TECHNISCHE UNIVERSITÄT MÜNCHEN

Fakultät für Medizin
Klinik und Poliklinik für Chirurgie des Klinikums rechts der Isar
Direktor: Univ.-Prof. Dr. Helmut Friess

Prädiktoren und Risikofaktoren für Malignität bei intraduktalen papillären muzinösen Neoplasien (IPMN)

Predictors and risk factors for malignancy in intraductal papillary mucinous neoplasms (IPMN).

Habilitationsschrift

vorgelegt von

Dr. med. Ilaria Pergolini

2024

CONTENTS

1. INTRODUCTION

As imaging constantly improves, incidental pancreatic cysts are increasingly detected and managing patients with intraductal papillary mucinous neoplasms (IPMNs) has become an everyday issue in our clinical practice. To understand the extent of the problem, it is important to know that the prevalence of incidentally discovered pancreatic cysts can even reach 19.6%, depending on the imaging modality (Zhang et al. 2002, Lee et al. 2010, Laffan et al 2008, De Jong et al. 2010, Zerboni et al. 2019), while the prevalence of pancreatic cysts consistent with IPMNs was estimated at 6.6% in adult population (Laurent et al. 2017).

As the natural history of IPMNs is still unknown, the management remains very challenging and continues to evolve. On one side, as pancreatic cancer precursors following a classic "adenoma-carcinoma sequence", IPMNs offer a great opportunity for early diagnosis and prevention of pancreatic cancer (PDAC). Here, performing a surgical resection at the right time point ensures an excellent prognosis for these patients (Woo et al. 2008, Koh al. 2014). On the other side, we are still not able to accurately estimate the exact risk and timing of progression of IPMNs to PDAC and which patients may benefit more from surgery. Since the first description in 1982 (Ohashi et al. 1982), several guidelines have tried to resolve this issue supporting clinicians in the management of IPMNs (Tanaka et al. 2017, Vege et al. 2015, European Study Group on Cystic Tumours of the Pancreas 2018, Ohtsuka et al. 2024). The guidelines provide relative and absolute indications for surgery, as well as recommendations for surveillance, however with consistent differences and controversies between them. In addition to that, many other important factors must be considered in the decision-making process. Since pancreatic surgery is still burdened by a high risk of morbidity and mortality, in the choice of the management physicians have to constantly balance between the risk of progression and the risk of complications associated with surgery (Lee et al. 2020). Here, age, comorbidities, risk of IPMN recurrence, risk of concomitant PDAC, and not least, quality of life and costs of surveillance play a relevant role (Marinelli et al. 2020).

In this complex setting, identifying clinical, morphological, and molecular predictors of malignant progression is crucial to pursuing the ultimate goal of early diagnosis and prevention of pancreatic cancer. Moreover, when PDAC occurs, the analysis of prognostic factors also represents an important step to improve the preoperative patients´ stratification and offer them the best treatment.

In the present habilitation, risk factors and predictors of malignant progression in IPMNs were investigated. To improve patients´ stratification and management, further prognostic predictors and surgical aspects were also analyzed.

2. RELATED PROJECTS AND THEIR RELEVANCE IN THE FIELD

2.1. Analysis of clinical, morphological, and molecular predictors of malignancy in IPMNs

2.1.1 Clinical predictors

It is well-known that IPMNs occur more frequently in the sixth or seventh decades, and in males (Crippa et al. 2010, Tanaka et al. 2017). As the incidence of IPMNs increases with time, age represents an important risk factor for the development of this disease (Marchegiani et al. 2018). Interestingly, in young patients (< 50 years) IPMNs are not only less common, but they present also a different histological and prognostic profile (**Morales et al. 2017**). In a cohort of 1693 resected patients, we found that the 90 patients (5%) younger than 50 years of age were harboring more frequently an IPMN of oncocytic and intestinal epithelial subtype located in the pancreatic head and uncinate process compared to older patients. Moreover, young patients were less likely to have an invasive carcinoma arising from IPMN, but when they had, this was frequently of the colloid type and showed a better prognosis. By contrast, no age-dependent differences in terms of symptoms, radiological worrisome features, or high-risk stigmata of malignancy were found. (**Morales et al. 2017**).

Other clinical risk factors, such as smoking, obesity, chronic pancreatitis, and a family history of PDAC demonstrated to predispose to the development of IPMN and its progression (Capurso et al. 2013, Capurso et al. 2020, Carr et al. 2017). It is widely known that IPMNs are frequently asymptomatic. In patients under surveillance, up to 80% of patients show no symptoms (**Pergolini et al. 2017**, **Petrone et al. 2018**). However, when symptoms are present, they may identify a more advanced disease. Accordingly, some of them, such as jaundice and acute pancreatitis, were included in the guidelines as absolute or relative indications for surgery, respectively (Tanaka et al. 2017). In this context, scant attention has been paid in the past to diabetes mellitus (DM) and other metabolic disorders (**Pergolini et al. 2021**). In the guidelines of the American Gastroenterological Association and the revised Fukuoka guidelines (Vege et al. 2015, Tanaka et al. 2017), DM and other metabolic factors were not mentioned. For the first time in 2018, the European guidelines for the management of IPMN

introduced new-onset diabetes as a relative indication for surgery, however with a low level of evidence (European Study Group on Cystic Tumours of the Pancreas et al. 2018). For this reason, in 2019 we decided to conduct a systematic review and meta-analysis to evaluate the role of DM in IPMNs (**Pergolini et al. 2021**). As main result, we found a big lack of knowledge on this topic in the literature, and overall, only two papers specifically focused on DM (**Morales et al. 2017**, Leal et al. 2015). In most papers, there are no details on the definition, onset, and assessment of DM or its association with other clinical and pathological features, such as type of IPMN or grade of dysplasia, and DM was just reported as a comorbidity or symptom. In our systematic review, the prevalence of DM among the included studies was globally 25% but with a wide range (15%-43%). The prevalence of DM was higher than in the general population of the United States and increased in presence of malignancy. The meta-analysis showed that patients with diabetes had an increased risk of harboring a more aggressive IPMN in comparison with non-diabetic patients. Patients with DM had more frequently MD-IPMN and invasive cancer, but most importantly, DM was significantly associated with the presence of high-grade dysplasia (HGD). This means that patients with IPMN and a history of DM should probably deserve closer observation to earlier detect HGD, and in presence of other worrisome features, more promptly receive surgical treatment. Given this lack of knowledge, we decided to analyze DM and its association with other clinical, morphological, and histological features in patients who underwent resection for a histologically confirmed IPMN at our institution (**Pergolini et al. 2021**). In our series, a higher proportion of patients with DM were male and older than non-diabetic ones. DM was significantly associated with main-duct involvement (odds ratio [OR], 2.827; 95% CI, 1.059–7.546; P = 0.038) and the presence of HGD or invasive carcinoma (OR, 2.692; 95% CI, 1.283–5.651; P = 0.009). This risk was even higher in patients with new-onset or worsening DM (OR, 4.615; 95% CI, 1.423–14.698; P = 0.011). In PDAC, DM has been investigated more extensively and seems to play a dual role, as risk factor but also as manifestation or consequence of it (Pannala et al. 2009, Magruder et al. 2011). Moreover, DM in patients with PDAC demonstrated to be "paradoxically" associated with weight loss and both seem to occur a lot of time before the cancer diagnosis (Hart et al. 2011, Sah et al. 2013, Pannala et al. 2009). Interestingly, also in our cohort of resected IPMNs, patients with weight loss at the time of diagnosis had more frequently DM compared to those with constant weight [weight loss: 18/31 (58%) vs constant weight: 31/97 (32%), P = 0.009]. In addition, this

association between DM and weight loss was still present when patients with invasive carcinoma were excluded and the analysis was limited only to IPMN with HGD and low-grade dysplasia (LGD). Here, 50% (8/16) of patients with weight loss at diagnosis had DM vs 21% (12/66) of patients without weight loss (P = 0.024). By contrast, DM and weight loss were no longer associated when the analysis was restricted only to patients with LGD [5/12 (42%) vs 9/44 (21%), P = 0.133]. In other words, in malignant IPMNs as in PDAC, DM is paradoxically associated with weight loss. But, most interestingly, this association occurs already in patients with HGD and is no longer present with benign IPMNs (LGD) **(Pergolini et al. 2021)**. Recently, new onset or recent exacerbation of DM, although the definitions of “new onset” and “acute exacerbation” of DM have not been determined yet, were introduced in the revised version of the international guidelines for the management of IPMNs as new worrisome feature (Ohtsuka et al. 2024).

2.1.2 Morphological predictors

In addition to clinical parameters, several morphological features are crucial in predicting the presence of malignancy and were included in the guidelines for the management of IPMNs. Here, the most important predictors of malignancy are main-duct dilatation and solid-enhancing nodules, in both resected IPMNs and those under surveillance (Tanaka et al. 2017, Marchegiani et al. 2018, Zhao et al. 2022, Del Chiaro et al. 2020, Sugimoto et al. 2017, Wu et al. 2021, **Petrone et al. 2018**). In a surgical series of 901 consecutive patients, del Chiaro et al. found that main duct (MPD) dilatation is the best predictor of HGD or invasion in resected IPMNs, identifying a cut-off of 5 to 7 mm to discriminate between malignant and benign lesions (Del Chiaro et al. 2020). This result was also confirmed in other series of resected IPMNs, as well as in systematic reviews and meta-analyses (Sugimoto et al. 2017, Wu et al. 2021). In addition, mural nodules and MPD dilatation >5 mm showed a significant association with malignancy also in branch-duct IPMNs (BD-IPMNs) under surveillance (**Petrone et al. 2018**). In our series of 167 patients, both features correlated significantly with malignancy in the univariate and multivariate analysis (P = 0.004 and P = 0.001, respectively) and MPD dilatation proved to be the strongest independent risk factor of progression (OR = 24.5) (**Petrone et al. 2018**). Accordingly, a recent systematic review and meta-analysis by Zhao et al. confirmed that enhanced solid component or mural

nodule and main duct dilation are the most important predictors of HGD/invasive cancer in BD-IPMNs under follow-up (Zhao et al. 2022).

Despite these findings, in IPMNs the risk of overlooking a malignant lesion or performing an unnecessary or too extensive resection unfortunately remains high. Considering the still high morbidity and mortality rate in pancreatic surgery, the risk of overtreatment represents certainly an important issue to investigate. In a series of 93 resected main duct-/mixed-type IPMNs (MD-IPMNs), where in principle a surgical resection is recommended, we found an overtreatment rate of 19% (18/93) (**Crippa et al. 2016**). In our study, overtreatment was registered in 10 patients who received a too extensive resection (malignancy was localized only in a part of the surgical specimen) and in 8 patients where the operation was even unnecessary (in the specimen other cystic lesions rather than IPMN, chronic pancreatitis or LGD-IPMNs were found). Twelve of these 18 patients who were overtreated had no symptoms or mild symptoms such as vague abdominal pain. Total pancreatectomy was the most common procedure (67%, 12/18) associated with overtreatment. Five of these 12 patients had severe postoperative complications, requiring a reoperation in 2 patients and causing death in 2 cases. In 21 patients who globally underwent upfront total pancreatectomy, a diffuse main duct involvement by IPMN was recognized only in the surgical specimen of 14 patients (67%). In the other 7 cases (33%), IPMN was located only in the head of the pancreas, while chronic pancreatitis was found in the rest of the gland. Of note, the median size of the main duct in the area with IPMN was 12 mm, while was 7 mm in the area where only chronic pancreatitis was found ($P < 0.05$) **(Crippa et al. 2016)**. Collectively, these results suggested that the risk of overtreatment is not negligible, even in MD-IPMNs, and each predictor of malignancy should be considered not singularly but together with other worrisome features. In this light, Marchegiani et al. (Marchegiani et al. 2018) showed that patients with MPD dilatation associated with other worrisome features had an increased 5-year cumulative incidence of malignancy compared to patients without a dilated duct (11% versus 1.2%; $P < 0.001$) or with a dilated main duct alone (4% versus 1.2%; $P = 0.448$). Therefore, especially in asymptomatic patients with mild MPD dilatation (5-7 mm) and without other worrisome features or high-risk stigmata, surgery should be carefully considered and partial pancreatic resection should be preferred.

In BD-IPMNs, as the cysts are mostly small and indolent, the choice of the optimal treatment and surveillance policy is even more difficult. Since our knowledge is mostly based on retrospective surgical series and observational studies with short median surveillance periods, the natural history of BD-IPMNs and the risk of progression over time are still unclear (Tanaka et al. 2017). The AGA guidelines in 2015 recommended stopping surveillance after 5 years in case of asymptomatic cystic lesions, while other guidelines have not provided any specific end-point to surveillance (Vege et al. 2015, Tanaka et al. 2017, European Study Group on Cystic Tumours of the Pancreas et al. 2018). This controversy generated a big debate, also considering the increasing incidence of incidentally discovered pancreatic cysts and the elevated cost of follow-up policies. In this setting, we decided to evaluate the risk of development of pancreatic cancer after 5 years of follow-up in a large cohort of patients with BD-IPMNs under surveillance, and, at the same time, to identify a subset of BD-IPMNs at low risk of progression where follow-up may safely forego after 5 years (**Pergolini et al. 2017**). In this study, 577 patients with a median follow-up time of 82 months (range, 6-329 months) were included; of these, 363 patients (63%) were followed for more than 5 years and 121 (21%) for more than 10 years. In this cohort, which to our knowledge remains one of the largest in the current literature, malignancy (HGD and invasive cancer) occurred after 5 years of follow-up in 5.5% of patients (20/363), and 35% of these were asymptomatic. In 5 patients (5/121, 4.1%), malignant progression occurred even after 10 years of surveillance. Among the 282 patients (282/363) without worrisome features or high-risk stigmata at the 5-year mark, in which for the AGA guidelines a discontinuation of surveillance can be considered, 12 (4.3%) developed HGD or invasive cancer afterward. In addition, the age-standardized incidence rate of pancreatic malignancy in these 282 patients was 18.8 times (95% confidence interval, 9.7-32.8; $P < 0.001$) higher than expected in the comparable US population. Overall, these results demonstrated that the risk of progression in BD-IPMNs under surveillance persists after 5 years and even after 10 years of follow-up. Even among the cystic lesions without signs of malignancy within the first 5 years of follow-up, the risk of pancreatic cancer cannot be excluded and is 18.8 times higher than in the general population. According to these results, we strongly suggested continuing surveillance after 5 years from the initial diagnosis, however, with an exception. In fact, we could identify a possible subgroup of BD-IPMNs in which the surveillance might be discontinued. In our cohort, among the 108 patients followed for more than 5 years and

with a cyst size stably ≤1.5 cm, only 1 patient (0.9%) developed malignancy 65 months after the initial diagnosis of BD-IPMN. By contrast, 19 patients (7.5%) with cysts >1.5 cm progressed to HGD/invasive carcinoma (P = 0.01), and in 5 cases this occurred even after 10 years of follow-up. Moreover, the 1.5 cm cutoff showed a negative predictive value for malignancy of 99% and a sensitivity of 95% (**Pergolini et al. 2017**). The validity and safety of this cut-off were confirmed by a subsequent study (Ciprani et al. 2020), in which patients under surveillance with cysts measuring <1.5 cm and without worrisome features at diagnosis had a rate malignancy of 1.7%, which is similar to the risk of pancreatic cancer in the general population (1.6%). Moreover, the analysis showed that the risk of malignancy in small pancreatic cysts (< 1.5 cm) is higher in the first 3 years and becomes considerably lower over time (Ciprani et al. 2020). Overall, if confirmed in other series, the discontinuation of follow-up in this specific subset of patients with very small and stable cysts, which represent the majority of incidentally discovered pancreatic cystic lesions, should be considered.

In the last years, clinical and morphological predictors were combined to develop prognostic tools, such as nomogram models, to assess the probability of high-risk IPMNs and to reduce the risk of overtreatment (**Attiyeh et al. 2018**, Lee et al. 2020, Wu et al. 2020). An example is the independently validated nomogram model presented by Attiyeh et al. (**Attiyeh et al. 2018**). Here, cyst size > 3.0 cm, solid component/mural nodule, pain symptoms, dilated duct > 10 mm, and weight loss were significantly associated with high-risk IPMNs and were included. This nomogram model demonstrated the ability to discriminate between low- and high-risk disease in 81% of cases.

2.1.3 Molecular predictors

To date, clinical and morphological predictors are still the most valuable factors used in clinical routine to predict the risk of malignancy in IPMNs and choose the management. For the near future, the identification of molecular predictors represents a great hope for achieving early prediction of pancreatic cancer. Accordingly, several research groups are investing their efforts in liquid biopsy and molecular analysis of pancreatic cyst fluid.

An important study that analyzed the circulating tumor-derived extracellular vesicles (EVs) in the plasma of patients resected for benign and malignant pancreatic disease was included in this habilitation. Here, a signature of five markers able to detect PDAC with an accuracy of 84% [95% confidence interval (CI), 69 to 93%] was indentified (**Yang et al. 2017**).

In another study, a novel murine monoclonal antibody called mAb Das-1 was developed and tested in intraoperatively aspirated cyst fluid samples by ELISA assay. The analysis of mAb Das-1 was able to identify high-risk pancreatic cystic lesions (cysts with invasive carcinomas, HGD, or intestinal-type IPMNs with intermediate-grade dysplasia) with 88% sensitivity and 98% specificity (**Das et al. 2019**).

Another multi-institutional study analyzed the cystic fluid of 149 IPMNs collected intraoperatively by a multianalyte bead array analysis (Luminex) for 4 protein markers. Here, 2 models based on the combinations of these markers were validated with high objective predictive ability for the identification of high-risk IPMNs (HGD or invasive cancer) (**Al Efishat et al. 2018**).

2.2. Prognostic predictors in malignant IPMNs

As described above, clinical, morphological, and molecular predictors in patients with IPMNs are critical in achieving early diagnosis and prevention of pancreatic cancer. When pancreatic cancer occurs, the proper prognostic stratification of patients is also very important to know which patient may benefit from surgery.

Despite the progress made thanks to neoadjuvant therapy, only a minority of patients with PDAC are resectable, and unfortunately, also in these patients the survival outcomes are negatively influenced by early recurrence due to unrecognized or rapidly progressive metastatic disease (Barugola et al. 2009). Overall, the diagnosis of occult metastases and the appropriate assessment of resectability in patients with PDAC is still troublesome (Wong et al. 2008, Callery et al. 2009, Farma et al. 2008, Kaneko et al. 2010). In this setting, identifying prognostic factors predictive of early recurrence and poor outcomes is important to offer the best treatment option, avoid useless resections, and consider neoadjuvant chemotherapy instead of upfront surgery.

2.2.1 Preoperative predictors

In this context, a useful tool could be (18)fluoro-deoxyglucose positron emission tomography/computed tomography (18FDG-PET/CT), that in different malignancies, such as prostate and lung cancers, is used for the diagnosis and staging (Lordick et al. 2010, Fischer et al. 2009, Ruers et al. 2002). As its role in pancreatic cancer is unclear, we decided to analyze the use of 18FDG-PET/CT as a prognostic tool to predict recurrence and survival in patients undergoing surgery for resectable pancreatic cancer and improve their preoperative stratification (**Pergolini et al. 2018**). In this study, 46 patients who preoperatively received 18FDG-PET/CT were included in the analysis. Interestingly, patients who recurred within 12 months showed a significantly higher preoperative median SUVmax (maximum standard uptake value) (8.1 vs 6.1, P = 0.039). Receiver operating characteristics (ROC) curves for disease-free survival (DFS) and disease-specific survival (DSS) identified SUVmax of 6.0 as the optimal cut-off. Patients with SUVmax ≥6.0 had a significantly worse median disease free survival (DFS) and disease specific survival (DSS) compared with those with SUVmax <6.0 (DFS: 13 vs 25 months, P = 0.003; DSS: 23 vs 44 months, P < 0.001). In a multivariate analysis, SUVmax ≥ 6.0 demonstrated to be an independent predictor of poor DFS (HR 2.288, P = 0.024) and DSS (HR 4.875, P < 0.001). Importantly, the prognostic potential of SUVmax was even higher when combined with elevated CA19.9 (≥ 200 U/ml). Patients with SUVmax ≥6.0 and CA19.9 ≥200 U/ml had significantly shorter DFS (8 vs 20 months, P < 0.001) and DSS (14 vs 33 months, P < 0.001) than those without concordantly increased values and, maybe, they can benefit more from neoadjuvant chemotherapy than upfront resection. According to the results of this analysis, but conscious of the high cost of this imaging procedure, we suggested to consider the use of 18FDG-PET/CT in selected patients in which the assessment of resectability is particularly difficult and, preferably in combination with other stigmata of a more aggressive tumor biology, like high levels of CA19.9.

2.2.2 Histological predictors

In the same light and with the same purpose, we evaluated the expression of Ki-67 and its prognostic value in a cohort of patients with PDAC who underwent surgical resection (**Pergolini et al. 2019**). The expression of Ki-67 is a well-known tumor proliferation marker that correlates with progression, risk of metastasis, and prognosis

of several tumors, such as prostate, lung, and breast cancers (Jalava et al. 2006, Scholzen et al. 2000, de Azambuja et al. 2007, Berlin et al. 2017, Wei at al. 2018). In pancreatology, Ki-67 is routinely used in the staging of pancreatic neuroendocrine neoplasms (PanNENs) and is recognized as an independent predictor of survival (Falconi et al. 2016, Klöppel et al. 2018, Lloyd et al. 2017). Some colleagues also reported the valuable use of Ki-67 index preoperatively in PanNEN samples obtained by endoscopic ultrasonography–fine-needle aspiration (EUS–FNA) to predict tumor aggressiveness (Hasegawa et al. 2014, Farrell et al. 2014, Weynand et al. 2014). In PDAC, the staining for Ki-67 in surgical specimens is not routinely performed and its prognostic value of Ki-67 is unclear. In our study, 170 patients, in which the immunohistochemical analysis of Ki-67 in the surgical specimen was additionally performed, were included in the analysis. Here, Ki-67 index demonstrated to be an independent predictor of poor DFS [hazard ratio (HR) 0.52, 95% CI 0.29 to 0.91; P = 0.022] and DSS (HR 0.53, 0.31 to 0.91; P = 0.022), as well as tumor grading, nodal and resection margin status. Interestingly, Ki-67 index was strongly associated with tumor grade (P < 0.001), and patients with a low differentiated tumor (G3) and a Ki-67 index above 50% showed worse survival outcomes compared with the rest of the cohort (P < 0.001 for both DFS and DSS). Overall, these results showed that the Ki-67 index is the expression of a more biologically unfavorable disease, and accordingly, might help to discriminate which patients should receive more aggressive adjuvant treatment. In the future, if the feasibility of preoperative assessment of the Ki-67 index by EUS–FNA will be proved in PDAC, as in PanNENs, this may also help to identify patients who may benefit more from neoadjuvant chemotherapy than upfront surgery.
The prognostic value of other histological parameters, such as perineural invasion and lymph node involvement, was also studied. In a multicenter retrospective study that included 778 patients undergoing PDAC resection, the impact of perineural invasion on tumor recurrence, especially in "early stage disease" (size ≤ 20 mm, R0, N0), was analyzed (**Crippa et al. 2022**). In the entire cohort, patients with perineural invasion had worse DFS at univariate analysis (median DFS: 20 vs 15 months, P < 0.01), but not in the multivariate analysis (P = 0.07). By contrast, the presence of perineural invasion was an independent predictor of overall survival (27 vs 50 months, P = 0.01), together with G3 tumors, pN1 status, CA19.9, and pain, but not of DFS. Most importantly, the presence of perineural invasion was the only independent predictor of DFS in R0/N0 tumors (HR: 2.2) and in PDAC smaller than 20 mm (HR: 1.8). This

finding supports the hypothesis that invasion of nerves by cancer cells is relevant and has a driving role in pancreatic cancer progression already at an early stage. Therefore, perineural invasion, especially in PDACs at early stages, might be an important discriminator to distinguish patients at higher risk of recurrence and deserving more aggressive adjuvant treatments.

