference between techniques used for accessing the MPD for aspiration. In conclusion, our study underscores the potential for NGS to provide valuable ancillary data for select patients with pancreatic lesions and to inform management decisions accordingly. Future studies should assess long-term outcomes for patients with negative NGS undergoing surveillance and explore the potential for NGS to improve cost-effectiveness of management for patients with MPD dilatation of unclear etiology.

DISCLOSURE

The following authors disclosed financial relationships: A. D. Singhi: Honorarium from Foundation Medicine, Inc. G. Khatri: Consultant for Guerbet LLC; stockholder with Goa Therapeutics. All other authors disclosed no financial relationships.

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Abbreviations: CP, chronic pancreatitis; FNA, fine-needle aspiration; IPMN, intraductal papillary mucinous neoplasm; MPD, main pancreatic duct; NGS, next-generation sequencing; PDAC, pancreatic ductal adenocarcinoma; UPMC, University of Pittsburgh Medical Center.

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