In another study, we investigated the different timing and patterns of recurrence of PDAC in relation to the lymph nodes involvement (**Honselmann et al. 2020**). In a cohort of 546 patients with PDAC who underwent surgery upfront or after neoadjuvant therapy, the presence of positive lymph nodes was found to be predictive of the timing of recurrence, but not of the location of recurrence. In fact, patients with positive lymph nodes developed more frequently tumor recurrence (upfront surgery: pN0 55% vs. pN1 77%, $P < 0.001$ and pN0 64% vs. pN1 78%, $P = 0.040$ in the neoadjuvant group) and even earlier (upfront surgery: median 16 mo pN0 vs. 10 mo pN1 $P < 0.001$; pN0 21 mo vs. 11 mo pN1, $P < 0.001$ in the neoadjuvant group). By contrast, no differences in the site of first recurrence (local vs. distant recurrence) were found depending on the nodal status. Moreover, regardless of nodal involvement, local recurrence was independently associated with R1 resection, suggesting a possible benefit of local tumor therapy in addition to systemic therapy in these patients. By contrast, both pN0 and pN1 patients are more likely to develop distant metastases, emphasizing the need for multi-modality systemic therapy in PDACs.

2.2.3. Translational models

In recent years, the treatment possibilities of patients with PDAC, before and after surgery, have increased significantly. Patients with borderline/locally advanced pancreatic cancer who underwent neoadjuvant chemotherapy with FOLFIRINOX showed after surgery even better survival outcomes than those with resectable tumors who received surgery upfront (**Michelakos et al. 2019**, Schorn et al. 2017). Also patients with resectable PDAC seem to benefit more from neoadjuvant treatments than upfront surgery (Versteijne et al. 2018, Versteijne et al. 2020). In addition to these findings, new protocols have enriched the possibilities of adjuvant treatment (Neoptolemos et al. 2017, Conroy et al. 2022). Despite all this, PDACs are often chemoresistant and only poor survival improvements have been achieved. As a possible explanation, recent advances in genomics have revealed great biological

heterogeneity in pancreatic cancer (Group Young Researchers In Inflammatory Carcinogenesis et al. 2021, Mi et al. 2022). Due to this heterogeneity, personalized therapies will probably become the new frontier of treatment in patients with PDAC in the future (Tesfaye et al. 2018). To study cancer biology, there are several *in vitro* and *in vivo* preclinical models, such as cell lines and xenograft tumors derived from them, genetically engineered mouse models, organoids, and also patient-derived xenograft (PDX) tumors. These latter seem to reproduce very faithfully the original tumor biology, mimicking the genetic complexity and the features of the microenvironment of human cancer, and for this reason, might be very useful in the study of pancreatic cancer. In a study, we subcutaneously implanted 133 primary and metastatic PDAC tumors into immunodeficient mice and evaluated the validity of this model to histologically reproduce pancreatic cancer (**Pergolini et al. 2017**). In addition, we analyzed the clinical and pathological features associated with successful tumor engraftment and xenograft growth rate, as well as its prognostic value, to better understand the mechanisms behind tumor engraftment and to get insights regarding PDAC tumor biology. The tumor was subcutaneously implanted into the flank of each mouse and then monitored weekly for tumor growth. When the tumor engraftment was successful (tumor growth > 1 cm), it was harvested and a little fragment was reimplanted into new mice, and so for more passages. Mice in which the tumor was not palpable after 6 months, as well as those removed from the study due to health reasons before, were categorized as no engraftment. Fifty-seven tumors (43%) were successfully engrafted. The histological analysis showed that the grade of differentiation and the stromal features of the human tumors were retained in each of the corresponding xenografts, even after extensive passaging of reimplantation (10x). Moreover, PDXs contained α-smooth muscle actin-expressing fibroblasts, as well as collagen composition and subtypes similar to the original patient tumors. Interestingly, the analysis of the clinicopathological features of patients who provided the implanted tumor revealed that more aggressive human tumors could engraft more frequently and more rapidly. Here, lymphovascular invasion (75% vs 50%, P = 0.001), worse recurrence-free survival (median, 7 vs. 16 months, log-rank P = 0.047), and overall survival (median, 13 vs. 21 months, log-rank P = 0.038) were significantly associated with the success of xenograft tumor engraftment. Human tumors with lymph node metastasis were also able to grow more rapidly (P = 0.022). Of note, in patients who developed recurrence during the follow-up, xenograft tumors developed a median of 166 days before the diagnosis of

recurrence. Overall, the results of this study showed that PDXs can faithfully reproduce the original pancreatic tumors and their aggressiveness. For this reason, this model can be considered a valuable tool in precision medicine, to study the biology of pancreatic cancer, to predict patients at very high risk of disease recurrence, and to test new therapeutic alternatives (Garrido-Laguna et al. 2011).

3. SUMMARY

In the management of IPMNs, there are still several unresolved problems and open issues. Given the increasing incidence of pancreatic cystic lesions, as clinicians, we will have to face this disease more and more frequently. On one hand, we pursue the goal of "saving" patients with IPMNs from the progression to PDAC and operating them at the right time-point, when they still harbor HGD. On the other hand, pancreatic surgery still has high morbidity and mortality, and performing unnecessary or too extensive resections should be avoided. The current guidelines are very helpful in the management of IPMNs, however, identifying which patients are harboring HGD and deserve a surgical resection remains difficult. Overall, amid this abundance of pancreatic cysts, the risk of failure is not negligible. In this context, the search for predictors of malignancy, as well as for prognostic factors, that could help in the decision-making process, continues unabated.

In the studies included in this work, several clinical, morphological, and molecular risk factors and predictors of malignancy in IPMNs were analyzed. Furthermore, surgical aspects, new prognostic predictors to improve the treatment choice before and after surgery, as well as new models to study the biology of pancreatic cancer were investigated.

Among the clinical risk factors, age certainly represents an important one. In fact, IPMNs occur more frequently in the sixth or seventh decades and the incidence increases with age. In a study, we analyzed whether young patients (<50 years) with IPMNs present a different profile of the disease (**Morales et al. 2017**). Interestingly, we found that younger patients had more often IPMNs of oncocytic and intestinal epithelial subtype located in the pancreatic head than older patients, without differences in terms of symptoms or radiological worrisome features or high-risk stigmata of malignancy. When invasive carcinoma from IPMN occurs, in younger patients the tumor was more frequently of the colloid type and showed a better prognosis.

Among clinical risk factors and predictors, the role of diabetes (DM) and other metabolic disorders in IPMNs is not well-known. In 2019, we performed a systematic review and meta-analysis on this topic and this revealed that only two papers until then were specifically focused on the role of DM in IPMNs. DM was reported in most papers as a symptom or comorbidity and a consensus on the definition of new-onset diabetes was lacking (**Pergolini et al. 2021**). To fill this gap, we analyzed DM in relation to other

clinical, morphological, and pathological features and to survival outcomes in our cohort of resected IPMNs, with a focus on its association with weight loss (**Pergolini et al. 2021**). First, we found that the prevalence of DM was pretty high (37%) reaching 50% in patients with invasive carcinoma arising from IPMN, similar to the prevalence reported in patients with PDAC. DM showed to be the expression of a more aggressive disease and was significantly associated with MPD involvement and the presence of HGD and invasive carcinoma in the surgical specimen. This association was even stronger with new-onset DM (onset < 2 years from IPMN diagnosis). In this series, as in PDAC, DM was “paradoxically” associated with weight loss. Most interestingly, this association between DM and weight loss was already present in IPMNs with HGD, and not only in those with invasive cancer arising from IPMN. By contrast, among benign lesions (IPMNs with LGD), this association was no longer recognized. Recently, new onset or recent exacerbation of DM was included as new worrisome feature in the revised international guidelines for the management of IPMNs (Ohtsuka et al. 2024). However, further investigations are needed to understand the mechanisms behind this association.

To date, in addition to clinical parameters, morphological predictors of malignancy have played a central role in the choice of management of IPMNs. Among them, the presence of dilation of the MPD and mural nodules represents the most relevant ones (**Petrone et al. 2018**). According to the guidelines, due to their higher malignant potential, MD-IPMNs undergo surgery more frequently. However, even in these patients, it is not difficult to incur misdiagnosis or overtreatment. We analyzed the risk of performing unnecessary operations (for cysts rather than IPMN, chronic pancreatitis, or LGD-IPMNs) or too extensive resections (when malignancy was localized only in a part of the surgical specimen) in a cohort of patients resected for a preoperatively presumed MD-IPMN. Here, we registered a rate of misdiagnosis or overtreatment of 19% (**Crippa et al. 2016**). Severe complications occurred in a relevant percentage of these patients (42%) and total pancreatectomy was the most common procedure associated with overtreatment (67%). Overall, only 50% of all performed pancreatectomies were appropriate for extension of the resection and indication.

In BD-IPMNs, as the natural history of these lesions and their real malignant potential are still unclear, the choice of management is even more difficult than in MD-IPMNs, and the risk of overtreatment is higher. In a large cohort of presumed BD-IPMNs under surveillance, we investigated the risk of malignant progression after 5 years of follow-

up and we sought to find a subset of patients in which the surveillance might be discontinued (**Pergolini et al. 2017**). In this series, which to our knowledge remains one of the largest in the current literature, we showed that the risk of malignancy (HGD and invasive cancer) persisted after 5 years of follow-up, and even after 10 years. Also in patients without worrisome features at 5 years from the diagnosis (in which AGA guidelines suggest surveillance discontinuation), the risk of progression was still present in the years ahead. According to these findings, we suggest that the surveillance should not be stopped after 5 years from diagnosis. However, in patients with a cyst stably smaller than 1.5 cm for 5 years of follow-up, we demonstrated that the risk of progression is negligible and the discontinuation of the surveillance might be probably considered.

In addition to clinical and morphological predictors, the identification of molecular predictors is certainly a goal that we must continue to pursue in the future to achieve early prediction of pancreatic cancer. Liquid biopsy, as well as the analysis of cystic fluid, and nomogram models that combine molecular factors with clinical and morphological parameters, showed interesting and promising results, as those included in this habilitation (**Yang et al. 2017, Das et al. 2019, Al Efishat et al. 2018**), and represent a field of research in which new efforts and further investigations should be invested.

When invasive carcinoma occurs in patients with IPMNs, identifying prognostic factors is also very important to stratify patients correctly and offer them the optimal treatment. Sometimes the assessment of resectability is challenging and it is difficult to establish which patients could benefit more from neoadjuvant therapy rather than upfront surgery. Also postoperatively, as the treatment protocols for adjuvant therapy are increasing and the cancer biology revealed to be very heterogeneous, prognostic factors additionally to the tumor stadium, and new models for personalized therapy could help to choose the most effective therapy for each patient.

In this setting, we evaluated if 18FDG-PET/CT can help predict recurrence and survival outcomes and improve the stratifications of patients with PDAC (**Pergolini et al. 2017**). In our series, patients in which the tumor recurred within 12 months after resection showed in the preoperative 18FDG-PET/CT a significantly higher median SUVmax. In particular, patients with a tumor with SUVmax ≥ 6.0 showed significantly worse survival outcomes than those with SUVmax <6.0. The prognostic value of SUVmax was even higher when combined with increased CA19.9 (≥ 200 U/ml). According to these

findings, but conscious of the high cost of the procedure, we suggested considering the use of 18FDG-PET/CT in selected patients in which the assessment of resectability, as well as the choice of the management, results more complex.

A study that evaluated the role of Ki-67 as prognostic predictor in pancreatic cancer was also included in this work (**Pergolini et al. 2019**). The expression of Ki-67 is a well-known tumor proliferation marker and, in pancreatology is routinely used in the staging of pancreatic neuroendocrine tumors. Moreover, the preoperative assessment of Ki-67 in EUS-FNA samples demonstrated to be able to predict the tumor aggressiveness and guide the treatment choice. In PDAC, the analysis of Ki-67 is not routinely performed and its prognostic value is still unknown. Therefore, we decided to perform this additional histological examination in the surgical specimen of resected PDACs and compare its expression to the survival outcomes. Interestingly, in our series, Ki-67 demonstrated to be an independent prognostic predictor, equal to other pathological parameters, like tumor grading, nodal, and resection margin status. Moreover, Ki-67 expression significantly correlated with tumor grading, and patients with G3 tumors and Ki-67> 50% showed the worst survival outcomes in the entire cohort. These interesting findings show that Ki-67 is the expression of biological aggressiveness and a valuable prognostic factor also in PDAC. Accordingly, Ki-67 index might help to discriminate which patients should receive more aggressive adjuvant treatments, or, when the feasibility of its preoperative assessment by EUS–FNA in PDAC will be proved, to identify those who may benefit more from neoadjuvant chemotherapy than upfront surgery. Of note, several studies showed that Ki-67 seems to be a predictor of malignancy also in IPMNs able to distinguish between LGD and HGD or invasive carcinoma (Shimura et al. 2016, Takeshita et al. 2012).

Other histological prognostic factors were also evaluated in this work. In a large multicentric study (**Crippa et al. 2022**), perineural invasion was shown to have an important prognostic value in predicting recurrence, especially in PDACs at early stage (with size ≤ 20 mm, R0, N0). In this subset of patients, perineural invasion was the only prognostic predictor of DFS, suggesting a possible driving role of perineural invasion in the pancreatic cancer progression already at an early stage and the need in these patients of a more aggressive systemic therapy. Next to the perineural invasion, in a large multicentric study, nodal involvement showed to have an important role in the prediction of the timing of recurrence, but not in the location of recurrence (**Honselmann et al. 2020**). Both pN0 and pN1 patients are more likely to develop

distant metastases, emphasizing, once again, the need for multi-modality systemic therapy in PDACs.

As explained above, new models are necessary to increase our knowledge of the complex biology of pancreatic cancer and develop new treatments, even in a personalized manner. In a study, we evaluated the feasibility of patient-derived xenograft (PDX) tumors to histologically reproduce pancreatic cancer. We also analyzed clinical and morphological tumor characteristics associated with successful tumor engraftment and xenograft growth speed (**Pergolini et al. 2017**). Fragments of tumor tissue harvested during surgery were implanted subcutaneously in a large cohort (N=133) of immunodeficient mice and reimplanted for several passages in other mice after successful growth within 6 months. PDACs successfully engrafted in 43% of cases. Here, the grade of differentiation and the stromal features of the human tumors were maintained in the corresponding xenografts, even after extensive passaging of reimplantation (10x). Engraftment and growth rapidity were associated with more aggressive human tumors and with worse survival outcomes. Interestingly, tumors harvested from patients who developed recurrence were able to engraft on average a long time before the recurrence. In our cohort, PDXs demonstrated to be a valuable model that reproduces faithfully the original tumor and, therefore, might be useful to study the biology of pancreatic cancer and to test new medications. Moreover, as tumors engrafted on average long before recurrence, PDXs might offer new perspectives for personalized therapy.

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5. ORIGINAL ARTICLES

5.1 First Authorships

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Surgery xxx (2020) 1–8

Contents lists available at ScienceDirect

Surgery

journal homepage: www.elsevier.com/locate/surg

Diabetes mellitus in intraductal papillary mucinous neoplasms: A systematic review and meta-analysis

Ilaria Pergolini, MD[a], Stephan Schorn, MD[a], Carsten Ja¨ger, MA, PhD[a], Rüdiger Go¨ß, MD[a], Alexander Novotny, MD[a], Helmut Friess, MD[c,d], Güralp Onur Ceyhan, MD[b], Ihsan Ekin Demir, MD[a,b,c,d,*]

[a] Department of Surgery, Technical University of Munich, Munich, Germany
[b] Department of General Surgery, HPB-Unit, School of Medicine, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkey
[c] German Cancer Consortium (DKTK), Partner Site, Munich, Germany
[d] CRC 1321 Modelling and Targeting Pancreatic Cancer, Munich Germany

ARTICLE INFO

Article history:
Accepted 3 July 2020
Available online xxx

ABSTRACT

Background: Our current knowledge of diabetes mellitus in intraductal papillary mucinous neoplasm is very limited and its prevalence and predictive value for malignant transformation are not clear. This study sought to systematically review the literature to define the prevalence of diabetes mellitus in intraductal papillary mucinous neoplasm and to evaluate the association of diabetes mellitus with the progression to high-grade dysplasia or invasive cancer.

Methods: A PubMed/Medline systematic search was performed to identify studies reporting data on preoperative diabetes mellitus in intraductal papillary mucinous neoplasm. Articles meeting the predefined inclusion criteria were analyzed and a meta-analysis was performed. The study was preregistered (PROSPERO ID: CRD42020153581).

Results: From the initially detected 827 studies, 27 studies including resected patients with histologically confirmed intraductal papillary mucinous neoplasm were included. The global prevalence of preoperative diabetes mellitus was 25% (1,112 of 4,412); whereas new-onset/worsening diabetes mellitus was reported in 6% of patients (68 of 1,202). The meta-analysis revealed that patients with pre-existing diabetes mellitus had an increased risk of harboring a main pancreatic duct involvement (risk ratio 1.43, 95% confidence interval: 1.21−1.69, $P < .001$), high-grade dysplasia (risk ratio 1.27, 95% confidence interval: 1.01−1.59, $P = .04$), and invasive cancer (risk ratio 1.61, 95% confidence interval: 1.33−1.95, $P < .001$).

Conclusion: The prevalence of diabetes mellitus in intraductal papillary mucinous neoplasm is high, and diabetic patients demonstrate an increased risk of a more aggressive disease. Therefore, diabetes mellitus should be increasingly considered in the stratification of patients with intraductal papillary mucinous neoplasm. Further investigations to determine the mechanisms behind the association with progression should be carried out.

Introduction

Intraductal papillary mucinous neoplasm (IPMN) is a common pancreatic disease with an increasing prevalence in the general population.[1–3] A small but relevant proportion of IPMNs evolves over time, following the classic pattern from adenoma to invasive carcinoma.[4–7] Determining whether patients are at higher risk of harboring or developing high-grade dysplasia (HGD) and invasive cancer and consequently requiring surgery remains a challenge in the management of IPMN. The detection of reliable risk factors in IPMN offers the unique opportunity of early diagnosis and prevention of pancreatic cancer, thus representing an important goal to pursue.

The revised International Consensus Fukuoka guidelines for the management of IPMN have reconfirmed and updated clinical and radiological worrisome features and high-risk stigmata

* Reprint requests: Ihsan Ekin Demir, MD, Department of Surgery, Technical University of Munich, Klinikum rechts der Isar, Ismaninger Straße 22, 81675 Munich, Germany.
E-mail address: ekin.demir@tum.de (I.E. Demir).

https://doi.org/10.1016/j.surg.2020.07.006
0039-6060/

of malignancy, which is helpful in the clinical decision-making of patients with IPMN; however, no attention has been drawn to metabolic risk factors, such as diabetes mellitus (DM).[8] By contrast, the European guidelines for pancreatic cystic lesions have recently introduced new-onset DM as a relative indication for surgery in IPMN, although with a low level of evidence.[9] In fact, our current knowledge regarding DM and other metabolic disorders in IPMN is very limited. The majority of studies have evaluated the impact of resection of IPMN on the postoperative development of DM.[10,11] In contrast, the association between preoperative DM and malignant progression of IPMN and its potential role as a predictor of malignancy have not been established. The role of DM is better acknowledged and widely investigated in patients with pancreatic ductal adenocarcinoma (PDAC). DM is present in around 50% of patients with pancreatic cancer and is associated with a two-fold higher risk of PDAC in comparison with the normal population.[12] This association seems to be even stronger in cases of new-onset DM.[13] Although IPMN may progress to invasive cancer, PDAC arising from IPMN appears to have distinguished genomic and morphologic features and it should be considered a distinct entity.[14-16] Therefore, established risk factors for PDAC cannot directly be translated into IPMN and a specific evaluation should be carried out.

This study sought to systematically review the literature to define the prevalence of DM in IPMNs and to evaluate the association of DM with malignant progression.

Methods

To perform this systematic review and meta-analysis, we adhered to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines (PRISMA checklist—Supplementary material 1).[17] As recommended by the PRISMA guidelines, this systematic review and meta-analysis were registered in the international register of systematic reviews (PROSPERO) (Registration ID: CRD42020153581).

Search strategy and study selection

A systematic search of the literature was conducted on April 10, 2019, in PubMed/Ovid-Medline, using the following search terms: “intraductal papillary mucinous neoplasm”, “intraductal papillary mucinous tumor”, “ipmn”, “pancreatic cyst”, “pancreatic cystic tumor”, “pancreatic cystic neoplasm”, “pancreatic cystic lesion”, “intraductal papillary mucinous carcinoma” filtered by “diabetes”, “diabetes mellitus”, “hyperglycemia”, “risk factor”, and “predictor”. The search was limited to the period from 2000 to April 2019. Figure 1 presents the search strategy. A review of titles and abstracts was conducted by 2 authors (I.P. and C.J.) through all identified references. Full papers were retrieved for all abstracts deemed potentially eligible, screened for relevance, and assessed against inclusion and exclusion criteria. Additional publications were included using the PubMed function “related articles” and screening the reference list of selected articles. To avoid data duplication, when multiple articles were published from the same study group, in case of overlapping of study periods or patients’ selection, only the most recent or the most informative article was included. These indications were followed in case of multicentric studies as well. Any disagreement during the search and selection process was resolved by consensus. The identified publications were screened and transferred to the program RevMan (Review Manager, v 5.3.5, Copenhagen: Nordic Cochrane Center, Cochrane Collaboration, Copenhagen, Denmark) for further analysis.

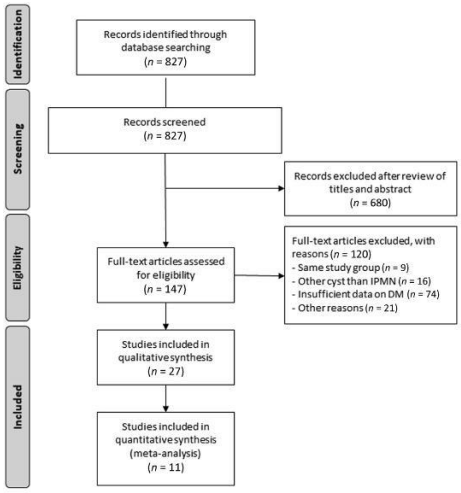

Fig. 1. Flow diagram of study selection. *IPMN*, intraductal papillary mucinous neoplasm; *DM*, diabetes mellitus.

Inclusion and exclusion criteria

We included original papers (eg, no review papers, no case reports) providing data on DM in resected patients with histologically confirmed IPMN. Only studies written in English, with full-text available and including more than 35 patients, were considered. Papers including pancreatic cystic lesions other than IPMN or selectively evaluating a surgical procedure or a risk subgroup of patients (ie, papers including only patients who underwent total pancreatectomy or only patients with Fukuoka-negative IPMN) were excluded. Because type I diabetes appears to not be associated with pancreatic cancer,[18,19] studies reporting data selectively on diabetes type I were also not considered.

Excluded studies were recorded. The number of excluded studies and the reasons for the exclusion are presented in Fig 1 and Supplementary material 2.

Data extraction

The following data were collected: author details, country, year of publication, study design, period of patient recruitment, sample size, mean/median age, sex, diabetes (as preexisting and new-onset or worsening), localization of IPMN (head-uncinate process, body-tail, or diffuse), surgical procedure (duodenopancreatectomy, distal pancreatectomy, total pancreatectomy or other types of resection), type of IPMN [branch duct (BD)- or main duct/mixed-IPMN (MD-IPMN)], presence of HGD, and invasive cancer. The definition and the modality of assessment of DM were also recorded, when available. For the meta-analysis, the rate of DM in relation to the type of IPMN and the progression to HGD and invasive cancer was obtained. According to a recent international consensus, we avoided the use of the term “malignancy” to refer to both HGD and invasive cancer, maintaining a clearly distinct definition (HGD/invasive cancer), even when considered in the same category by a paper’s authors.[8]

Table I
Basic characteristics of studies included in systematic review and meta-analysis

Study	Country	Year of publication	Recruitment period	Sample *N*	Age (Y)	Gender (M/F)	Operation DP/distal/ total/other	Pre-existing diabetes	Definition of pre-existing diabetes	New-onset or worsening diabetes	Definition of new-onset or worsening diabetes	BD-/MD-IPMN *N*	LGD/ HGD+ invasive *N*	LGD+HGD/ invasive *N*
Taouli et al[22]	France	2000	1993–1999	36	61 (mean)	19/17	24/6/5/1	7 (19%)	NR	NR	NR	10/26	20/16	27/9
Wiesenauer et al[23]	USA	2003	1988–2002	64	NR	33/31	39/18/7/5	NR	NR	7 (11%)	Glucose intolerance treated with diet or medication <2 years	NR	44/20	NR
Nagai et al[34]	Japan	2007	1984–2006	72	63 (median)	44/28	37/20/10/5	NR	NR	21 (29%)	New-onset/ worsening diabetes as presenting symptoms	49/23	28/44	42/30
Niedergethmann et al[42]	Germany	2008	1996–2006	97	NR	NR	78/14/5/0	37 (38%)	Pre-existing diabetes	NR	NR	NR	NR	29/68
Woo et al[38]	South Korea	2009	1998–2005	85	63 (mean)	50/35	39/NR/NR/NR	20 (24%)	NR	NR	NR	85/0	71/14	76/9
Lubezky et al[43]	Israel	2010	2002–2008	62	NR	24/38	32/16/13/1	NR	NR	11 (18%)	NR	NR	NR	39/23
Mimura et al[44]	Japan	2010	1998–2009	82	69 (median)	49/33	50/17/14/1	29 (35%)	Long-standing diabetes (>24 years)	1 (1%)	Diagnosis <2 years	43/39	28/54	53/29
Hwang et al[46]	South Korea	2010	1994–2008	187	63 (mean)	114/73	78/81/14/14	45 (24%)	NR	NR	NR	118/69	129/58	144/43
Park et al[45]	South Korea	2011	1995–2009	103	61 (mean)	76/27	57/34/12/0	34 (35%) of 98	NR	NR	NR	34/69	64/39	68/35
Okabayashi et al[47]	Japan	2012	2000–2011	100	NR	69/31	48/34/6/10	43 (43%)	NR	NR	NR	67/33	65/35	82/18
Winner et al[24]	USA	2013	1994–2011	183	67 (mean)	76/107	68/54/31/27	38 (21%)	History of diabetes	NR	NR	53/121 of 174	121/62	155/28
Sturm et al[48]	USA	2013	1992–2012	274	67 (mean)	136/138	NR	41 (15%)	Self-report, medication, increased blood glucose, or increased glycated hemoglobin	NR	NR	140/134	189/85	222/52
Kawakubo et al[25]	Japan	2014	1995–2011	59	66 (mean)	42/17	37/22/0/0	18 (31%)	History of diabetes	NR	NR	45/14	NR	39/20
Nguyen et al[39]	USA	2014	1996–2012	66	69 (median)	24/42	47/12/3/4	16 (24%)	NR	1 (1,5%)	NR	66/0	51/15	62/4
Daudé et al[35]	France	2015	1998–2012	74	61 (mean)	46/28	52/15/6/1	12 (16%)	NR	NR	NR	0/74	22/52	33/41
Chang et al[26]	Taiwan	2015	1994–2014	106	62 (mean)	53/53	NR	44 (42%)	NR	NR	NR	66/40	45/61	80/26
Jang et al[37]	South Korea	2016	2004–2014	74	66 (mean)	56/18	33/27/8/6	21 (28%)	NR	NR	NR	0/74	63/11	42/32
Sugimoto et al[36]	USA	2016	1996–2015	103	71 (median)	59/44	74/23/6/0	26 (25%)	NR	NR	NR	0/103	39/64	62/41
Ridtitid et al[40]	USA	2016	2001–2013	135	65 (mean)	71/64	NR	20 (15%)	NR	NR	NR	135/0	117/18	128/7
Leal et al[27]	USA	2016	2000–2013	103	64 (mean)	35/68	60/31/0/12	17 (17%)	Diabetes in medical record, medication, diabetes diagnosed at preoperative workup	NR	NR	57/45 of 102	88/NR	101/NR

(*continued on next page*)

Table I (*continued*)

Study	Country	Year of publication	Recruitment period	Sample *N*	Age (Y)	Gender (M/F)	Operation DP/distal/total/other	Pre-existing diabetes	Definition of pre-existing diabetes	New-onset or worsening diabetes	Definition of new-onset or worsening diabetes	BD-/MD-IPMN *N*	LGD/ invasive *N*	HGD+ invasive *N*	LGD+HGD/ invasive *N*
Hirono et al[28]	Japan	2017	1999–2015	286	71 (median)	162/124	185/54/26/21	91 (32%)	NR	NR	NR	109/177	94/192		190/96
Morales-Oyarvide et al[29]	USA	2017	1990–2016	454	67 (mean)	231/223	NR	154 (34%)	Self-reported or preoperative fasting plasma glucose ≥126 mg/dL	NR	New-onset <1 year, recent-onset <5 years	134/155	240/214		351/103
Pérez-Cuadrado-Robles et al[41]	Belgium/ Spain	2018	2006–2017	60	66 (mean)	30/30	44/15/1/NR	9 (15%)	NR	8 (13%)	NR	60/0	36/24		52/8
Marchegiani et al[31]	Italy	2018	2006–2016	272	NR	158/114	NR	57 of 227 (25%)	NR	NR	NR	66/206	125/147		201/71
Khoury et al[30]	USA	2018	2014	478	66 (mean)	232/246	266/168/NR/NR	115 (24%)	NR	NR	NR	NR	370/108		381/97
Aronsson et al[32]	Sweden	2019	2010–2016	251	69 (median)	115/136	132/68/40/1	50 of 249 (20%)	NR	NR	NR	NR	153/84 of 237		227/24
Del Chiaro et al[33]	Sweden/ USA	2019	2004–2017	796	69 (median)	383/413	463/228/88/17	168 (21%)	NR	19 (2%)	New-onset <1 year	361/363 of 724	476/320		661/135

NR, not reported; *LGD/HGD + invasive*, low-grade IPMN versus high-grade IPMN pooled together with invasive IPMN; *LGD + HGD/invasive*, low-grade IPMN pooled together with high-grade IPMN versus invasive IPMN; *M*, male; *F*, female; *DP*, duodenopancreatectomy.

Outcomes

In this systematic review, we evaluated the prevalence of pre-existing and new-onset/worsening DM in resected IPMN. Subgroup analyses with stratification according to the type of IPMN (BD- or MD-IPMN) were performed. The primary outcome of this meta-analysis was to examine the impact of pre-existing and new-onset/worsening DM on the progression of IPMN to HGD/invasive cancer. Secondarily, we analyzed DM in relation to the involvement of the main pancreatic duct.

Evaluation of study quality

In accordance with methods recommended by the Cochrane Collaboration[20] and using the RevMan risk-of-bias tool, we assessed the risk of bias of each included study against the following key criteria: selection bias, blinding of participants, incomplete outcome data, selective outcome reporting, and other sources of bias. The following judgments were used: low risk, high risk, or unclear (either lack of information or uncertainty over the potential bias).

Statistical analysis

Data were collected in an Excel database (Microsoft Excel 2016 for Windows; Microsoft Corp, Redmond, WA). Papers were pooled in various subgroups depending on the provided data and the sought outcome. Continuous variables were reported as mean and standard deviation. Categorical variables were presented as numbers and percentages. Meta-analysis was carried out using Review Manager software (RevMan, Cochrane Collaboration), as discussed earlier in this report, and performed using the Mantel-Haenszel method for random effects. Results were reported as risk ratio (RR) and 95% confidence interval (95% CI). Heterogeneity was evaluated using the inconsistency statistic (I^2), which represents the percentage of total variation across studies that is attributable to heterogeneity rather than chance.[21] A low-moderate level of heterogeneity was assumed with I^2 less than 50%, and I^2 $\geq$ 50% defined a high level of heterogeneity. According to the recommendations of the Cochrane Collaboration, tests for funnel plot asymmetry were not performed because the meta-analyses included fewer than 10 studies.[20] A P value <.05 was accepted as statistically significant.

Results

From the initially identified 827 studies, 27 studies provided data regarding DM and met the inclusion criteria for the systematic review for a total of 4,662 patients.[22–48] Table I presents the descriptive characteristics of the 27 included studies.

Evaluation of study quality

All the included studies entailed a high risk of selection bias, as they were all retrospective (Supplementary Material 3). In addition, as retrospective studies, most of them did not specifically focus on the evaluated topic, therefore we had to work with incomplete and selective outcome data. Patient blinding (performance bias, detection bias) could not be evaluated, as this is not possible with retrospective studies. Additional bias was encountered for the following: indications for surgery not completely stated, guidelines for surgery or surveillance changed over time, and varying inclusion criteria (Supplementary Material 3,Supplementary Figure 3).

Table II
Studies reporting data on diabetes with distinction of the IPMN-Type

Study	BD-/MD-IPMN *n*	DM in BD-/MD-IPMN *n* (%)
Mimura et al[44]	43/39	11 (26%)/18 (46%)
Okabayashi et al[47]	67/33	27 (40%)/16 (49%)
Daude´ et al[35]	0/74	NR/12 (16%)
Sugimoto et al[36]	0/103	NR/26 (25%)
Jang et al[37]	0/74	NR/21 (28%)
Hirono et al[28]	109/177	25 (23%)/66 (37%)
Morales-Oyarvide et al[29]	134/155	39 (29%)/72 (46%)
Woo et al[38]	85/0	20 (24%)/NR
Nguyen et al[39]	66/0	16 (24%)/NR
Ridtitid et al[40]	135/0	20 (15%)/NR
Pe´rez-Cuadrado-Robles et al[41]	60/0	9 (15%)/NR

IPMN, intraductal papillary mucinous neoplasm; *BD*, branch duct; *MD*, main duct; *DM*, diabetes mellitus; *NR*, not reported.

Table III
Studies reporting data on pre-existing DM with distinction of grade of dysplasia

Study	Sample, *N*	Pre-existing diabetes: Yes/no	Grade of dysplasia: LGD/HGD/invasive *n*	LGD-IPMN: DM/non-DM *n*	HGD-IPMN: DM/non-DM *n*	LGD+HGD-IPMN: DM/non-DM *n*	Invasive-IPMN: DM/non-DM *n*	HGD+Invasive-IPMN: DM/non-DM *n*
Taouli et al[22]	36	7/29	20/7/9	NR*	NR*	2/25	5/4	NR
Mimura et al[44]	82	29/53	28/25/29	5/23	12/13	17/36	12/17	24/30
Chang et al[26]	106	44/62	45/35/26	NR*	NR*	31/49	13/13	NR
Hirono et al[28]	286	91/195	94/96/96	22/72	30/66	52/138	39/57	69/123
Morales-Oyarvide et al[29]	454	154/300	240/111/103	62/178	40/71	102/249	52/51	92/122
Khoury et al[30]	478	115/363	370/11/97	83/287	NR*	NR	NR*	32/76
Marchegiani et al[31]	272	57/170 of 227	125/76/71	NR*	NR*	40/129 of 169	17/41 of 58	NR
Aronsson et al[32]	251	50/199 of 249	153/60/24 of 237	NR*	NR*	42/183 of 225	8/16 of 24	NR
Del Chiaro et al[33]	796	168/628	476/185/135	93/383	36/149	129/532	39/96	75/245

IPMN, intraductal papillary mucinous neoplasm; *LGD + HGD-IPMN*, low-grade IPMN pooled together with high-grade IPMN; *HGD + invasive-IPMN*, high-grade IPMN pooled together with invasive IPMN; *DM, diabetes mellitus; non-DM, non-diabetes mellitus; NR*, not reported.
* Not reported, only available as pooled data (LGD + HGD or HGD + invasive).

Prevalence of DM in IPMN

The global prevalence of preoperative DM obtained from 24 studies for a total of 4,412 patients was 25% (1,112 of 4,412) with a range of 15% to 43% (Table I).[22,24,25-33,35–42,44,45–48] Data on new-onset/worsening DM were extracted from 7 studies (1,202 patients) and, by contrast, the prevalence was 6% (68 of 1,202) with a range of 1.5% to 29% (Table I).[23,33,34,39,41,43,44] Of note, symptoms of exocrine insufficiency, such as diarrhea and bloating, were reported only in 3 papers by 8% to 17% of patients.[22-24] The prevalence of preexisting DM in resected MD–IPMN was 35% (231 of 655, 7 studies) with a range of 16% to 49%,([28,29,35-37,44,47] and in BD-IPMN was 24% (167 of 699, 8 studies) with a range of 15% to 40% (Table II).[28,29,38–41,44,47] Of note, only 4 studies included both BD- and MD-IPMN and reported DM rate with distinction to the type of IPMN.[28,29,44,47] The prevalence of DM in MD-IPMN was significantly higher than in BD-IPMN ($P < .001$).

Focusing on the grade of dysplasia, DM was present in 33% (292 of 888, 5 studies) of patients with HGD or invasive cancer, with a range of 23% to 44%,[28–30,33,44] whereas it was 28% (118 of 417, 4 studies) with a range of 37% to 49%[28,29,33,44] and 22% (265 of 1,208, 5 studies) with a range of 18% to 31%[28-30,33,44] in IPMN with HGD and low-grade dysplasia (LGD), respectively (Table III). When patients with invasive cancer were separately considered, the prevalence of DM reached 39% (185 of 480, 8 studies) with a range of 29% to 56%[22,26,28,29,31–33,44] (Table III) and was significantly higher than IPMN with HGD or LGD (39% vs 28% vs 22%, $P < .001$).

DM and malignant progression of IPMN

A meta-analysis of diabetic versus non-diabetic patients was conducted for (1) HGD plus invasive cancer (Fig 2, A); (2) for invasive cancer alone (Fig 2, B); and (3) for HGD alone (Fig 2, C).

The meta-analysis demonstrated that DM was significantly associated with the presence of HGD/invasive (RR 1.29, 95% CI: 1.16–1.43, $P < .001$; DM: 292 of 557 [52.42%]; non-diabetes mellitus [non-DM]: 596 of 1539 [38.73%]; Fig 2, A). When malignant cases were separately pooled according to the grade of invasiveness, diabetic patients demonstrated a significantly higher risk of harboring invasive cancer (DM: 185 of 600 [30.83%]; non-DM: 295 of 1636 [18.03%]; RR 1.61, 95% CI: 1.33–1.95, $P < .001$; Fig 2, B), but also HGD (DM: 118 of 300 [39.33%]; non-DM: 299 of 955 [31.32%]; RR 1.27, 95% CI: 1.01–1.59, $P = .04$) compared with patients who did not have diabetes (Fig 2, C).

Because IPMNs with an associated dilatation of the MPD present a higher risk of malignant progression,[8,9] we also analyzed DM in relation to the type of IPMN. A total of 4 studies reported data on DM with a distinction between BD- and MD-IPMN for a total of 922 patients (Table II). The meta-analysis revealed that diabetic patients present more frequently with involvement of the MPD in comparison with non-diabetic patients (DM: 172 of 317 [54.26%]; non-DM: 232 of 605 [38.35%]; RR 1.43, 95% CI: 1.21–1.69, $P < .001$; Fig 3).

New-onset DM in regard to HGD/invasive cancer was evaluated only in 3 studies and with various definitions of the time of onset.[23,29,33] Morales-Oyarvide et al[29] described recent-onset DM as having occurred <5 years from the diagnosis of IPMN. Of note, the authors demonstrated that diabetic patients had a 6.9-fold significantly higher risk of invasive cancer, but not of HGD, compared with non-diabetic patients; however, data were not extractable for the meta-analysis.[29] Moreover, Wiesenauer et al[23] defined new-onset DM as the occurrence of glucose intolerance within 2 y before the diagnosis of IPMN, and Del

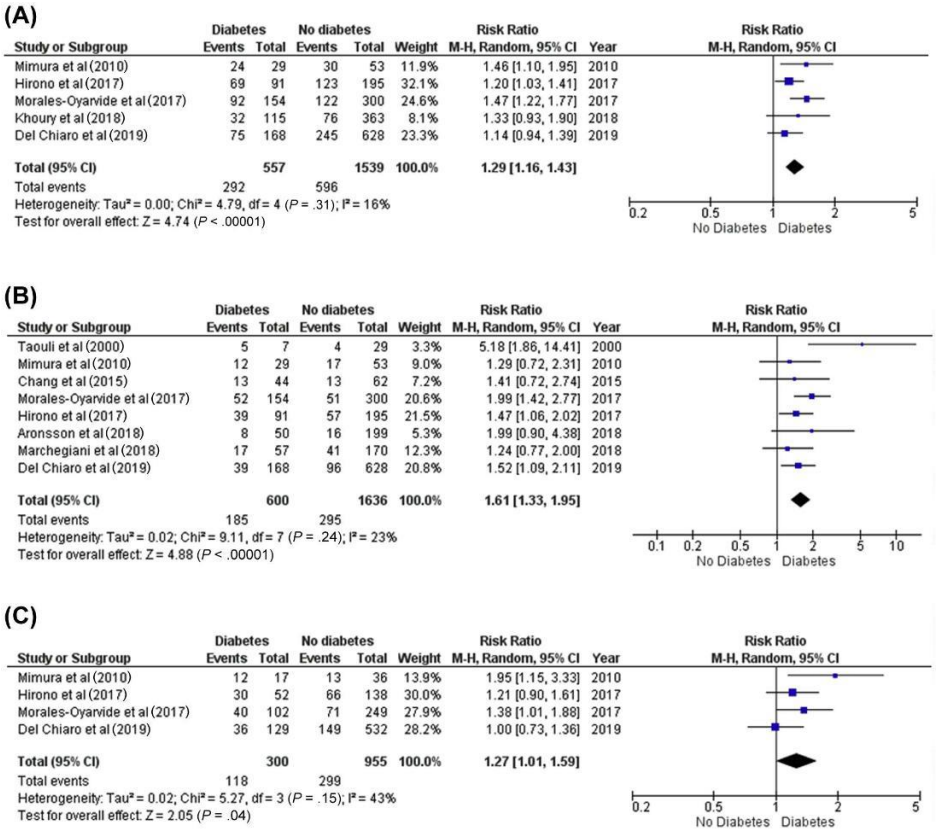

Fig. 2. Forest plot of diabetic versus non-diabetic patients in the comparison of (A) high-grade dysplasia/invasive cancer rate, (B) invasive cancer rate, and (C) high-grade dysplasia. The Forest plots revealed a higher risk of harboring (A) high-grade dysplasia/invasive cancer (RR 1.29, 95% CI: 1.16–1.43, $P < .001$), but also (B) invasive cancer (RR 1.61, 95% CI: 1.33–1.95, $P < .001$), and (C) high-grade dysplasia (RR 1.27, 95% CI: 1.01–1.59, $P = .04$) separately, for patients with diabetes in comparison with non-diabetic patients. *M-H*, Mantel-Haenszel test; *random*, random effect.

Chiaro et al[33] as DM occurred <1 year.[33] Because of these discrepancies, we decided against performing a meta-analysis on this point.

Heterogeneity of study population

Heterogeneity analysis demonstrated low heterogeneity for the analysis of MPD involvement ($I^2 = 24\%$, $P = .27$), HGD/invasive cancer and invasive cancer alone ($I^2 = 16\%$, $P = .31$ and $I^2 = 23\%$, $P = .24$ respectively). For the analysis of HGD, the heterogeneity was more pronounced but not significant ($I^2 = 43\%$, $P = .15$).

Discussion

In patients with IPMN, identifying preinvasive lesions is of paramount importance, as it allows for a timely surgical treatment resulting in an excellent long-term prognosis. In the last decades, the interest in IPMN has increased; however, several questions such as the role of DM in IPMN remain unanswered. To our knowledge, this is the first systematic review and meta-analysis to date on this subject.

In this systematic review, we observed a wide variation in the prevalence of DM among 24 studies on patients undergoing resection for IPMN (15%–43%). Such variations likely reflect differences in patient selection, size and composition of study groups, and methods of DM assessment. Overall, a diagnosis of DM was present in 25% of resected patients with IPMN, which is higher compared with the general population of the United States, where the prevalence in subjects age 65 years and older reaches 20.8%.[49] Among patients with invasive carcinoma arising from IPMN prevalence of DM was 39%. In 3 studies DM even affected half of the study cohorts,[22,26,29] similarly to the reported prevalence of DM in patients with PDAC.[12,50] In contrast, a diagnosis of DM was reported in 28% of patients with HGD-IPMN, rather than 22% of patients with LGD.[28-30,33,44]

Although new-onset DM was included in the European guidelines as a relative indication for surgery, data on new-onset or worsening DM were reported only in 7 studies with absent or

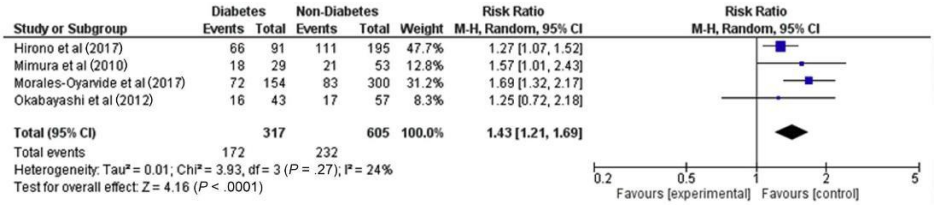

Fig. 3. Forest plot of diabetic versus non-diabetic patients in the comparison of MPD involvement rate. The Forest plot demonstrated that diabetic patients present more frequent involvement of the MPD in comparison with non-diabetic patients.

heterogeneous definitions of the onset timing and very little information in relation to the histologic grade. Therefore, further investigations on new-onset DM in IPMN are certainly needed. Moreover, it is evident from the analysis of the literature that a consensus on the definition of DM and the timing of diagnosis is also necessary in this context.

Our meta-analysis demonstrated that diabetic patients with IPMN had an increased risk of a more aggressive disease compared with non-diabetic patients. As expected, and based on the literature on PDAC, we found an association between DM and invasive pancreatic cancer[12,13]; however, even more noteworthy, our meta-analysis demonstrated that DM was also significantly associated with a higher risk of HGD. Therefore, patients with an IPMN and a history of DM could potentially benefit from further diagnostic workup to earlier detect HGD. Moreover, in the presence of other worrisome issues, a diagnosis of DM may be helpful in the decision-making process to a more prompt surgical approach. In fact, the assessment of HGD is crucial given the excellent life expectancy of patients with preinvasive lesions that are resected early in comparison with patients whose lesions have evolved to invasive cancer.[51–53]

Moreover, a subgroup analysis distinguishing between the types of IPMN demonstrated that patients with pre-existing DM present more frequent involvement of the MPD, which is also expression of a more aggressive disease. The cause-effect relationship between DM and the involvement of the MPD is unclear. DM could represent a consequence of the obstruction of the ductal system by mucin production and associated chronic pancreatitis,[54] but may also represent a risk factor for the development of MD-IPMN as suggested by Capurso et al.[55] It is well-known that morphologic subtypes of IPMN correlate with distinct genomic alterations, different clinical presentation, and prognosis. Of note, Morales-Oyarvide et al[29] reported that patients with DM were more likely to harbor intestinal-type IPMN and colloid carcinomas. In this setting, it seems very worthwhile to further investigate the association of DM with specific epithelial subtypes and histologic types of invasive cancer arising from IPMN to comprehend the underlying mechanisms.

This study has several limitations. As first, we observed a notable lack of knowledge on this topic in the literature. As mentioned earlier in this report, only two papers specifically focused on DM as an end point,[27,29] and most papers reported DM just as a comorbidity or symptom, with no detailed information on the definition, onset, and assessment of DM, and with no data on DM in relation to the type of IPMN and the grade of dysplasia. A consensus on the definition of new-onset and worsening DM is also lacking. This issue represents not only a limitation, but also a major finding of this systematic review. Accordingly, we support the importance of further investigations to better clarify the role of diabetes in IPMN and to achieve more robust conclusions. Another limitation of this study is that all the included papers were retrospective. Commonly, retrospective analyses lead to a high level of heterogeneity. However, in the performed analysis of heterogeneity, the studies achieved an acceptable heterogeneity level. Moreover, we decided to include only papers reporting data on patients with a histologically confirmed IPMN and not presumed IPMN as in observational cohorts. On the one hand, this allows the avoidance of bias as the inclusion of other pancreatic cysts with lower risk of progression. On the other hand, only surgically fit patients underwent resection and for the most part had IPMN with worrisome features and accordingly an intrinsic higher risk of malignancy.

In summary, the prevalence of DM in IPMN is high, and diabetic patients with IPMN demonstrate an increased risk of a more aggressive disease, such as involvement of the MPD and disease progression. Therefore, DM should be further investigated and considered in the stratification risk of patients with IPMN.

Conflict of interest/Disclosure

The authors have no conflict of interest to declare.

Funding/Support

The authors have no funding to report.

Supplementary data

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.1016/j.surg.2020.07.006.

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Diabetes and Weight Loss Are Associated With Malignancies in Patients With Intraductal Papillary Mucinous Neoplasms

Ilaria Pergolini,[*] Carsten Jäger,[*] Okan Safak,[*] Rüdiger Göß,[*] Alexander Novotny,[*] Güralp O. Ceyhan,[‡] Helmut Friess,[*,§,‖] and Ihsan Ekin Demir[*,‡,§,‖]

[*]Department of Surgery, Klinikum Rechts der Isar, School of Medicine, Technical University of Munich, Munich, Germany; [‡]Department of General Surgery, HPB-Unit, School of Medicine, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkey; [§]German Cancer Consortium (DKTK), Partner Site Munich, Munich Germany; and [‖]Collaborative Research Center (CRC) 1321 Modelling and Targeting Pancreatic Cancer, Munich Germany

BACKGROUND & AIMS: **The role of diabetes in intraductal papillary mucinous neoplasms (IPMNs) is not known. We investigated the prevalence of diabetes among patients with resected IPMNs and the association between diabetes, clinical and morphological features, and high-grade dysplasia or invasive cancer.**

METHODS: **We collected clinical, pathology, laboratory, and demographic data from 134 patients who underwent pancreatic resection for IPMN from a referral center in Germany. We identified 50 patients with diabetes (37%).**

RESULTS: **Higher proportions of patients with diabetes were male and older, but did not have increased body mass index, compared to patients without diabetes. Diabetes was significantly associated with main-duct involvement (odds ratio [OR], 2.827; 95% CI, 1.059–7.546; $P = .038$) and high-grade dysplasia or invasive carcinoma (OR, 2.692; 95% CI, 1.283–5.651; $P = .009$). Risk of high-grade dysplasia or invasive cancer was even higher in patients with new-onset or worsening diabetes (OR, 4.615; 95% CI, 1.423–14.698; $P = .011$). Fifty-eight percent of patients (18/31) with weight loss at diagnosis had diabetes vs 32% of patients (31/97) without weight loss ($P = .009$). However, when the analysis was restricted to IPMNs with low-grade dysplasia, weight loss and diabetes were no longer associated (42% [5/12] vs 21% [9/44]; $P = .133$).**

CONCLUSIONS: **In patients with IPMNs, diabetes is associated with increased risk of main duct involvement and high-grade dysplasia or invasive carcinoma. Studies are needed to determine the relationship between diabetes and progression of IPMNs, which might lead to strategies for early detection and prevention of invasive cancer. Findings from this study should be considered in the guidelines for management of IPMN.**

Key Words: Metabolic Disorders; IPMN; Malignant Progression.

Intraductal papillary mucinous neoplasms (IPMNs) are cystic tumors of the pancreas that can vary in extension in the pancreatic gland and variable malignant potential.[1,2] IPMN as precursor of pancreatic ductal adenocarcinoma (PDAC) offers a great opportunity for understanding the mechanisms behind the cancerization.[3] Determining risk factors involved in the progression to cancer is of paramount importance to achieve earlier diagnosis and prevention of pancreatic cancer.[3]

The European guidelines for the management of IPMN have recently introduced new-onset diabetes as a relative indication for surgery but with a low level of evidence.[4] Previous guidelines, such as American Gastroenterological Association and revised Fukuoka guidelines, do not mention diabetes mellitus (DM) and other metabolic factors in the management of IPMN.[1,5] In fact, several meta-analyses have identified DM as a risk factor for IPMN and pancreatic cancer.[6] However, knowledge on this topic is limited. Previous studies examined mostly overweight as a potential risk factor for developing an IPMN and progression, and DM as long-

Abbreviations used in this paper: BD-IPMN, branch-duct intraductal papillary mucinous neoplasm; CI, confidence interval; DM, diabetes mellitus; HGD, high-grade dysplasia; IPMN, intraductal papillary mucinous neoplasm; LGD, low-grade dysplasia; MD-IPMN, main-duct/mixed intraductal papillary mucinous neoplasm; OR, odds ratio; PDAC, pancreatic ductal adenocarcinoma.

Most current article

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https://doi.org/10.1016/j.cgh.2020.04.090

term complication depending on the extension of resection.[6–9] Only a few papers focused on the prevalence of preoperative DM among patients with IPMN and its potential role in the progression to high-grade dysplasia (HGD) and invasive cancer.[7,10–14] By contrast, DM has been much better investigated in PDAC, where it seems to play a double role: as risk factor for PDAC, but also as manifestation/consequence of pancreatic cancer.[15,16] Interestingly, several studies showed that DM induced by PDAC is paradoxically associated with weight loss (eg, as paraneoplastic phenomena preceding the onset of cancer-specific symptoms by several months).[15,17] We have questioned if these metabolic changes also occur in IPMN with HGD and may help for earlier diagnosis and prevention of invasive cancer.

Therefore, this study aimed to examine the prevalence of DM in a cohort of resected IPMNs in a referral center for pancreatic surgery and to evaluate the association of preoperative DM with other clinical-morphological features, with a focus on weight loss, and with the progression to HGD and invasive cancer.

What You Need to Know

Background

The association between diabetes mellitus in intraductal papillary mucinous neoplasm (IPMN) is not clear.

Findings

In 134 patients who underwent pancreatic resection for an IPMN, the overall prevalence of preoperative diabetes was 37%. Diabetes was significantly associated with weight loss, main-duct involvement, and progression to high-grade dysplasia or invasive carcinoma.

Implications for patient care

Diabetes, in patients with IPMNs, appears to be associated with a more aggressive course of neoplasia and should be considered in management of IPMNs.

Materials and Methods

Study Population

A cohort of consecutive patients who underwent pancreatic resection for histologically confirmed IPMN between July 2007 and December 2018 at the Klinikum Rechts der Isar, Technical University of Munich, Germany, were retrospectively reviewed. Patients' demographic and clinicopathologic features were obtained from medical records. Variables included sex, age at initial diagnosis and surgery, personal medical history, preoperative daily medications, symptoms at the time of diagnosis, serum tumor markers (carcinoembryonic antigen, normal value <5 ng/mL; and CA 19.9, normal value 0–37 U/mL), cyst morphology, main pancreatic duct (MPD) features, indication for surgery, date and type of surgery, postoperative course, and final pathological findings. Patients with IPMN associated with pancreatic or duodenal neuroendocrine tumors at pathology were excluded. As well, patients with distinct concomitant PDAC were not considered in the analysis. The study was approved by the Ethics Committee of the Technical University of Munich (approval nr. 118/19s). All authors had access to the study data and reviewed and approved the final paper.

Preoperative DM

The prevalence of preoperative DM, the association of DM with other clinical-morphological features and with the progression to HGD and invasive cancer were the main endpoints of this study. A diabetic status was determined based on the medical reports of the preoperative surgical consultation in case of self-reported history of DM and intake of hypoglycemic medications or insulin. Patients were further categorized as diabetic in case of fasting plasma glucose $\geq$126 mg/dl at the preoperative workup, according to the current American Diabetes Association guidelines.[18] Patients with type I diabetes were excluded from the study.

DM was defined as new onset when diagnosed within 2 years from the diagnosis of IPMN or in conjunction with the preoperative laboratory tests. The presence of worsening DM at the moment of IPMN diagnosis was also collected. In the remaining cases, DM was considered as long-standing.

Clinical-Morphological Features of IPMNs

Patients were defined as asymptomatic when the diagnosis was made in absence of the following symptoms: jaundice, upper abdominal pain, acute pancreatitis, worsening or new-onset DM, unjustified weight loss, or steatorrhea. Weight loss was quantified by the patient at the time of the preoperative visit and was considered as positive when reported as significant ($\geq$5%), unjustified and occurred in the last 6 months/1 year. The diagnosis was achieved by computed tomography, magnetic resonance imaging/magnetic resonance cholangiopancreatography, or endoscopic ultrasonography or endoscopic retrograde cholangiopancreatography. Endoscopic ultrasonography with or without fine needle aspiration was performed at the discretion of the physician; when performed, cytological characteristics and fluid carcinoembryonic antigen levels were collected. The following IPMN characteristics were recorded: predominant location, MPD diameter, cyst size, communication of the cyst with the MPD, presence in the cyst of septations, wall thickening, and solid component with or without enhancement. When patients underwent

primary surveillance for a certain time before surgery, we collected the largest size and the worse features developed by the cyst and the MPD during this time. The presence of associated chronic pancreatitis, clinically or radiologically recognized, was also recorded. IPMN was preoperatively considered as branch-duct IPMN (BD-IPMN) in presence of cysts >5 mm in communication with the MPD but in absence of MPD dilation. IPMNs with MPD dilation ≥5 mm with or without associated side-branch cystic lesions were classified as main-duct/mixed IPMN (MD-IPMN).

Moreover, all patients were retrospectively reviewed for worrisome features and high-risk stigmata according to the revised International Consensus Guidelines.[1] Briefly, worrisome features were acute pancreatitis, cyst size ≥3 cm, thickened or enhancing cyst walls, nonenhancing mural nodules, MPD size of 5–9 mm, abrupt change in caliber of the MPD, lymphadenopathy, and an elevated serum level of CA 19.9. High-risk stigmata were defined by the presence of obstructive jaundice in case of a cystic lesion of the head of the pancreas, MPD ≥10 mm, enhancing solid mass or mural nodule, or cytology positive for HGD or adenocarcinoma.

Surgical Indications

Surgery was performed in patients with signs of malignancy and for patients' decision. The type of resection was planned according to the dimension and the site of the tumor. The final extension of the resection was determined based on the intraoperative findings and the results of the frozen section analysis of the resection margin.

Pathology

At pathology, IPMN were defined as BD-IPMN in presence of cysts >5 mm without the involvement of the MPD. IPMN with MPD involvement with/without associated cystic lesions were classified as MD-IPMN.[1] According to the Baltimore Consensus, IPMNs were classified as (1) IPMNs with low-grade dysplasia (LGD-IPMN), (2) IPMNs with high-grade dysplasia (HGD-IPMN), and (3) invasive ductal carcinoma arising from IPMN (invasive-IPMN).[19] This last was defined as invasive carcinoma originated within the area with the known pancreatic cyst and extended contiguously to the IPMN; by contrast, pancreatic cancer arising away and separately from the IPMN was considered a concomitant or distinct pancreatic ductal carcinoma and these patients were excluded from our analysis.

Statistical Analysis

Categorical variables are presented as frequencies and percentages, and continuous variables as median and range. Comparisons of categorical variables were conducted using the chi-square or Fisher exact tests depending on the number of observations. Odds ratio (ORs) and 95% confidence intervals (CIs) were calculated using an unadjusted and multivariable-adjusted binomial logistic regression. Continuous variables were compared by Mann-Whitney U test or Kruskal-Wallis test, as appropriate. All hypothesis tests were 2-sided; statistical significance was set at $P < .05$. Statistical analyses were performed using SPSS Statistics 22 (IBM Corporation, Armonk, NY). This study was designed according to the REMARK (REporting recommendations for tumor MARKer prognostic studies) and STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) guidelines.[20,21] All authors had access to the study data and reviewed and approved the final paper.

Results

Of the 148 patients who underwent pancreatic resection for IPMN during the study period, 134 met the selection criteria and were included in the analysis (Figure 1).

Patient characteristics and surgical and pathological data regarding the entire cohort are presented in Tables 1 and 2. Invasive pancreatic cancer arising from IPMN was recognized in 58 (43%) patients, while HGD was present in an additional 16 (12%) patients. The global prevalence of preoperative DM consisted of 37% (n = 50 of 134). In 20 (15%) of them, DM was defined as new-onset or worsening DM: 10 patients showed positive preoperative laboratory tests within 1 month before surgery, while in the remaining 10 cases new or worsening DM led to the diagnosis of IPMN. Overall, 38 (28%) patients were followed for >6 months before surgery. A total of 51% of patients underwent resection because of high-risk stigmata, while only 6 (4.5%) patients without worrisome features or high-risk stigmata at diagnosis or during follow-up were resected for other reasons. In 25 patients of our cohort, new worrisome features or high-risk stigmata or a worsening of them were detected during follow-up. Thirty-five (26%) patients

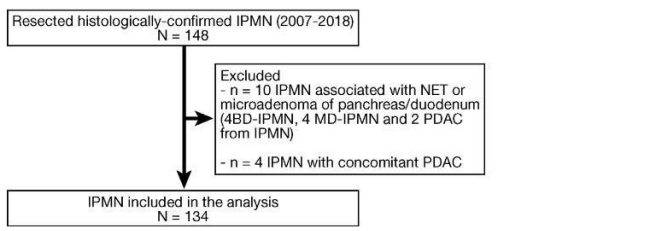

Figure 1. Patient Selection. BD-IPMN, branch-duct intraductal papillary mucinous neoplasm; IPMN, intraductal papillary mucinous neoplasm; MD-IPMN, main-duct/mixed intraductal papillary mucinous neoplasm; NET, neuroendocrine tumor; PDAC, pancreatic ductal adenocarcinoma.

Table 1. Clinical-Morphological Features Based on Diabetes Mellitus

	Entire cohort (N = 134)	Nondiabetic patients	Diabetic patients	*P* value
Male	67 (50)	36 (43)	31 (62)	.032[a]
Age at diagnosis, *y*	68 (34–86)	66.0 (34–85)	70.5 (35–86)	.002[a]
Age at surgery, *y*	69 (35–86)	67.5 (36–85)	71 (35–86)	.009[a]
BMI, kg/m^2	24.6 (16.4–41.0), n = 133	24.5 (18–41)	25,3 (16–41)	.115
Comorbidities				
Prostate hypertrophy	7 (5)	2 (2)	5 (10)	.055
Hypertension	72 (54)	40 (48)	32 (64)	.066
Hypercholesterolemia	25 (19)	12 (14)	13 (26)	.092
Cardiovascular disease	18 (13)	11 (13)	7 (14)	.882
Hepatopathy	13 (10)	7 (8)	6 (12)	.488
Dysthyroidism	39 (29)	28 (33)	11 (22)	.162
Autoimmune disease	6 (5)	3 (4)	3 (6)	.511
History of transplantation	3 (2)	3 (2)	0 (0)	.177
Previous cancers	28 (21)	18 (21)	10 (20)	.844
Chronic pancreatitis	29 (22)	18 (21)	11 (22)	.938
Clinical presentation				
Symptoms at diagnosis				
No	45 (35)	30 (38)	15 (30)	.355
Yes	84 (65)	49 (62)	35 (70)	
Abdominal pain				
No	80 (62)	45 (57)	35 (70)	.137
Yes	49 (38)	34 (43)	15 (30)	
Jaundice				
No	111 (85)	71 (89)	40 (80)	.169
Yes	19 (15)	9 (11)	10 (20)	
Weight loss				
No	97 (76)	66 (84)	31 (63)	.009[a]
Yes	31 (24)	13 (16)	18 (37)	
History of acute pancreatitis				
No	102 (80)	60 (76)	42 (86)	.182
Yes	26 (20)	19 (24)	7 (14)	
Steatorrhea				
No	118 (91)	75 (94)	43 (89)	.237
Yes	11 (9)	5 (6)	6 (11)	
Nausea				
No	116 (91)	71 (90)	45 (92)	.711
Yes	12 (9)	8 (10)	4 (8)	
Preoperative CA 19.9, *ng/mL*	22.5 (0–8667), n = 82	13.5 (0–2370)	35 (1–8667)	.042[a]
Preoperative CEA, *ng/mL*	2.3 (0.5–621.0), n = 66	2.3 (0.5–10.2)	2.4 (0.8–621)	.146
Morphological features				
Multifocal localization	57 (43)	28 (33)	29 (58)	.005[a]
Localization				.001[a]
Head	59 (44)	35 (42)	24 (48)	
Body/tail	36 (27)	31 (37)	5 (10)	
Diffuse	39 (29)	18 (21)	21 (42)	
Cyst size max, *mm*	30 (0–100), n = 120	30 (0–100)	32 (11–80)	.064
Main duct size max, *mm*	7 (2–30), n = 130	6 (2–30)	8 (2–22)	.006[a]
MPD 5–9 *mm*				
No	76 (57)	44 (52)	32 (65)	.146
Yes	57 (43)	40 (48)	17 (35)	
History of acute pancreatitis				
No	102 (80)	60 (76)	42 (86)	.182
Yes	26 (20)	19 (24)	7 (14)	
Abrupt change of caliber				
No	120 (90)	75 (89)	45 (92)	.663
Yes	13 (10)	9 (11)	4 (8)	
Cyst $size \geq 3\,cm$				
No	65 (49)	43 (51)	22 (45)	.484
Yes	68 (51)	41 (49)	27 (55)	
Wall Thickening				
No	120 (91)	80 (96)	40 (82)	.004[a]
Yes	12 (9)	3 (4)	9 (18)	

Table 1. Continued

	Entire cohort (N = 134)	Nondiabetic patients	Diabetic patients	*P* value
Nodule/solid component				
No	72 (54)	53 (64)	19 (39)	.005[a]
Yes	60 (46)	30 (36)	30 (61)	
Lymphadenopathy				
No	106 (80)	69 (83)	37 (75)	.287
Yes	26 (20)	14 (17)	12 (25)	
Obstructive jaundice				
No	111 (85)	71 (89)	40 (80)	.169
Yes	19 (15)	9 (11)	10 (20)	
Enhanced solid component				
No	96 (72)	69 (82)	27 (55)	.001[a]
Yes	37 (28)	15 (18)	22 (45)	
MPD ≥10 mm				
No	93 (70)	67 (80)	26 (53)	.001[a]
Yes	40 (30)	17 (20)	23 (47)	
Positive cytology				
No	126 (95)	79 (94)	47 (96)	.641
Yes	7 (5)	5 (6)	2 (4)	

Values are n (%) or median (range).
BMI, body mass index; CEA, carcinoembryonic antigen; MPD, main pancreatic duct.
[a]Significant *P* value (< .05)

underwent total pancreatectomy; however, pancreaticoduodenectomy was the most frequent procedure (44%). At pathology, 94 (75%) patients presented MD-IPMN, while 31 (25%) IPMNs were described as BD. In 9 patients, the type of IPMN was undetermined or unknown.

DM and Clinical-Morphological Features

Comparison results between diabetic and nondiabetic patients are shown in Tables 1 and 2. Patients with DM were more frequently male and older, with no differences regarding other comorbidities. Surprisingly, median body mass index did not correlate with the presence of a diabetic status (median body mass index: 25.3 kg/m^2 in diabetic patients vs 24.5 kg/m^2 in nondiabetic patients; $P = .115$), neither with progression to malignancy. By contrast, at the time of diagnosis, diabetic patients reported more frequently weight loss as a reason for medical consultation and diagnosis of IPMN (37% in diabetic patients vs 16% in nondiabetic patients; $P = .009$). Seven of the 10 patients in whom new-onset or worsening DM was determined for the diagnosis of the IPMN also presented weight loss at diagnosis, but none of them had jaundice. Jaundice showed no association with DM or new-onset DM. In addition, history of acute and chronic exocrine dysfunction of the pancreatic gland did not correlate with the simultaneous presence of endocrine dysfunction. Interestingly, median CA 19.9 was significantly higher in patients with DM (35 mg/dL in diabetic patients vs 13.5 mg/dL in nondiabetic patients; $P = .042$). Of note, in our series higher CA 19.9 was significantly associated with HGD or invasive cancer (12 mg/dl in LGD vs 39.5 mg/dL HGD or invasive cancer; $P = .023$).

Regarding the morphological features of IPMN, the presence of multifocal disease (58% in diabetic patients vs 33% in nondiabetic patients; $P = .005$) and diffuse location in the pancreatic gland (42% in diabetic patients vs 21% in nondiabetic patients; $P = .001$) was significantly associated with DM, but no differences in median cyst size were found (32 mm in diabetic patients vs 30 mm in nondiabetic patients; $P = .064$). However, 63% of patients with a cyst ≥4 cm had DM ($P = .003$). By contrast, MPD diameter was significantly bigger in diabetic patients (8 mm in diabetic patients vs 6 mm in nondiabetic patients; $P = .006$).

DM and Worrisome Features or High-Risk Stigmata

Within the worrisome features and high-risk stigmata of the updated Fukuoka guidelines, a diabetic status was significantly associated with a MPD ≥10 mm and the presence of a nodule or solid component. Diabetic patients had more frequently a high-risk stigmata than nondiabetic patients (74% in diabetic patients vs 38% in nondiabetic patients; $P < .001$).

DM and Surgical Outcomes

Patients underwent surgery after a median of 2 (range, 0–123) months after diagnosis. In diabetic patients a total pancreatectomy was more frequently carried out (42% in diabetic patients vs 17% in nondiabetic patients; $P = .002$). As well, vascular resections were more frequently performed in patients with DM (30% in diabetic patients vs 12% in nondiabetic patients; $P = .009$). No differences in the complication and mortality

Table 2. Surgical and Pathological Outcomes Based on Diabetes Mellitus

	Entire cohort (N = 134)	Nondiabetic patients	Diabetic patients	*P* value
Surgery				
Type of resection				.002[a]
Duodenopancreatectomy	59 (44)	37 (44)	22 (44)	
Distal pancreatectomy	33 (25)	28 (33)	5 (10)	
Total pancreatectomy	35 (26)	14 (17)	21 (42)	
Enucleation	3 (2)	2 (2)	1 (2)	
Middle pancreatectomy	2 (1.5)	2 (2)	0 (0)	
other	2 (1.5)	1 (1)	1 (2)	
Vascular resection	25 (19)	10 (12)	15 (30)	.009[a]
Complications	77 (58)	49 (58)	28 (56)	.792
Pancreatic fistula	18 (13)	15 (18)	3 (6)	.052
Biliary fistula	4 (3)	3 (4)	1 (2)	.605
Enteric fistula	3 (2)	2 (2)	1 (2)	.885
Abdominal fluid collection	18 (13)	15 (18)	3 (6)	.052
Bleeding	3 (2)	2 (2)	1 (2)	.885
Delayed gastric emptying	16 (12)	8 (10)	8 (16)	.264
Ileus	7 (5)	4 (5)	3 (6)	.755
Wound infection	14 (10)	10 (12)	4 (8)	.475
Systemic complications	42 (31)	25 (30)	17 (34)	.609
Readmission	8 (7)	5 (7)	3 (8)	.765
Pathology				
Type of IPMN				.033[a]
MD-IPMN	94 (75)	56 (69)	38 (86)	
BD-IPMN	31 (25)	25 (31)	6 (14)	
Grade of dysplasia				.018[a]
LGD	60 (45)	45 (54)	15 (30)	
HGD	16 (12)	10 (12)	6 (12)	
Invasive cancer from IPMN	58 (43)	29 (35)	29 (58)	
Malignant IPMN (HGD/invasive)				.008[a]
No	60 (45)	45 (54)	15 (30)	
Yes	74 (55)	39 (46)	35 (70)	
Pathological cyst size, *mm*	30 (4–90), n = 118	29 (4–70)	35 (7–90)	.120
Pathological duct size, *mm*	10 (2–40), n = 56	9.5 (2–40)	10 (2–24)	.554
Epithelial type				
Intestinal	42 (38)	27 (38)	15 (38)	.138
Gastric	54 (49)	38 (53)	16 (41)	
Pancreatobiliary	13 (12)	5 (7)	8 (21)	
Oncocytic	2 (2)	2 (3)	0 (0)	
Presence of chronic pancreatitis				.100
No	47 (36)	34 (42)	13 (27)	
Yes	83 (64)	48 (58)	35 (73)	

Values are n (%) or median (range).
BD-IPMN, branch-duct intraductal papillary mucinous neoplasm; HGD, high-grade dysplasia; IPMN, intraductal papillary mucinous neoplasm; LGD, low-grade dysplasia; MD-IPMN, main-duct/mixed intraductal papillary mucinous neoplasm ; PDAC, pancreatic ductal adenocarcinoma.
[a]Significant *P* value ($< .05$)

rate between diabetic and nondiabetic patients were recognized.

DM and Type of IPMN, Epithelial Subtypes, and Grade of Dysplasia

At pathology, 40% of patients with MD-IPMN vs 19% of patients with BD-IPMN were diabetic ($P = .033$). DM was significantly associated with a higher risk of MPD involvement. (OR, 2.827; 95% CI, 1.059–7.546; $P = .038$). In our cohort, DM did not show an association with specific epithelial subtypes. Gastric and intestinal subtypes were predominant, with no significant differences between diabetic and nondiabetic patients. Of note, in 23 patients, the epithelial type was unknown or undetermined.

Overall, 50% of patients with invasive cancer were diabetic vs 38% and 25% of those with HGD and LGD, respectively ($P = .018$). DM was associated with a higher risk of HGD or invasive cancer in unadjusted analysis (OR, 2.692; 95% CI, 1.283–5.651; $P = .009$). This association remained after adjusting for worrisome features (OR, 2.380; 95% CI, 1.021–5.550; $P = .045$) but was not found after adjusting for high-risk stigmata (OR, 1.546; 95% CI, 0.605–3.948; $P = .363$) (Table 3).

Regarding patients with new-onset or worsening DM, 80% of them harbored a malignant lesion vs 63% and

Table 3. ORs for High-Grade Dysplasia and Invasive Carcinoma by Diabetes Mellitus

	High-Grade Dysplasia/Invasive Cancer From IPMN		
Diabetes	Patients	OR (95% CI)	*P* Value
Yes	50	1.00 (reference)	
No	84		
Unadjusted		2.692 (1.283–5.651)	.009
Adjusted for worrisome features		2.380 (1.021–5.550)	.045
Adjusted for high-risk stigmata		1.546 (0.605–3.948)	.363

Worrisome features: history of acute pancreatitis, cyst size ≥3 cm, thickened cyst wall, nodule/solid component, abrupt change in caliber of main pancreatic duct, lymphadenopathy, and main pancreatic duct diameter 5–9 mm. High-risk stigmata: jaundice, enhancing solid component, and main pancreatic duct dia meter ≥1 cm. CI, confidential interval; IPMN, intraductal papillary mucinous neoplasm; OR, odds ratio.

46% of patients with long-standing DM and without DM, respectively. On the one hand, patients with new-onset or worsening DM showed a significantly higher risk of HGD or invasive cancer in contrast to patients without DM in an unadjusted analysis (OR, 4.615; 95% CI, 1.423–14.698; $P = .011$), and even higher after adjusting for worrisome features (OR, 8.165; 95% CI, 1.967–33.886; $P = .004$) (Table 4). On the other hand, long-standing DM was not associated with an increased risk of HGD or invasive cancer compared with patients without DM (OR, 1.993; 95% CI, 0.845–4.698; $P = .115$) and with new-onset or worsening-DM (OR, 0.432; 95% CI, 0.115–1.622; $P = .214$).

Table 4. Odds Ratios for High-Grade Dysplasia and Invasive Carcinoma by New-Onset/Worsening Diabetes Compared With Nondiabetic Patients

	High-Grade Dysplasia/Invasive Cancer From IPMN		
New-onset/worsening diabetes	Patients	OR (95% CI)	*P* value
Yes	20	1.00 (reference)	
No	84		
Unadjusted		4.615 (1.423–14.698)	.011
Adjusted for worrisome features		8.165,1.967–33.886)	.004
Adjusted for high-risk stigmata		3.853 (0.886–16.747)	.072

Worrisome features: history of acute pancreatitis, cyst size ≥3 cm, thickened cyst wall, nodule/solid component, abrupt change in caliber of main pancreatic duct, lymphadenopathy, and main pancreatic duct diameter 5–9 mm. High-risk stigmata: jaundice, enhancing solid component, and main pancreatic duct CI, confidential interval; IPMN, intraductal papillary mucinous neoplasm; OR, odds ratio.

DM and Weight Loss

In our study, DM but not weight loss was associated with HGD or invasive carcinoma. However, we noticed that 58% (n = 18 of 31) of patients who presented at diagnosis with weight loss had a DM status vs 32% (n = 31 of 97) of those without weight loss ($P = .009$). Importantly, a consistently higher percentage of patients with weight loss showed new-onset or worsening DM in comparison with patients with constant weight (26% [n = 8 of 31] vs 11% [n = 11 of 97]; $P = .026$). Overall, patients with DM had an increased risk of showing associated weight loss at diagnosis (OR, 2.948; 95% CI, 1.284–6.769; $P = .011$). This association persisted even when the analysis was conducted excluding patients with invasive carcinoma and limited to IPMN with LGD and HGD. In this case, 50% (n = 8 of 16) of patients with weight loss at diagnosis were diabetic vs 21% (n = 12 of 66) of patients who maintained the usual weight ($P = .024$). By contrast, when the analysis was restricted only to patients with LGD, DM and weight loss were no longer associated [42% [n = 5 of 12] vs 21% [n = 9 of 44]; $P = .133$).

Discussion

In our retrospective cohort, the prevalence of DM was pretty high, and patients with a previous history of DM revealed a more aggressive disease. Thirty-seven percent of our study population was diabetic at diagnosis, in line with previous studies (15%–43%),[8–11,14,22,23] reaching 50% in patients with invasive carcinoma arising from IPMN, similar to the prevalence reported in patients with PDAC.[17] As a possible explanation of this high prevalence, a case-control by Capurso et al[24] showed that a previous history of DM represents a risk factor for the development of IPMN (OR, 1.79; 95% CI, 1.08–2.98; $P = .025$), even more for MD-IPMNs. In our cohort, DM was significantly associated with the involvement of the MPD, and diabetic patients showed a 2.7-fold higher risk of harboring a malignant IPMN (HGD or invasive cancer). These results are in line with other series investigating predictors of malignant progression in IPMN.[10,11,14,25] However, the possible mechanisms behind this association are still unknown. In fact, in the present literature, only a few papers specifically focus on the role of DM in IPMN,[7,14,24] and it is important to underline a gap of knowledge on this topic.

DM has been more extensively investigated in PDAC. Several authors support the existence of a bidirectional association between DM and PDAC: (1) DM represents a risk factor for the development of PDAC; (2) DM can be induced by pancreatic cancer and be paradoxically associated with weight loss.[15,16] Interestingly, PDAC-induced diabetes, which by definition is new-onset DM, and weight loss precede the onset of other cancer-specific symptoms by several months.[15,26] Indeed, a

reduction of weight seems to begin as early as 3 years before the diagnosis of cancer. Despite this reduction, the glycemic control worsened over time in these patients, in contrast to patients with type 2 diabetes, in whom glycemia improves with weight loss.[26,27] IPMN represents a different entity than PDAC and the association between weight loss and DM has not been investigated yet. In our study, we found that a higher percentage of patients with weight loss at diagnosis were also diabetic in comparison with patients with constant weight (58% vs 32%; $P = .009$). Moreover, these patients who lost weight had more frequently a new-onset or worsening DM. When we excluded patients with invasive cancer, in whose DM and weight loss were expected to be associated, we saw that this association was still present. In other words, this association was already existing in presence of HGD, whereas it was absent among patients with LGD-IPMNs (42% in diabetic patients vs 21% in nondiabetic patients; $P = .151$).

In our series, we also showed that DM remained associated with progression to HGD or invasive cancer even after adjusting for worrisome features (OR, 2.380; 95% CI, 1.021–5.550; $P = .045$) but not after adjusting for high-risk stigmata ($P = .363$). On the same line, Morales-Oyarvide et al[14] found that in patients with MPD <10 mm, DM significantly correlates with a higher risk of HGD but not of invasive cancer. A previous publication focused specifically on low-risk IPMNs (asymptomatic IPMNs without worrisome features) showed that DM, male sex, and recent weight loss are associated with a higher risk of developing HGD and invasive cancer.[12] In addition, in another series, 67% of male patients with Fukuoka-negative BD-IPMN and recent DM (<1 year) harbored a malignant lesion.[13]

Overall, these results suggest that DM appears to be helpful for early prediction of progression, notably in association with weight loss and in low-risk IPMNs. In this setting, further investigations are certainly worthwhile for understanding the pathogenesis of these disorders in IPMN and for including diabetes as worrisome feature in the guidelines for the management of IPMN. Identifying factors predictive for HGD is crucial in the management of IPMN to achieve early diagnosis and prevention of pancreatic cancer and to offer an excellent prognosis to these patients.

Particular emphasis should be placed on new-onset DM. Although included in the European guidelines as relative indication for surgery, the role of new-onset DM in IPMN is considered controversial. In our study, patients with new-onset or worsening DM showed a significantly higher risk of HGD or invasive cancer compared with patients without DM, even after adjusting for worrisome features. By contrast, other series demonstrated that recent-onset DM (diagnosed within 12 months) was not associated with an increased risk of IPMN[24] and of progression to HGD or invasive cancer.[10] It is also necessary to note that in the current literature there is no clear definition of new-onset or recent DM. The onset timing of new-onset DM is often not defined or greatly varies from 1 to 5 years before diagnosis of IPMN.[10,13,14,24] Thus, a consensus on the definition of new-onset DM is needed, and its role should be further investigated.

Limitations of this study include its retrospective nature and the limited sample size that increases the risk of selection and data collection bias and limits the possibility of making definite conclusions. In addition, we are conscious that most of our study population underwent surgery because of worrisome features or high-risk stigmata selecting cases with more aggressive disease. However, even though the management of patients changed over time, patients' selection and surgical treatment were carried out in the same institution by an established multidisciplinary team.

In conclusion, we advise surgeons to give greater importance to DM in IPMN and to be aware that diabetic patients may harbor a more aggressive disease requiring surgery. Further investigations focused on the role of DM and weight loss in larger series of patients are needed to include DM in the guidelines for the management of IPMN.

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Reprint requests
Address requests for reprints to: Ihsan Ekin Demir, MD, PhD, Department of Surgery, Klinikum rechts der Isar, School of Medicine, Technical University of Munich, Ismaninger Straße 22, 81675 Munich, Germany. e-mail: ekin.demir@tum.de; fax: + 49 089-4140-6038.

Conflicts of interest
The authors disclose no conflicts.

Original article

BJS Open Open Access

Prognostic impact of Ki-67 proliferative index in resectable pancreatic ductal adenocarcinoma

I. Pergolini[1], S. Crippa[3], M. Pagnanelli[3], G. Belfiori[1], A. Pucci[1], S. Partelli[3], C. Rubini[2], P. Castelli[4], G. Zamboni[4,5] and M. Falconi[3]

Departments of [1]Surgery and [2]Pathology, Università Politecnica delle Marche, Ospedali Riuniti, Ancona, [3]Pancreatic Surgery Unit, Pancreas Translational and Clinical Research Center, Università Vita e Salute, IRCCS San Raffaele Scientific Institute, Milan,[4]Department of Pathology, Ospedale Sacro Cuore – Don Calabria, Negrar, and [5]Department of Pathology, Università di Verona, Verona, Italy

Correspondence to: Professor M. Falconi, Department of Surgery, Division of Pancreatic Surgery, IRCCS San Raffaele Scientific Institute, Via Olgettina 60, 20132 Milan, Italy (e-mail: falconi.massimo@hsr.it)

Background: Pancreatic ductal adenocarcinoma (PDAC) is an aggressive disease characterized by complex biological features and poor prognosis. A prognostic stratification of PDAC would help to improve patient management. The aim of this study was to analyse the expression of Ki-67 in relation to prognosis in a cohort of patients with PDAC who had surgical treatment.

Methods: Patients who had pancreatic resection between August 2010 and October 2014 for PDAC at two Italian centres were reviewed retrospectively. Patients with metastatic or locally advanced disease, those who received neoadjuvant chemotherapy, patients with PDAC arising from intraductal papillary mucinous neoplasm and those with missing data were excluded. Clinical and pathological data were retrieved and analysed. Ki-67 expression was evaluated using immunohistochemistry and patients were stratified into three subgroups. Survival analyses were performed for disease-free (DFS) and disease-specific (DSS) survival outcomes according to Ki-67 expression and tumour grading.

Results: A total of 170 patients met the selection criteria. Ki-67 expression of 10 per cent or less, 11–50 per cent and more than 50 per cent significantly correlated with DFS and DSS outcomes ($P = 0{\cdot}016$ and $P = 0{\cdot}002$ respectively). Ki-67 index was an independent predictor of poor DFS (hazard ratio (HR) 0·52, 95 per cent c.i. 0·29 to 0·91; $P = 0{\cdot}022$) and DSS (HR 0·53, 0·31 to 0·91; $P = 0{\cdot}022$). Moreover, Ki-67 index correlated strongly with tumour grade ($P < 0{\cdot}001$). Patients with PDAC classified as a G3 tumour with a Ki-67 index above 50 per cent had poor survival outcomes compared with other patients ($P < 0{\cdot}001$ for both DFS and DSS).

Conclusion: Ki-67 index could be of use in predicting the survival of patients with PDAC. Further investigation in larger cohorts is needed to validate these results.

Presented as a poster to Pancreas 2018, Baltimore, Maryland, USA, April 2018, and to the European Pancreatic Club, Berlin, Germany, June 2018; published in abstract form as *Pancreatology* 2018; **18**: S84

Funding information
No funding

Paper accepted 26 March 2019
Published online 10 May 2019 in Wiley Online Library (www.bjsopen.com). **DOI:** 10.1002/bjs5.50175

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive disease characterized by complex biological features and a poor prognosis[1,2]. Recent literature[3–5] in this field has focused on molecular biomarkers and targets to improve staging, treatment and, consequently, patient survival.

The expression of Ki-67 in tumour tissue is a well known marker associated with tumour proliferation and correlated with the progression, risk of metastasis and prognosis of several tumours, including breast and prostate cancers[6–10]. In pancreatic neuroendocrine neoplasms (PanNENs), Ki-67 has been documented to play an essential role in defining tumour grading and classification (WHO 2017/ENETS criteria), and is recognized as an independent predictor of survival[11–16]. Moreover, some authors[17–20] have reported that the Ki-67 index could be determined from selected PanNEN samples

obtained by endoscopic ultrasonography–fine-needle aspiration (EUS–FNA), thereby demonstrating its value in the preoperative phase. In PDAC, the prognostic value of Ki-67 has not yet been established[21–23]. The aim of this study was to analyse the expression of Ki-67 as a prognostic factor in a cohort of patients with resected PDAC, in relation to survival outcomes.

Methods

This study was designed according to the REMARK[24] and STROBE[25] guidelines. It was not preregistered with an analysis plan in an independent institutional registry.

Patients who had a pancreatic resection for histologically confirmed PDAC between August 2010 and October 2014 at the Ospedale Sacro Cuore – Don Calabria (Negrar, Verona, Italy), a teaching hospital affiliated to the University of Verona, and at the Ospedali Riuniti Ancona Università Politecnica delle Marche (Ancona, Italy), a university hospital and referral centre for hepatobiliopancreatic surgery in the Marche Region, were reviewed retrospectively. Surgical resections were performed in both centres by the same surgeons. Both institutions were documented as high-volume centres for pancreatic surgery (more than 100 pancreatic resections annually) at the time of the study.

Patients with metastatic or locally advanced disease, those who had received neoadjuvant chemotherapy, patients with PDAC arising from intraductal papillary mucinous neoplasms, and patients with missing data or follow-up were excluded. Written informed consent for use of their personal data and tissue for research purposes was obtained from all patients included in the study. Institutional review board approval was not required owing to the retrospective nature of the study.

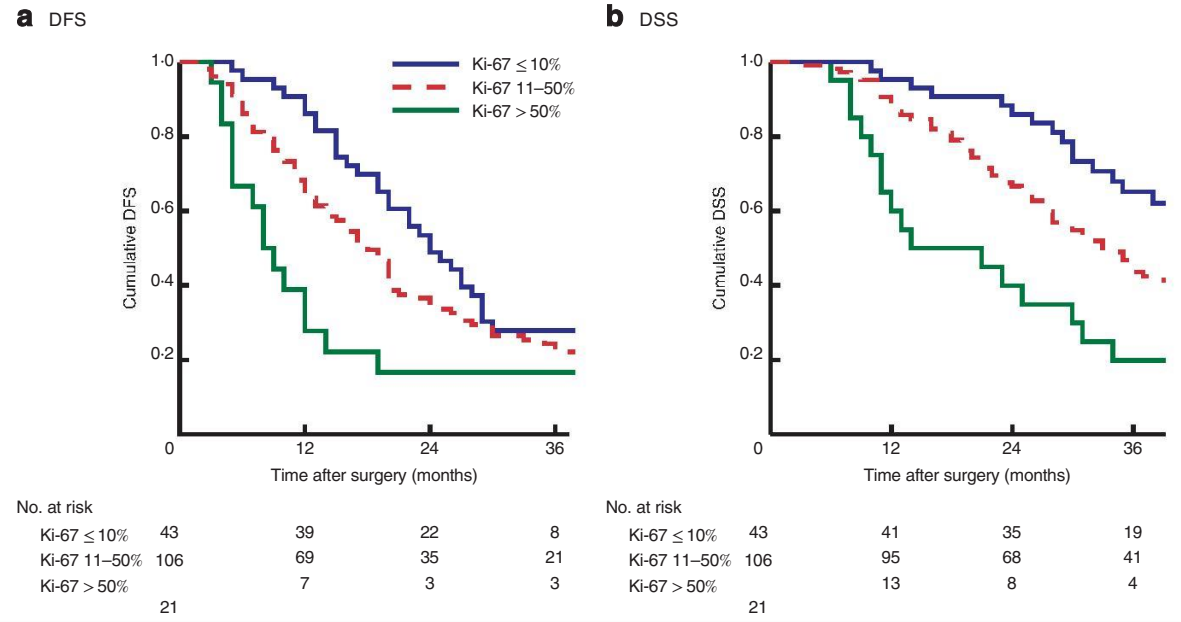
Data on patient demographics, clinical presentation, tumour marker levels (serum carbohydrate antigen (CA) 19-9 and carcinoembryonic antigen (CEA)), preoperative treatments, surgical and postoperative data, including delivery of adjuvant treatment, were recorded. In the absence of jaundice, the preoperative concentration of CA19-9 was recorded; in patients with abnormal serum bilirubin values at the time of diagnosis, the CA19-9 level was determined after biliary drainage and jaundice resolution. Pathology data included tumour size and grade, number of resected/positive lymph nodes, TNM staging, lymphatic and vascular invasion, perineural invasion and margin status. Glandular differentiation and mitotic activity were evaluated in the entire tumour specimen and the more severe grades were recorded. TNM staging was done in accordance with the 7th AJCC system[26], and margin status was determined according to the 2010 WHO definition[27].

Table 1 Details of patients who had upfront surgery

	No. of patients* (*n* = 170)
Age (years)†	70 (44–85)
Sex ratio (M : F)	92 : 78
Preoperative tumour marker levels†	
CEA (ng/ml)	2 (0–90)
CA19-9 (units/ml)	36 (0–2689)
Jaundice at diagnosis	113 (66·5)
Duration of surgery (min)†	343 (120–575)
Postoperative complications	86 (50·6)
Pancreatic fistula	46 (27·1)
Biliary fistula	12 (7·1)
Duration of hospital stay (days)†	11 (5–70)
Readmission	50 (29·4)
Ki-67 index (%)†	30 (2–95)
≤ 10	43 (25·3)
11–50	106 (62·4)
> 50	21 (12·4)
Tumour size (mm)†	25 (2–70)
Grade of differentiation	
G1	15 (8·8)
G2	86 (50.6)
G3	69 (40·6)
T category	
T1	12 (7·1)
T2	10 (5·9)
T3	148 (87·1)
T4	0 (0)
N category	
N0	48 (28·2)
N1	122 (71·8)
Resection margin	
R0	120 (70·6)
R+	50 (29·4)
Lymphatic invasion	126 (74·1)
Vascular invasion	115 (67·6)
Perineural invasion	145 (85.3)
Stage	
Ia	7 (4·1)
Ib	2 (1·2)
IIa	38 (22·4)
IIb	123 (72·4)
Adjuvant treatment	166 (97·6)
Recurrence	135 (79·4)
Died	
Yes, from other cause	6 (3·5)
Yes, from pancreatic cancer progression	109 (64·1)

*With percentages in parentheses unless indicated otherwise; †values are median (range). CEA, carcinoembryonic antigen; CA, carbohydrate antigen.

Fig. 1 Kaplan–Meier analysis of survival in Ki-67 index subgroups. a Disease-free (DFS) and b disease-specific (DSS) survival in patients with a Ki-67 index of 10 per cent or less, 11–50 per cent and more than 50 per cent. a $P = 0{\cdot}016$, b $P = 0{\cdot}002$ (log rank test)



Ki-67 expression

Formalin-fixed specimens were processed into paraffin according to standard practice. Sections (5 μm) were stained with haematoxylin and eosin for conventional histological examination, and used for Ki-67 immunohistochemical analysis. For Ki-67 immunohistochemical staining, after deparaffinization in xylene for 30–40 min, the specimen slides were rehydrated in a descending alcohol series, from absolute ethanol to distilled water. Before staining, in order to retrieve antigen epitopes, the samples were heated in an aqueous sodium citrate solution in a microwave oven (temperature 98°C, pH 6) for 20 min. After microwave treatment, the sections were cooled down for a further 20 min. Endogenous peroxidase was blocked by 0·3 per cent hydrogen peroxide for 7 min. After washing in Tris-buffered saline (TBS), the slides were incubated at room temperature for 30 min with the primary antibody for Ki-67. The primary antibody was a monoclonal mouse antihuman Ki-67 antigen (MIB-1; Dako, Glostrup, Denmark) used at a dilution of 1 : 80. After incubation, the primary antibody was washed away with TBS. The slides were then incubated at room temperature for 20 min, using the visualization system EnVision™ FLEX/HRP (Dako) containing the secondary antimouse/rabbit antibody. Final staining was done with diaminobenzidine tetrahydrochloride (DAB) solution for 10 min at room temperature. Slides were then transferred through an ascending ethanol series, finally through xylene, and then mounted.

Two tissue blocks for each patient were selected from the most representative area of the tumour (the region of the tumour with highest grade). A section of each block was immunolabelled for Ki-67 using the above protocol. Counting of tumour cells was done manually using a Nikon Eclipse 80i microscope (Nikon Instruments, Amsterdam, the Netherlands), at 40× magnification. A counting protocol of 1000 cells was chosen to overcome the marked cellular heterogeneity for each carcinoma, as the number of high-power fields could be variable. The percentage of Ki-67-positive cells was determined by scoring a minimum of 1000 cells within a hotspot area (defined as the area in which the 1000-cell count provided the highest percentage of Ki-67-positive nuclei). Of note, the Ki-67 index was counted in hotspot areas that did not necessarily parallel the histological grade field by field.

Outcome measure

Primary outcome measures were disease-free survival (DFS), the first recurrence of cancer after surgery, and disease-specific survival (DSS), death from the disease.

Table 2 Univariable and multivariable analyses of predictors of disease-free survival

	Univariable analysis			Multivariable analysis	
	n	Median DFS (months)	*P*	Hazard ratio	*P*
Age (years)			0·101		
≤ 70	93	20			
> 70	77	18			
Sex			0·821		
M	92	19			
F	78	20			
Jaundice			0·665		
No	57	19			
Yes	113	19			
Preoperative CA19·9 (units/ml)			0·052		
≤ 200	135	17			
> 200	35	24			
Preoperative CEA (ng/ml)			0·562		
≤ 2	109	18			
> 2	61	20			
Postoperative complications			0·894		
No	84	20			
Yes	86	18			
Ki-67 index (%)			0·016	0·52 (0·29, 0·91)	0·022
≤ 10	43	24			
11–50	106	18			
> 50	21	8			
Tumour size (mm)			0·783		
≤ 25	98	19			
> 25	72	17			
Grade of differentiation			0·039	0·77 (0·54, 1·12)	0·169
G1	15	29			
G2	86	20			
G3	69	13			
T category			0·732		
T1–2	22	20			
T3	148	19			
N category			< 0·001	2·28 (1·48, 3·53)	< 0·001
N0	48	26			
N1	122	16			
Margin status			0·026	1·55 (1·06, 2·28)	0·024
R0	120	20			
R+	50	13			
Vascular invasion			0·756		
No	55	19			
Yes	115	20			
Perineural invasion			0·452		
No	25	19			
Yes	145	19			
Stage			< 0·001		
Ia, Ib, IIa	47	29			
IIb	123	16			
Adjuvant treatment			0·842		
No	4	26			
Yes	166	19			

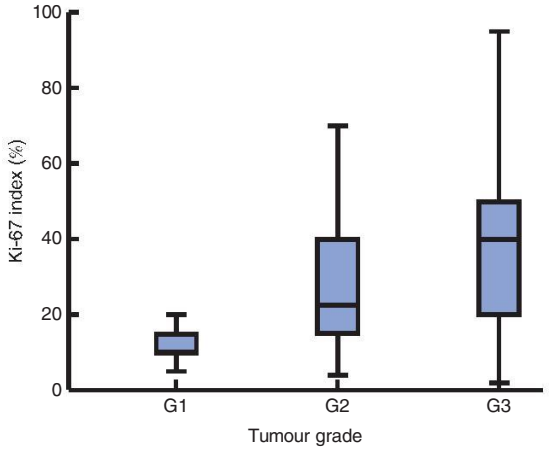
Values in parentheses are 95 per cent confidence intervals. DFS, disease-free survival; CA, carbohydrate antigen; CEA, carcinoembryonic antigen.

Table 3 Univariable and multivariable analyses of predictors of disease-specific survival

	Univariable analysis			Multivariable analysis	
	n	Median DSS (months)	*P*	Hazard ratio	*P*
Age (years)			0·573		
≤ 70	93	33			
> 70	77	35			
Sex			0·705		
M	92	35			
F	78	34			
Jaundice			0·597		
No	57	32			
Yes	113	35			
Preoperative CA19·9 (units/ml)			0·344		
≤ 200	135	33			
> 200	35	37			
Preoperative CEA (ng/ml)			0·626		
≤ 2	109	33			
> 2	61	36			
Postoperative complications			0·918		
No	84	36			
Yes	86	34			
Ki-67 index (%)			0·002	0·53 (0·31, 0·91)	0·022
≤ 10	43	47			
11–50	106	33			
> 50	21	14			
Tumour size (mm)			0·697		
≤ 25	98	35			
> 25	72	33			
Grade of differentiation			0·001	0·63 (0·43, 0·94)	0·022
G1	15	n.r.			
G2	86	38			
G3	69	25			
T category			0·106		
T1–2	22	56			
T3	148	33			
N category			< 0·001	3·37 (1·42, 3·94)	0·001
N0	48	n.r.			
N1	122	30			
Margin status			0·003	1·93 (1·28, 2·89)	0·002
R0	120	41			
R+	50	27			
Vascular invasion			0·337		
No	55	34			
Yes	115	35			
Perineural invasion			0·128		
No	25	66			
Yes	145	33			
Stage			< 0·001		
Ia, Ib, IIa	47	n.r.			
IIb	123	30			
Adjuvant treatment			0·406		
No	4	56			
Yes	166	34			

Values in parentheses are 95 per cent confidence intervals. DSS, disease-specific survival; CA, carbohydrate antigen; CEA, carcinoembryonic antigen; n.r., not reached.

Fig. 2 Box-and-whisker plot of Ki-67 index according to tumour grade of differentiation. Median Ki-67 index values, interquartile ranges and ranges are denoted by horizontal bars, boxes and error bars respectively. $P < 0{\cdot}001$ (Kruskal–Wallis test)

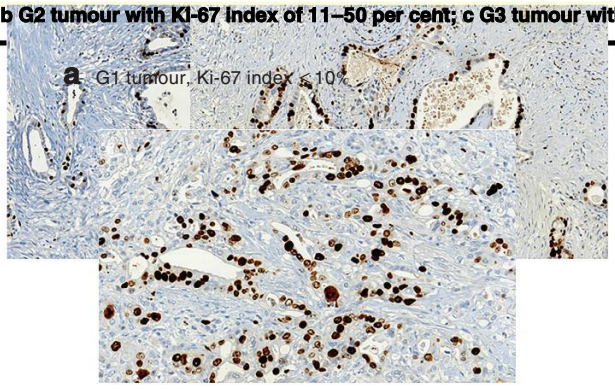


Follow-up was done on a regular basis by clinical evaluation or telephone interview, and patients were censored at the last available contact date.

Statistical analysis

Continuous variables are reported as median (range) values, and categorical variables as numbers with percentages. Continuous variables were dichotomized around the median value, except for CA19-9, for which a cut-off value of 200 units/ml or more was previously documented[28,29] to correlate with tumour burden, spread and early recurrence after resection of PDAC. Student's *t* test was used to compare normally distributed continuous variables; non-parametric analyses included Mann–Whitney *U* and Kruskal–Wallis tests. Survival analysis was done with the Kaplan–Meier method and log rank test using the following Ki-67 cut-off values: 10, 20, 30, 40, 50 and 60 per cent, tertiles and quartiles. Patients were also stratified according to Ki-67 index and tumour grades, and survivals were calculated accordingly.

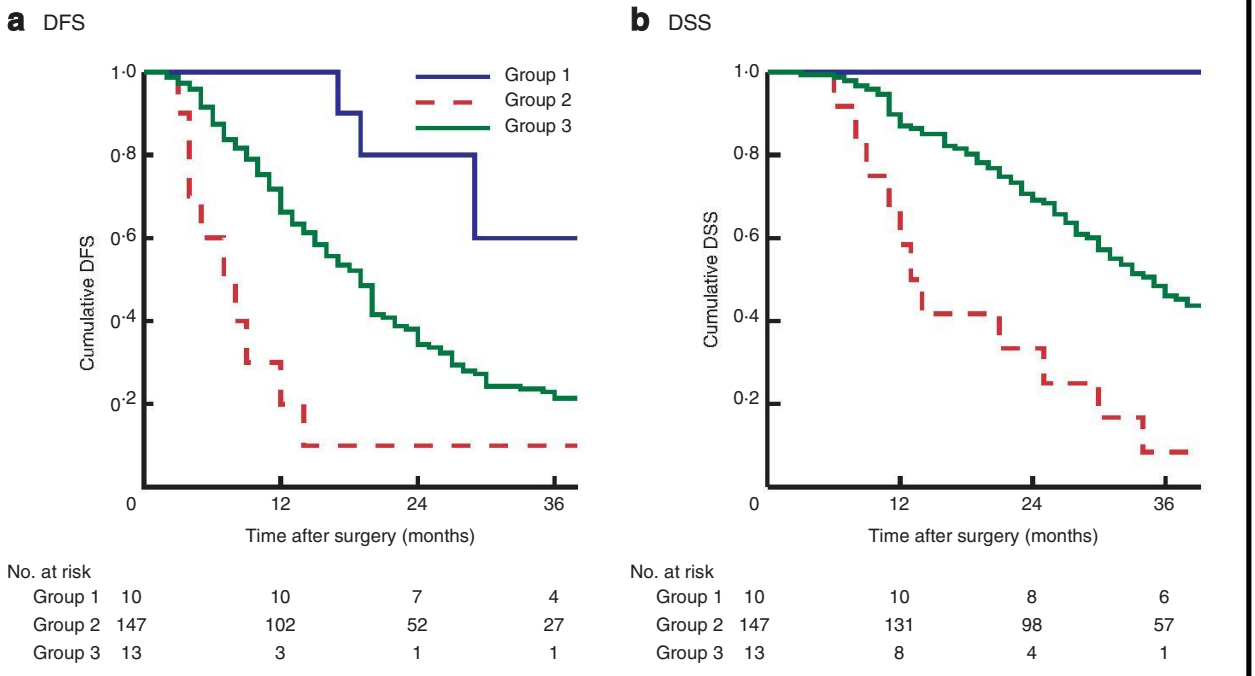
Fig. 3 Ki-67 immunohistochemical staining in pancreatic ductal adenocarcinoma. a G1 tumour with Ki-67 index of 10 per cent or less; b G2 tumour with Ki-67 index of 11–50 per cent; c G3 tumour with Ki-67 index above 50 per cent



b G2 tumour, Ki-67 index 11–50%

c G3 tumour, Ki-67 index > 50%

Fig. 4 Kaplan–Meier analysis of survival according to Ki-67 and tumour grade. a Disease-free (DFS) and b disease-specific (DSS) survival in patients with G1 tumours and Ki-67 index of 10 per cent or less (group 1), G3 tumours and Ki-67 index above 50 per cent (group 2), and all other patients (G1 tumours and Ki-67 index above 10 per cent, G2 tumours with any Ki-67 value and G3 tumours with Ki-67 index of 50 per cent or less) (group 3). a,b $P < 0{\cdot}001$ (log rank test)



Multivariable analysis was performed using the Cox regression model to evaluate significant predictors of DFS and DSS. Significant variables in the≤univariable analysis were included as co-variables; *P* 0·050 was considered significant. Statistical analyses were performed in SPSS® version 22.0 for Windows® (IBM, Armonk, New York, USA).

Results

Of 272 patients who underwent resection for PDAC during the study period, 170 met the selection criteria (*Fig. S1*, supporting information).

Patient characteristics, surgical and pathological data are presented in *Table 1*. PDACs were poorly differentiated (grade G3) in 40·6 per cent of patients, assessed as having T3 status in 87·1 per cent, with lymph node metastasis in 71·8 per cent of the cohort. Lymphatic invasion was documented in 100 per cent of the tumours with a positive N status, but was present in only 8 per cent (4 of 48) of N0 tumours. Some 67·6 per cent of tumours showed microvascular invasion and 85·3 per cent had perineural invasion. Stage IIb tumours were found in 72·4 per cent of patients. The median Ki-67 index was of 30 (range 2–95) per cent.

Survival outcomes

Median follow-up was 32 (range 0–76) months. Some 135 patients (79.4 per cent) had a recurrence. Median DFS was 19 (i.q.r. 35–10) months, and median DSS was 35 (not reached to 21) months. Ki-67 expression of 10 and 50 per cent were the only cut-off values significantly associated with DFS and DSS. On this basis, survival analysis was determined using the following Ki-67 intervals: 10 per cent or less, 11–50 per cent and more than 50 per cent. Median DFS was 24, 18 and 8 months for these respective Ki-67 index values ($P = 0.016$) (*Fig. 1a* and *Table 2*). Cox regression analysis showed that Ki-67 index (hazard ratio (HR) 0·52, 95 per cent c.i. 0·29 to 0·91; $P = 0{\cdot}022$), N status (HR 2·28, 1·48 to 3·53; $P < 0{\cdot}001$) and resection margin status (HR 1·55, 1·06, 2·28; $P = 0{\cdot}024$) were independent predictors of DFS (*Table 2*).

DSS decreased significantly in the 10 per cent or less, 11–50 per cent and more than 50 per cent subgroups (47

versus 33 *versus* 14 months respectively; $P = 0{\cdot}002$) (*Fig. 1b*; *Table 3*). Cox regression analysis identified Ki-67 index (HR 0·53, 95 per cent c.i. 0·31 to 0·91; $P = 0{\cdot}022$), tumour grade (HR 0·63, 0·43 to 0·94; $P = 0{\cdot}022$), N status (HR 3·37, 1·42 to 3·94; $P = 0{\cdot}001$) and resection margin status (HR 1·93, 1·28 to 2·89; $P = 0{\cdot}002$) as independent predictors of DSS (*Table 3*).

Stage and lymphatic invasion were not considered in the Cox regression analysis because of the overlap with N status.

Ki-67 and grading

Median Ki-67 was significantly higher in G3 tumours (*Fig. 2*). Tumours with a Ki-67 index above 50 per cent showed more aggressive grading: 62 per cent (13 of 21) had a pathological grade consistent with G3, whereas none was assessed as G1. By contrast, G3 tumours showed a more heterogeneous Ki-67 expression (*Fig. S2*, supporting information). In patients with G3 tumours, a Ki-67 index above 50 per cent was associated with significantly worse median survival than a Ki-67 index of 50 per cent or less (DFS: 7 *versus* 15 months respectively, $P = 0{\cdot}035$; DSS: 13 *versus* 29 months, $P = 0{\cdot}038$). There was no association between Ki-67 index and other pathological parameters, including T status, N status, tumour size, vascular or perineural invasion.

Patients were categorized into three subgroups: patients with G1 tumours with a Ki-67 index of 10 per cent or less (group 1); patients with G3 tumours with a Ki-67 index above 50 per cent (group 2); all other patients (those with G1 tumours with a Ki-67 index above 10 per cent, G2 tumours with any Ki-67 index value and G3 tumours with a Ki-67 index of 50 per cent or less) (group 3) (*Fig. 3*). Patients in group 2 had poor median survival outcomes compared with those in groups 1 and 3 (DFS: 7 months *versus* median survival not reached *versus* 19 months respectively, $P < 0{\cdot}001$; DSS: 13 months *versus* median survival not reached *versus* 35 months, $P < 0{\cdot}001$) (*Fig. 4*).

Discussion

Surgical resection followed by adjuvant chemotherapy/chemoradiotherapy is considered the standard of care for localized and resectable pancreatic cancer; however, the majority of patients develop tumour recurrence, and up to 30 per cent die within 1 year after surgery[29–31]. Early recurrences are related to aggressive tumours, probably associated with micrometastatic disease undetected at operation[30,31]. There is therefore a need to identify more aggressive subtypes of PDAC in order to improve their management.

Ki-67 is a well known marker of cellular proliferation[7]. Previous experience[3,22] focusing on PDAC showed that high Ki-67 expression was associated with poor pathological features, including poor tumour differentiation and presence of lymph node metastasis.

The present study evaluated the prognostic role of Ki-67 in a series of 170 patients with PDAC and found that patients with a Ki-67 index above 50 per cent had median DFS and DSS approximately threefold lower than those with a Ki-67 index of 10 per cent or less (DFS: 8 *versus* 24 months respectively; DSS: 14 *versus* 47 months). In contrast, past reports showed no association between Ki-67 and overall survival[3,21], although Ki-67 index was associated with the risk of recurrence within 1 year after resection[23].

In the present study a strong association between Ki-67 index and tumour grade was also found. As expected, the combination of Ki-67 index above 50 per cent and G3 grade was associated with a greater risk of recurrence and poor survival.

The present results may have clinical implications for patients' prognostic stratification. The Ki-67 index, as an expression of a more biologically unfavourable disease, might help to discriminate which patients should receive more aggressive adjuvant treatment. Currently, neoadjuvant chemotherapy is recommended for patients with anatomically borderline resectable pancreatic cancer at increased risk of early recurrence[30,32,33]. Preoperative assessment of the Ki-67 index by EUS–FNA may help to identify patients with marginally resectable tumours based on clinical criteria, who may benefit more from neoadjuvant chemotherapy than upfront surgery, given the high risk of early postoperative recurrence (those with a Ki-67 index above 50 per cent), although the feasibility of this should be investigated further.

Limitations of this study include its retrospective design and some issues relating to Ki-67 analysis, including intratumoral and intertumoral heterogeneity[18,21,34]. In addition, the immunohistochemistry protocol may have involved some interobserver variability in determining the percentage of Ki-67-positive cells[12,34]. To limit the lack of uniformity and consistency in quantification, several imaging methods have been developed to be used in routine practice[12,18]. However, standardization is needed to enable wider use of the index. Further investigations in larger cohorts are needed to validate these results.

Acknowledgements

I.P. and S.C. contributed equally to this paper.
Disclosure: The authors declare no conflict of interest.

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Digestive and Liver Disease xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Digestive and Liver Disease

journal homepage: www.elsevier.com/locate/dld

Oncology

SUVmax after (18)fluoro-deoxyglucose positron emission tomography/computed tomography: A tool to define treatment strategies in pancreatic cancer

Ilaria Pergolini[a,1], Stefano Crippa[b,1], Matteo Salgarello[c], Giulio Belfiori[a], Stefano Partelli[b], Giacomo Ruffo[d], Alessandro Pucci[a], Giuseppe Zamboni[e], Massimo Falconi[b,*]

[a] Department of Surgery, Universita' Politecnica delle Marche, Ancona, Italy
[b] Pancreatic Surgery Unit, Pancreas Translational & Clinical Research Center, IRCCS San Raffaele Scientific Institute, Milan, Italy
[c] Department of Nuclear Medicine, Ospedale Sacro Cuore-Don Calabria, Negrar, Italy
[d] Department of Surgery, Ospedale Sacro Cuore-Don Calabria, Negrar, Italy
[e] Department of Pathology, Ospedale Sacro Cuore-Don Calabria, Negrar, Italy

ARTICLE INFO

Article history:
Received 23 July 2017
Received in revised form
13 September 2017
Accepted 14 September 2017
Available online xxx

Keywords:
Pancreatic tumor
Positron emission tomography
Recurrence
Survival

ABSTRACT

Background: (18)fluoro-deoxyglucose positron emission tomography/computed tomography (18FDG-PET/CT) might be a useful tool in the management of pancreatic ductal adenocarcinoma (PDAC).
Aims: The aim of this study was to analyze maximum standard uptake value (SUVmax) after 18FDG-PET/CT as predictor of survival outcomes and method to determine treatment strategies.
Methods: A consecutive series of patients who underwent preoperative 18FDG-PET/CT and subsequent resection for PDAC were retrospectively reviewed. Patients who underwent neoadjuvant chemotherapy were excluded.
Results: 46 patients were included in the analysis. Median follow-up was 27 months (4–67). Patients who recurred within 12 months showed a significantly higher preoperative median SUVmax (8.1 vs 6.1, $p = 0.039$). Receiver operating characteristics (ROC) curves for disease-free survival (DFS) and disease-specific survival (DSS) identified SUVmax of 6.0 as optimal cut-off. Multivariate analysis showed that SUVmax ≥ 6.0 was an independent predictor of poor DFS (HR 2.288, $p = 0.024$) and DSS (HR 4.875, $p < 0.001$). The combination of SUVmax ≥ 6.0 with CA19.9 ≥ 200 U/ml was significantly associated with survival outcomes in comparison to patients without concordantly elevated values.
Conclusion: SUVmax ≥ 6.0 is an independent predictor of DFS and DSS in resected PDAC. 18FDG-PET/CT might be considered in the preoperative evaluation of patients with pancreatic cancer.

1. Introduction

Despite significant advances in cancer diagnosis and treatment, pancreatic ductal adenocarcinoma (PDAC) still has a very poor prognosis. Surgical resection remains the only treatment with curative intent with a 5-year survival rate of 20–25% [1]. Survival outcomes are negatively influenced by early recurrence following pancreatic resection, because of unrecognized or rapidly progressive metastatic disease [2]. The diagnosis of occult metastases and the appropriate assessment of resectability of PDAC is still challenging [3–6]. Therefore, identification of prognostic factors associated with early recurrence and poor outcomes in patients with resectable PDAC is of paramount importance in order to avoid useless resections and consider neoadjuvant chemotherapy instead of upfront surgery in these patients [7].

Whole body 2-(fluorine-18)fluoro-deoxy-D-glucose-positron emission tomography in combination with computed tomography (18FDG-PET/CT) is considered a useful tool for the diagnosis and staging of different malignancies [8–10]. However, the role of preoperative 18FDG-PET/CT in patients with potentially resectable PDAC remains debated [11–13]. Although some studies showed

* Corresponding author at: Department of Surgery, Division of Pancreatic Surgery, San Raffaele Scientific Institute, Via Olgettina 60, 20132 Milan, Italy.
E-mail address: falconi.massimo@hsr.it (M. Falconi).
[1] Ilaria Pergolini and Stefano Crippa contributed equally to the paper.

http://dx.doi.org/10.1016/j.dld.2017.09.122
1590-8658/

that PET/CT might improve the management of patients with pancreatic cancer [5,11,14], we previously analyzed the impact of 18FDG-PET/CT as a staging procedure in resectable PDAC supporting that the systematic use of the procedure was not justified [15]. Finally, some Authors showed that maximum standardized uptake value (SUVmax) was associated with survival in resectable pancreatic cancer [14,16,17].

In the present study, we aimed to evaluate the role of SUVmax after 18FDG-PET/CT as a preoperative prognostic predictor of recurrence and survival in patients undergoing surgery for resectable PDAC, and a tool to detect patients that may benefit more from surgery instead of neoadjuvant treatments.

2. Methods

In this retrospective work, the study population was represented by all consecutive patients who underwent 18FDG-PET/CT followed by pancreatic resection for histologically-confirmed PDAC between May 2011 and July 2012 at the Ospedale Sacro Cuore-Don Calabria (Negrar, Italy). Patients who underwent neoadjuvant treatment prior to surgery, were not included in the analysis. In these patients, 18FDG-PET/CT was carried out after neoadjuvant chemotherapy, missing a baseline evaluation; therefore, to avoid any bias on SUVmax by treatment and tumor-downstaging, we excluded these patients.

Patients' demographics, clinical presentation and tumor markers levels (serum carbohydrate antigen 19.9, CA19.9, and carcinoembryonic antigen, CEA) were collected for all patients. The value of CA19.9 was recorded in absence of jaundice. In those patients with abnormal values of serum bilirubin at the time of diagnosis, CA19.9 level was collected after biliary drainage and jaundice resolution. Preoperative diagnostic work-up and staging procedures as well as criteria for surgical resectability were discussed in details in a previous paper [15]. Briefly, the resectability was based on the following criteria: (1) absence of abutment/encasement of portal vein, superior mesenteric vein, hepatic artery, superior mesenteric artery, celiac trunk; (2) absence of infiltration of peripancreatic organs with the exception of common bile duct and duodenum; (3) absence of distant metastases. Operative and postoperative data, complications and pathology data were prospectively gathered. Formal pancreatic resections were carried out with standard lymphadenectomy as described elsewhere.

Classification and grading of pancreatic ductal adenocarcinoma was based on the WHO 2010 criteria [18]. Tumor (T), nodal status (N) and grade (G) were determined using standard TNM classification according to AJCC classification [19]. Intraoperative evaluation of the resection margins was performed routinely. The absence or presence of residual tumor after surgery (R0/R1/R2 resection) was defined based on the AFIP criteria [20].

2.1. PET-TC study

All patients underwent 18FDG-PET/CT within a week before the day of surgery. They were asked to fast for at least 6 h before the examination. The blood glucose level of each patient was determined before the examination. Scanning of patients with diabetes mellitus was not performed until the blood glucose level was less than 140 mg/dl. All examinations were carried out by a single, highly experienced, nuclear medicine physician (MS). All the tests were performed using a hybrid PET/CT scanner (Siemens mCT Biograph, Germany). The whole-body CT scanning was performed using a continuous spiral technique on a 64-slice helical CT, and the PET scanner had three detector rings. No contrast medium was administered during CT scanning. After CT scan, an emission scan was performed from the head to the thigh after the intravenous injection of 0.08 mC/kg (2.96 MBq/kg) FDG. PET scanning is performed with a 16 cm bed for about 7/8 bed per patient. CT and PET scan data were co-registered. The standardized uptake value (SUV) was acquired using the attenuation-corrected images, the amount of injected FDG, the body weight of the patient, and the cross-calibration factors between PET and the dose calibrator. Maximum SUV (SUVmax) was evaluated 60 min after FDG injection.

Table 1
Patient characteristics at diagnosis and neoadjuvant treatment details.

	N = 46	%
Sex		
Male	23	50
Female	23	50
Median age, years (range)	67	43–81
Symptomatic patients	40	87
Presence of diabetes	10	22
Symptoms		
Jaundice	28	61
Weight loss	18	39
Abdominal pain	19	41
Occlusion/vomiting	1	2
Acute pancreatitis	0	0
Tumor markers		
CEA[a], ng/ml, median (range)	1.8	0.5–19.9
CA19-9[b], U/ml, median (range)	63.3	0.8–1805.0
SUVmax60 after 18FDG-PET/CT[c], median (range)	6.7	2.5–24.5
Adjuvant treatment	42	91

[a] CEA carcinoembryonic antigen.
[b] CA19.9 serum carbohydrate antigen 19.9.
[c] SUVmax after 18FDG-PET/CT maximum standard uptake value after (18)fluoro-deoxyglucose positron emission tomography/computed tomography at 60 min.

2.2. Statistical analysis

Distributions of continuous variables are reported as median and minimum/maximum range. Categorical variables are presented as numbers and percentages. Survival time was calculated starting from the date of surgery. A receiver operating characteristics (ROC) curve was constructed to determine the optimal cutoff value of SUVmax. ROC curve analyses were conducted for disease free survival (DFS) and disease specific survival (DSS). Continuous variables were dichotomized around the median value. Since CA19.9 $\geq$200 U/ml seems to be correlate with tumor burden, tumor spread and early recurrence after resection in PDAC [2,21–23], this value was assigned as cut off in the survival analysis of our study. When comparing two groups, normally distributed continuous variables were analyzed using a two-sample Student t test, while Mann-Whitney U test was used for non-normally distributed variables. Survival analysis was conducting using the Kaplan–Meier method and log-Rank test. Disease specific survival was measured from the time of surgery. Multivariate analysis was performed by the Cox regression model to evaluate significant predictors of DFS and DSS. P values were considered significant when less or equal than 0.05. Statistical analyses were performed in SPSS 22.0 for Windows software (SPSS Inc, Chicago, IL).

3. Results

Forty six of the 60 consecutive patients who underwent 18FDG-PET/CT followed by pancreatic resection were included in the study (50% female; median age 67 years, range 43–81); 14 patients (23%) underwent neoadjuvant chemotherapy and were excluded from the analysis.

Patients' characteristics were listed in Table 1. Median SUVmax was 6.7 (range 2.5–24.5). Median CA19.9 value was 63.3 U/ml (range 0.8–1805.0). Table 2 shows operative procedures, postoper-

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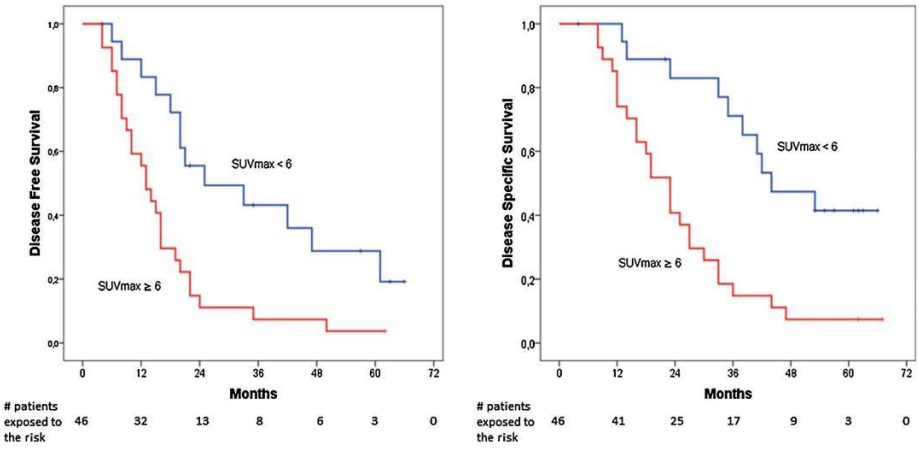

Fig. 1. Kaplan–Meyer curves. Patient with SUVmax (maximum standard uptake value) ≥6.0 had a significantly worse median disease free survival (DFS) and disease specific survival (DSS) compared with those with SUVmax <6.0 (DFS: 13 vs 25 months, p = 0.003; DSS: 23 vs 44 months, p < 0.001).

Table 2
Operative procedures, postoperative complications and pathology data.

	N	%
Pancreatic resection	46	100
Pancreaticoduodenectomy	35	76
Left pancreatectomy and splenectomy	7	15
Total pancreatectomy	4	9
Overall morbidity	28	47
Pancreatic fistula	11	24
Abdominal collection	10	22
Sepsis	6	13
Delayed gastric emptying	6	13
Bleeding	4	9
Chilous fistola	4	9
Postoperative mortality	0	0
Median tumor size (mm), range	25	10–50
Ki67 (%), range	30	5–70
Grading		
G1	0	0
G2	23	50
G3	23	50
Presence of microvascular invasion	44	96
Presence of perineural invasion	41	89
R status		
R0	38	83
R1	8	17
T stage		
T1	0	0
T2	1	2
T3	45	98
N status		
N0	9	20
N1	37	80

ative complications and pathology data. There was no in-hospital or 90-day mortality. Nodal metastases were found in 80%. Median follow-up was 27 months (range 4–67). Two patients (4%) died during follow-up for no disease-related causes, respectively 4 and 22 months after resection. Both patients did not experience recurrence. Recurrence rate was 85% (39 patients) and 77% of these patients developed metastatic disease. Early post-operative recurrence, defined as recurrence within 6 months from surgery, occurred in 5 (11%) patients. After 12 months from surgery, the rate of patients that had tumor recurrence increased up to 35% and these patients showed a significantly higher preoperative median SUVmax (8.1 vs 6.1, p = 0.039). Median DFS was 16 months (IQR 10–33) for the entire cohort, while median DSS was 30 months (IQR 16–47).

The ROC curves for DFS and DSS, with an area under the curve (AUC) of 0.705 and 0.727 respectively, identified SUVmax of 6.0 as the optimal cut off (DFS: sensibility 67%, specificity 86%; DSS: sensibility 71%, specificity 82%). The median DFS time was 25 months (IQR 18–61) in patients with SUVmax <6.0 and 13 months (IQR 8–20) in patients with SUVmax ≥6.0 (p = 0.003). Furthermore, SUVmax <6.0 was associated with a better median DSS compared with patients with SUV max ≥6.0 (23 vs 44 months, p < 0.001).

Table 3 shows the univariate and multivariate analysis for DFS. Independent predictors of worse DFS were SUVmax ≥6.0 (HR 2.288, p = 0.024), CA19.9 ≥200 U/ml (HR 2.935, p = 0.011) and N status (HR 7.249, p = 0.002). The multivariate analysis identified SUVmax ≥ 6.0 (HR 4.875, p < 0.001) and onset of recurrence within 12 months after surgery (HR 0.191, p < 0.003) as independent predictors of DSS (Table 4). CA19.9 ≥200 U/ml was associated with decreased DSS only at univariate analysis.

Interestingly, even though there was no relationship between them, the combination of SUVmax ≥6.0 and CA19.9 ≥200 U/ml was significantly associated with poorer DFS (8 vs 20 months, p < 0.001) and DSS (14 vs 33 months, p < 0.001) in comparison to patients without concordantly elevated values.

4. Discussion

Currently, the standard of care for resectable pancreatic cancer is surgery followed by adjuvant therapy. However, this disease maintains a poor prognosis due to an early appearance of metastases [2]. These outcomes support the systemic nature of PDAC since the early-stage, suggesting a systemic approach to this disease since its onset [24]. Therefore, accurate preoperative diagnosis and staging for pancreatic cancer are crucial not only to detect local invasion and distant metastases in order to assess resectability, but also to select those patients with more aggressive tumor biology and at higher risk of early recurrence after surgery who might benefit more from neoadjuvant therapy instead of upfront surgery [1,2].

In a previous study, we showed that 18FDG-PET/CT in the preoperative setting could change the therapeutic planning in about

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Table 3
Univariate and multivariate analyses of predictors of disease free survival DFS ($n = 46$).

Variable	Univariate analysis			Multivariate analysis		
	n	Median (months)	P value	Hazard ratio	95% CI	P
Sex						
Male	23	16	0.971			
Female	23	20				
Age (years)						
≤67	23	16	0.886			
>67	23	20				
Asymptomatic patient						
No	40	16	0.712			
Yes	6	15				
Diabetes						
No	36	16	0.529			
Yes	10	16				
Jaundice						
No	18	15	0.523			
Yes	28	18				
Weight loss						
No	28	18	0.454			
Yes	18	15				
Abdominal pain						
No	27	19	0.995			
Yes	19	16				
Occlusion/vomiting						
No	45	18	0.228			
Yes	1	10				
Acute pancreatitis						
No	46	16	NR			
Yes	0	NR				
SUVmax60 after 18FDG-PET/CT[a]						
<6.0	19	25	**0.003**	2.288	1.117–4.685	**0.024**
≥6.0	27	13				
CA19.9[b] (U/ml)						
<200	35	20	**0.026**	2.935	1.275–6.755	**0.011**
≥200	11	8				
CEA[c] (ng/ml)						
<5	41	18	0.299			
≥5	5	10				
Tumor size (mm)						
<25	27	20	0.118			
≥25	19	15				
Post-op complications						
No	18	20	0.439			
Yes	28	15				
Grading						
G1	0	0	0.133			
G2	23	20				
G3	23	13				
Vascular invasion						
No	2	16	0.296			
Yes	44	16				
Perineural invasion						
No	5	14	0.273			
Yes	41	18				
N status						
No	9	NR	**0.001**	7.249	2.109–24.918	**0.002**
Yes	37	15				
R status						
No	38	16	0.703			
Yes	8	12				
Adjuvant therapy						
No	4	16	0.125			
Yes	42	NR				

[a] SUVmax after 18FDG-PET/CT maximum standard uptake value after (18)fluoro-deoxyglucose positron emission tomography/computed tomography at 60 min.
[b] CA19.9 serum carbohydrate antigen 19.9.
[c] CEA carcinoembryonic antigen. In bold, significant p values ($p < 0.05$).

11% of patients. However, we supported its application in selected patients, considering (i) the cost of this procedure and (ii) its controversial reputation in providing a real advantage for the identification of liver and lymph node metastasis compared to conventional imaging [13,15,25]. In the present study, we aimed to evaluate its impact in providing preoperative survival predictors that could help clinicians in the recurrence risk stratification of patients with resectable PDAC and in the choice of the most appropriate treatment (neoadjuvant treatment versus upfront surgery).

Of note, our results demonstrated that SUVmax after preoperative 18FDG-PET/CT is a strong predictor of recurrence and survival in patients with resectable pancreatic cancer. In fact, multivariate analysis showed that SUVmax ≥6.0 represents an independent poor prognostic factor of both DFS and DSS after curative resection of

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Table 4
Univariate and multivariate analyses of predictors of disease specific survival DSS ($n = 46$).

Variable	Univariate analysis			Multivariate analysis		
	n	Median (months)	Pvalue	Hazard ratio	95% CI	P
Sex						
Male	23	33	0.54			
Female	23	25				
Age (years)						
<67	23	27	0.81			
>67	23	30				
Asymptomatic patient						
No	40	30	0.223			
Yes	6	25				
Diabetes						
No	36	30	0.94			
Yes	10	27				
Jaundice						
No	18	23	0.514			
Yes	28	33				
Weight loss						
No	28	33	0.147			
Yes	18	27				
Abdominal pain						
No	27	33	0.623			
Yes	19	23				
Occlusion/vomiting						
No	45	30	0.193			
Yes	1	16				
Acute pancreatitis						
No	46	30	NR			
Yes	0	NR				
SUVmax60 after 18FDG-PET/CT[a]						
< 6.0	19	44	**<0.001**	4.875	1.847-12.865	**0.001**
> 6.0	27	23				
CA19.9[b](U/ml)						
< 200	35	33	**0.011**			0.193
> 200	11	14				
CEA[c](ng/ml)						
< 5	41	30	0.179			
> 5	5	16				
Tumor size (mm)						
<25	27	36	0.062			
>25	19	23				
Post-op complications						
No	18	35	0.706			
Yes	28	25				
Grading						
G1	0	NR	**0.022**			0.905
G2	23	41				
G3	23	19				
Vascular invasion						
No	2	23	0.444			
Yes	44	30				
Perineural invasion						
No	5	19	0.652			
Yes	41	31				
N status						
No	9	NR	**0.013**			0.058
Yes	37	23				
R status						
No	38	30	0.309			
Yes	8	23				
Adjuvant therapy						
No	4	27	0.302			
Yes	42	27				
Recurrence in 6 months						
Yes	5	9	**0.01**			0.684
No	41	33				
Recurrence in 12 months						
Yes	16	13	<0.001	0.191	0.063–0.580	**0.003**
No	30	41				

[a] SUVmax after 18FDG-PET/CT maximum standard uptake value after (18)fluoro-deoxyglucose positron emission tomography/computed tomography at 60 min.
[b] CA19.9 serum carbohydrate antigen 19.9.
[c] CEA carcinoembryonic antigen. In bold, significant p values ($p < 0.05$).

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PDAC. Other previous studies have evaluated the prognostic role of SUVmax for patients with pancreatic cancer. Yamamoto et al., in a series of 128 resected patients, also identified SUVmax ≥6.0 as an independent predictor of poor survival [26]. Choi et al. supported similarly that a higher SUVmax is a strong predictive discriminator for overall survival (OS) and DFS in resected patients, but they used a different cut off of 3.5 [16]. Chirindel et al. observed a statistically significant difference in progression free survival, but not in OS, with SUVmax below and above the median cut-off point of 4.9 [27]. In other studies, metabolic parameters other than SUVmax after 18FDG-PET/CT, including metabolic tumor volume (MTV) and total lesion glycolysis (TLG), strongly correlated with tumor recurrence and survival after resection [27–29]. In addition, several Authors showed an association between 18FDG-PET/CT-parameters and survival also in patients with unresectable PDAC [17,30,31].

However, it would be very important to establish not only the predictive value of SUVmax for recurrence in general, but especially in the early postoperative period [7]. In our study, we observed that SUVmax <6.0 correlated with better DFS (25 vs 13 months, $p = 0.001$) and recurrence within 12 months was an independent predictor of poor DSS (HR 0.191, $p < 0.003$). Whereas, a relationship between SUVmax and early recurrence, defined as tumor recurrence in the first 6 months after surgery, was not found in our study; this may be related to the low number of patients that developed early recurrence. But unlike our experience, Okamoto et al., in a series of 56 patients, demonstrated that SUVmax >5.5 in the preoperative workup was strongly predictive of cancer recurrence within the first 6 months after radical resection [32]. In keeping with this results, Yamamoto et al. showed that the rate of early recurrence was significantly higher in patients with SUVmax ≥6.0 than in those with SUVmax <6.0 (49% vs 5%, $p < 0.001$) [26].

On the whole, SUVmax seems to reflect the tumor burden and the metabolic activity of the tumor and, as well as in other malignancies, represents the aggressiveness of pancreatic cancer, considering its strong association with DFS and DSS [9,12,26]. Therefore, 18FDG-PET/CT may play an important role in the preoperative planning of the treatment strategy, in particular in identifying those patients with a more aggressive tumor that could benefit from neoadjuvant treatment in advance of surgery.

In this light, preoperative CA19.9 values seems to have a similar role. It also may correlate with tumor burden, tumor spread, postoperative early recurrence and survival [2,21–23,33,34]. A recent study analyzing 10,806 patients with early-stage pancreatic cancer from the National Cancer Database (NCDB) supported that patients with elevated CA 19-9 levels (≥37 U/ml) at diagnosis are biologically borderline resectable regardless of anatomic resectability, and suggested neoadjuvant systemic therapy in these patients [34]. Interestingly, in the present study, we found that the combination of SUVmax ≥6.0 and CA19.9 ≥200 U/ml correlated significantly with worse DFS (8 vs 20 months, $p < 0.001$) and DSS (14 vs 33 months, $p < 0.001$). Therefore, we support the combined use of SUVmax and CA19.9 in the preoperative setting to identify more aggressive tumors at higher risk of micrometastatic disease. Since 18FDG-PET/CT is an expensive modality and its cost-effectiveness is still controversial [11,13], we support the use of 18FDG-PET/CT at least in patients with (i) borderline-resectable when an upfront surgery represent a possible first choice or (ii) resectable pancreatic cancer at imaging but with features associated with increased risk of early recurrence (i.e. high levels of CA19.9).

This study has several limitations. Certainly, the retrospective nature of the study and the small sample size limited the possibility of making robust conclusions. However, patients' selection, diagnostic workout by PET and surgical treatment were carried out in the same institution by an established multidisciplinary team, and PET schedule was accurately planned and combined with surgery. It is well known that the value of SUVmax after neoadjuvant therapy can be influenced by treatment [14], therefore patients who underwent neoadjuvant chemotherapy were excluded from the analysis because of missing baseline PET. On the other hand, the adjuvant chemotherapy was not uniformly performed in the study population, however, the univariate analysis did not show any influence on DFS and DSS by adjuvant treatments. Finally, in the present study, ROC curves showed a SUVmax ≥6.0 as the best cut off value to predict DSS and DFS. However, no consensus has yet been established in literature about the optimal SUVmax cut off, with a wide range of different values [16,17,26,27,32]. This is a reasonable concern about the use of 18FDG-PET/CT and further investigations in larger series are needed to achieve a standardization of SUVmax cut-off value.

In conclusion, our data show that preoperative SUVmax ≥6.0 is an independent predictor of poor DFS and DSS after surgical resection of PDAC. We support the use of 18FDG-PET/CT in the preoperative evaluation of patients with potentially resectable pancreatic cancer in combination with other stigmata of a more aggressive tumor biology like high levels of CA19.9. In this subgroup of patients, 18FDG-PET/CT may help to identify those who can benefit from a systemic approach with neoadjuvant treatment instead of an upfront surgery.

Conflict of interest

None declared.

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Please cite this article in press as: Pergolini I, et al. SUVmax after (18)fluoro-deoxyglucose positron emission tomography/computed tomography: A tool to define treatment strategies in pancreatic cancer. Dig Liver Dis (2017), http://dx.doi.org/10.1016/j.dld.2017.09.122

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Please cite this article in press as: Pergolini I, et al. SUVmax after (18)fluoro-deoxyglucose positron emission tomography/computed tomography: A tool to define treatment strategies in pancreatic cancer. Dig Liver Dis (2017), http://dx.doi.org/10.1016/j.dld.2017.09.122

Gastroenterology 2017;153:1284–1294

CLINICAL—PANCREAS

Long-term Risk of Pancreatic Malignancy in Patients With Branch Duct Intraductal Papillary Mucinous Neoplasm in a Referral Center

Ilaria Pergolini,[1,2] Klaus Sahora, [1] Cristina R. Ferrone, [1] Vicente Morales-Oyarvide, [1,3] Brian M. Wolpin,[3] Lorelei A. Mucci, [4] William R. Brugge, [5] Mari Mino-Kenudson, [6] Manuel Patino, [7] Dushyant V. Sahani, [7] Andrew L. Warshaw, [1] Keith D. Lillemoe, [1] and Carlos Fernández-del Castillo [1]

[1]Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts; [2]Department of Surgery, Universita' Politecnica delle Marche, Ancona, Italy; [3]Department of Medical Oncology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts; [4]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, Massachusetts; [5]Department of Gastroenterology, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts; [6]Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, and [7]Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts

See Covering the Cover synopsis on page 1174.

BACKGROUND& AIMS: Little is known about the development of branch duct intraductal papillary mucinous neoplasms (BD-IPMNs). We evaluated long-term outcomes of a large cohort of patients with BD-IPMNs to determine risk of malignancy and define a subset of low-risk BD-IPMNs. METHODS: We performed a retrospective analysis of data from 577 patients with suspected or presumed BD-IPMN under surveillance at the Massachusetts General Hospital. Patients underwent cross-sectional imaging analysis at 3 months or later after their initial diagnosis. The diagnosis of BD-IPMN was based on the presence of unilocular or multilocular cysts of the pancreas and a non-dilated main pancreatic duct (<5 mm). We collected demographic, clinical, and pathology data. Cysts were characterized at the time of diagnosis and during the follow-up period. Follow-up duration was time between initial cyst diagnosis and date of last visit or death for patients without development of pancreatic cancer, date of surgery for patients with histologically confirmed malignancy, or date of first discovery of malignancy by imaging analysis for patients with unresectable tumors or who underwent neoadjuvant treatment before surgery. The primary outcome was risk of malignancy, with a focus on patients followed for 5 years or more, compared with that of the US population, based on standardized incidence ratio. RESULTS: Of the 577 patients studied, 479 (83%) were asymptomatic at diagnosis and 363 (63%) underwent endoscopic ultrasound at least once. The median follow-up time was 82 months (range, 6À329 months) for the entire study cohort; 363 patients (63%) underwent surveillance for more than 5 years, and 121 (21%) for more than 10 years. Malignancies (high-grade dysplasia or invasive neoplasm) developed after 5 years in 20 of 363 patients (5.5%), and invasive cancer developed in 16 of 363 patients (4.4%). The standardized incidence ratio for patients with BD-IPMNs without worrisome features of malignancy at 5 years was 18.8 (95% confidence interval, 9.7À32.8; P < .001). One hundred and eight patients had cysts ○°1.5 cm for more than 5 years of follow-up; only 1 of these patients (0.9%) developed a distinct ductal adenocarcinoma. By contrast, among the 255 patients with cysts >1.5 cm, 19 (7.5%) developed malignancy (P ¼ .01). CONCLUSIONS:In a retrospective analysis of patients with BD-IPMNs under surveillance, their overall risk of malignancy, almost 8%, lasted for 10 years or more, supporting continued surveillance after 5 years. Cysts that remain ○°1.5 cm for more than 5 years might be considered low-risk for progression to malignancy.

Keywords: Pancreas; IPMN; Cancer Risk; Pancreatic Cancer.

Two decades after the first definition of intraductal papillary mucinous neoplasm (IPMN) of the pancreas by the World Health Organization,[1] its management remains controversial. The increasing detection of this tumor, the imperfect preoperative prediction of malignancy by imaging and endoscopic modalities, and the balance between the risk of malignant progression and overtreatment make these neoplasms challenging to manage. These concerns are mostly germane to branch duct IPMNs (BD-IPMNs), because main duct and mixed-type IPMNs are more often managed with surgical resection due to their increased likelihood of malignancy (high-grade dysplasia and invasive cancer) and higher frequency of symptoms.[2]

Our current understanding of BD-IPMNs is under the bias of retrospective surgical series and observational

Abbreviations used in this paper: BD, branch duct; CT, computed tomography; EUS, endoscopic ultrasound; FNA, fine-needle aspiration; HRS, high-risk stigmata; IPMN, intraductal papillary mucinous neoplasm of the pancreas; MPD, main pancreatic duct; MRI/MRCP, magnetic resonance imaging/magnetic resonance cholangiopancreatography; PDAC, pancreatic ductal adenocarcinoma; SIR, standardized incidence ratio; WF, worrisome feature.

Most current article

0016-5085/$36.00
https://doi.org/10.1053/j.gastro.2017.07.019

EDITOR'S NOTES

BACKGROUND AND CONTEXT

Little is known about development of branch duct intraductal papillary mucinous neoplasms (BD-IPMNs).

NEW FINDINGS

Overall risk of malignancy, almost 8%, lasted for 10 years or more for patients with BD-IPMNs, supporting continued surveillance after 5 years. Cysts that remain 1.5 cm or smaller for more than 5 years might be considered low-risk for progression to malignancy.

LIMITATIONS

Limitations include the lack of histological confirmation of the diagnosis of BD-IPMN in patients that did not undergo resection, the retrospective nature of the study, and the small population size.

IMPACT

Continued surveillance after 5 years from the initial diagnosis is encouraged. These findings may help reduce costs related to surveillance and improve patients' quality of life.

studies, mostly with short median surveillance periods, and consequently there is limited knowledge of the natural history and malignant potential of pancreatic cysts beyond 5 years, and practically none after 10 years.[3–5] This information is important because the recent guideline from the American Gastroenterological Association recommends stopping surveillance after 5 years,[6] while other guidelines, giving recommendations regarding indications for surgical resection and frequency of follow-up on patients managed non-operatively, have not given an end point to surveillance.[2] In this study, we evaluated long-term outcomes in a large cohort of patients undergoing primary surveillance for BD-IPMN in a single institution, with a focus on the risk of development of pancreatic cancer after 5 years of follow-up. In addition, we sought to identify whether any subset of low-risk BD-IPMNs can safely forego surveillance after 5 years of follow-up.

Methods

Patient Selection, Inclusion, and Exclusion Criteria

Consecutive patients with a clinical diagnosis of BD-IPMN followed and treated at the Massachusetts General Hospital with a minimum of 6 months of follow-up were included in the study. Patients had at least one cross-sectional imaging study (computed tomography [CT], magnetic resonance imaging/magnetic resonance cholangiopancreatography [MRI/MRCP], and/or endoscopic ultrasound [EUS]) done 3 months or longer after the initial diagnosis. Diagnosis of BD-IPMN was based on the presence of unilocular or multilocular cysts of the pancreas and a non-dilated main pancreatic duct (MPD) (<5 mm). All patients with a dilation of the MPD ≥5 mm or with cysts suspicious for another diagnosis rather than BD-IPMN (eg, serous cystadenoma, mucinous cystic neoplasm, cystic neuroendocrine tumor, solid pseudopapillary tumor, or pseudocyst) were excluded from the study. Among patients who underwent resection during follow-up, 6 months before undergoing surgery was the minimum follow-up required to be included. Patients with a prior diagnosis of pancreatic cancer were specifically excluded.

A retrospective review of the prospectively collected data was performed for this cohort study. Patients' demographic and clinicopathologic features were recorded. Variables included sex, age at initial diagnosis and at surgery, personal medical history, family history, presence or absence of symptoms at the time of diagnosis, cyst morphology at the initial diagnosis and during follow-up, indication for surgery, date and type of surgery, final pathologic findings, postoperative course, recurrence, and subsequent surgery.

Moreover, we classified patients at the initial diagnosis as having either suspected or presumed BD-IPMN. "Suspected IPMN" was used when communication between the cyst and the main pancreatic duct was documented or when cysts were multifocal or had a cyst fluid carcinoembryonic antigen >192 ng/mL. All remaining cysts were classified as "presumed BD-IPMN."[7] The study protocol was approved by our local Institutional Review Board.

Diagnostic Workup

The cystic lesion was defined as incidentally discovered when the diagnosis was made in absence of the following symptoms: upper abdominal pain, acute pancreatitis, worsening or new-onset diabetes, jaundice, steatorrhea, or unexplained weight loss. The imaging modalities used to diagnose and follow-up BD-IPMNs included CT, MRI/MRCP, and EUS. We collected data on indication, date, and type of imaging study used for the initial diagnosis. EUS with or without fine-needle aspiration (FNA) was performed at the discretion of the treating physician or surgeon. For patients who underwent EUS and FNA of the cyst, we recorded the cytologic characteristics, fluid features, and, when available, fluid carcinoembryonic antigen levels. Cyst characteristics were evaluated primarily with CT and MRI/MRCP, and included location, size, septations, wall thickening, communication with the MPD, and solid component with or without enhancement. We recorded these morphologic data at 2 time points at least: at the initial diagnosis and during follow-up. In patients with multiple lesions, we considered the features of the largest cyst or the most concerning one, and in case of more examinations performed contemporarily, we collected the largest size and the worse feature described. Regarding cyst size, we collected it at the first cyst discovery, at the last imaging, and also the maximum value reported. We also recorded the emergence of new MPD dilation and maximum duct diameter reached during follow-up.

Reports of each patient were retrospectively reviewed for high-risk stigmata (HRS) and worrisome features (WFs) according to the revised International Consensus Guidelines.[2] Briefly, HRS were defined by the presence of obstructive jaundice in a patient with cystic lesion of the head of the pancreas, main pancreatic duct ≥10 mm, enhancing solid component within the cyst, or cytology positive for high-grade dysplasia or adenocarcinoma. WFs were acute pancreatitis, cyst size ≥3 cm, thickened/enhancing cyst walls, non-enhancing

mural nodules, MPD size of 5À9 mm, abrupt change in caliber of the MPD and lymphadenopathy.

Surgical Indications

Indications for surgery varied over time. Mostly we followed the recommendations of the 2006 International Consensus Guideline (Sendai)[8] and from 2012 onward the revised version.[2] Surgery was also performed when cysts showed rapid growth or because of patient decision. Some patients who met criteria for resection were not operated on because of severe comorbidities or patient preference.

Pathology

IPMNs were classified according to the recommendations from the Baltimore consensus meeting[9] as IPMNs with low-grade dysplasia, IPMNs with high-grade dysplasia, and IPMNs with an associated invasive ductal carcinoma. High-grade dysplasia (formerly referred to as carcinoma in situ) and invasive carcinoma were both considered as malignant.[10] Pancreatic cancer was considered derived from IPMN when it originated within the area with the known pancreatic cyst and the invasive solid mass extended continuously to the IPMN, and was considered a concomitant or distinct pancreatic ductal carcinoma when the invasive component was located separately from the IPMN (Figures 1 and 2). In cases where neoadjuvant chemoradiotherapy was given and the presence of IPMN was no longer recognized, but the mass grew in the same site of the known cyst, we considered the invasive ductal carcinoma as derived from IPMN (Figure 1).

Follow-Up

Follow-up duration was recorded as the time in months between initial cyst diagnosis (the first cross-sectional imaging on which the cyst was detected) and one of the following end points: date of last visit or death for patients without progression to pancreatic cancer; date of surgery for resected patients with histologically confirmed malignancy; and date of first discovery of malignancy on imaging for patients with an unresectable tumor or who underwent neoadjuvant treatment before resection. Patients continued surveillance after surgery, and recurrence in the remnant pancreas was assumed if imaging studies demonstrated new cystic lesions suspicious for IPMN.

Statistical Analysis

Categorical variables were reported as frequencies and percentages, and continuous variables as median and range. Comparisons of categorical variables were conducted using the c^2 or Fisher exact tests, as appropriate. Continuous variables were compared by MannÀWhitney U test. The optimal cutoff level for cyst size to discriminate low-risk BD-IPMNs was determined by receiver operating characteristic curves. Sensitivity, specificity, and positive and negative predictive values were also calculated. The relative risk of malignancy in patients who reached more than 5 years of follow-up without developing WF or HRS was compared with that of the US population using a standardized incidence ratio (SIR).[11] The SIR is the ratio of the number of observed cancers to the number expected. We calculated the expected number of cancers by multiplying the observed person-years at risk in our cohort (stratified by 5-year age groups) by stratum-specific incidence rates of pancreatic cancer obtained from the Surveillance, Epidemiology, and End Results Program of the National Cancer Institute.[12] Observed person-years at risk in our cohort were accrued starting on the 5-year mark; hence, the SIR reflects the risk of malignancy after 5 years of surveillance. We calculated 95% confidence intervals for the SIR based on the assumption that observed cases followed a Poisson distribution. All hypothesis tests were 2 two-sided; statistical significance was set at $P < .05$. Statistical analyses were performed using SPSS software (SPSS Inc, Chicago, IL).

Results

Study Population

Five hundred and seventy-seven patients (median age at initial diagnosis, 66 years; range, 21À90 years; 59% were female) with suspected or presumed BD-IPMN were included. Median follow-up for the entire cohort was 82 months (range, 6À329 months). Three hundred and sixty-three patients (63%) underwent surveillance for more

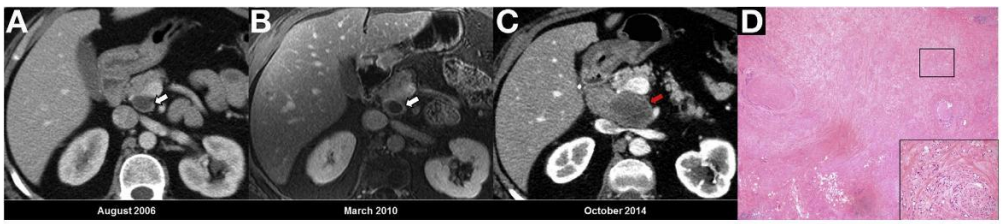

Figure 1. PDAC arising from an IPMN. (A) Axial contrast-enhanced CT image from August 2006 shows a 1.9 Â 1.4 cm hypoattenuating cystic lesion in the uncinate process of the pancreas (white arrow), representing a BD-IPMN. (B) Axial contrast-enhanced MR image from March 2010 shows that the lesion is hypointense, with thick wall and without significant change in size (2.2 Â 1.4 cm), compared to the initial imaging. (C) Axial CT image in the pancreatic phase of contrast from October 2014 shows a predominantly solid, hypoattenuating lesion with significant increase in size (6 Â 3 cm) (red arrow), abutting adjacent vascular structures (eg, superior mesenteric artery). (D) Histopathologic image shows small nests and glands in an infiltrating pattern consistent with invasive ductal carcinoma. There was no remaining cystic lesion in the resected pancreas.

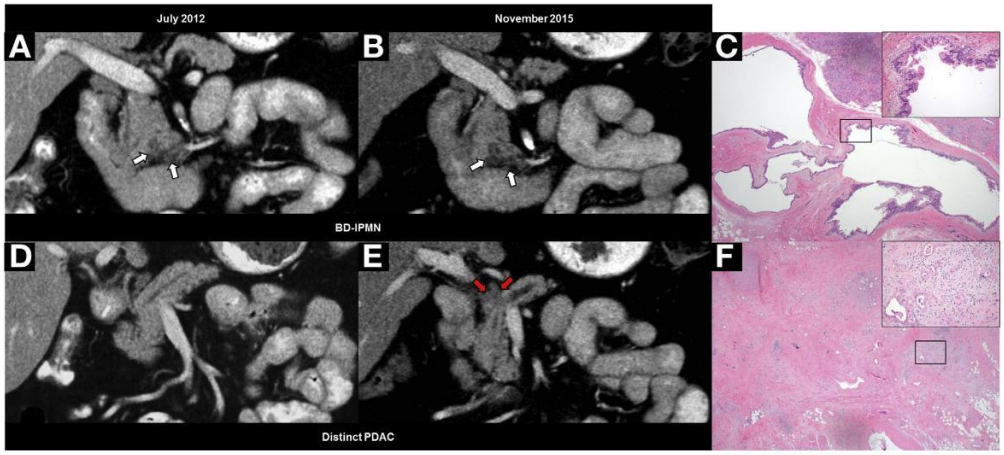

Figure 2. Concomitant PDAC and BD-IPMN. (A, B) Coronal CT images showing a 2 cm hypoattenuating cystic lesion in the uncinate process of the pancreas (white arrows) representing a BD-IPMN, without significant change in the interval time. (D) Coronal CT image from July 2012 shows a mildly prominent pancreatic duct without evidence of a solid pancreatic mass, and (E) coronal CT image from November 2015 shows dilatation of the main pancreatic duct (9.5 mm) with sudden change in caliber at the level of the pancreatic neck, associated with a hypoattenuating mass (red arrows), not seen in the previous examination. (C, F) Histopathologic images from the 2 different lesions. The cyst is lined by gastric-type epithelium of low to intermediate grade consistent with branch-duct IPMN (C) and is located away from the mass that consists of infiltrating atypical ducts consistent with concomitant PDAC (F).

than 5 years (median, 107 months; range, 60À329 months), and 121 (21%) were followed for more than 10 years (median, 147 months; range, 120À329 months). Patient characteristics are listed in Table 1. A total of 479 patients (83%) were asymptomatic at the initial diagnosis and, of these, 48 (10%) became symptomatic during follow-up.

Diagnostic Workup

For the initial diagnosis, CT, MRI/MRCP, and EUS were performed in 86%, 46%, and 37% of cases respectively; 17% of patients were studied with all these 3 modalities. FNA was performed in 33% of patients and in 2 patients was suspicious for carcinoma; neither of them developed changes suspicious for malignancy during follow-up. They did not undergo surgical resection because of advanced age/comorbidities. During follow-up, the median number of imaging and endoscopic studies was 4 for each patient (0.86 per year). Thirty-eight percent of patients underwent EUS/FNA and cytology was positive in 32 (14%); 8 of them had a prior EUS/FNA at the time of diagnosis that was negative. Overall, 94% and 80% of patients underwent CT and MRI/MRCP, respectively, during the study period, while 63% of patients had at least 1 EUS (95% with FNA); FNA showed a positive predictive value of 59%.

Morphologic Data at Diagnosis and During Follow-Up

At diagnosis, median cyst size was 14 mm (range 2À54 mm) and 53 patients (9%) had a cyst ≥3 cm. During follow-up, an additional 76 patients developed a lesion >3 cm and the median cyst diameter had increased to 20 mm (range, 3À90 mm). At the end of the observational period, 319 patients (55%) had increased cyst size; median increase was 0.9 mm per year of follow-up. Morphologic features of BD-IPMNs at diagnosis and during follow-up are described in Table 2. Patients who developed malignancy had a significantly larger cyst size at diagnosis compared with those without malignant transformation (19 vs 14 mm; P ¼ .047), as well as a greater maximum diameter during follow-up (32 vs 19 mm; P < .001) (Supplementary Figure 1).

Worrisome Features and High-Risk Stigmata

Seventy-two (12%) and 3 (1%) patients showed WF and HRS, respectively, at the initial diagnosis. During follow-up, an additional 77 (13%) and 53 (9%) patients developed new WF and HRS, respectively, and in 10 patients (2%) they underwent regression (ie, a patient showing a nodule initially that is no longer seen on follow-up). Overall, 205 patients (36%) at some point had cysts with WF/HRS during the study period.

Surgery

One hundred and eighteen patients (20%) underwent surgery during the follow-up period. Table 3 shows operative procedures, postoperative complications, and pathology results. The median duration of follow-up before surgery was 34 months (range, 6À241 months). Operative mortality was 1.7%. Four of the 118 patients (3%) underwent palliative

Table 1.Patients' Characteristics at Diagnosis (N ¼ 577)

Characteristic	Data
Sex	
Male	237 (41)
Female	340 (59)
Age at diagnosis, y, median (range)	66 (21À90)
Follow-up, mo, median (range)	82 (6À329)
Follow-up >5 y	363 (63)
Follow-up >10 y	121 (21)
Family history of pancreatic cancer	63 (11)
ACE-27 score ○83	35 (6)
Charlson score, median (range)	3 (0À10)
Charlson score ○84	201 (35)
Alcohol	248 (43)
Smoking	224 (39)
BMI, kg/m^2, median (range)	26.8 (12.6À48.7)
Incidental diagnosis	479 (83)
Symptoms leading to diagnosis	98 (17)
Jaundice	0 (0)
Weight loss	21 (4)
Abdominal pain	84 (15)
Steatorrhea/diarrhea	5 (1)
Acute pancreatitis	16 (3)
Diabetes	0 (0)
Presence of diabetes	137 (24)
Long-standing diabetes	76 (13)
New onset (by 2 y from diagnosis of IPMN)	12 (2)
During follow-up	34 (6)
Unknown	15 (3)
History of other cancers	238 (41)

NOTE. Data are n (%) unless otherwise noted.
ACE-27, Adult Comorbidity Evaluation-27; BMI, body mass index.

surgery after intraoperative discovery of liver or peritoneal metastasis, or for planned bypass and intraoperative radiotherapy because of an unresectable tumor. In addition, 5 patients were deemed inoperable when malignancy was diagnosed and no surgical procedure was performed.

Post-Operative Follow-Up and Recurrence

Median time of follow-up after resection was 54 months (range, 0À205 months). The 4 patients who underwent palliative treatment died 15, 7, 4, and 3 months after surgery, respectively. Twelve patients showed stability of known cystic lesions in the remnant pancreas, 1 case had an increased known cyst, while in 7 patients discovery of new cysts was observed. Malignant recurrence occurred in 11 patients. In 9 cases, the previously resected lesion was malignant and none underwent secondary surgery. In 2 patients, the prior resected lesion was a benign BD-IPMN and malignancy was discovered in the remnant pancreas 100 and 104 months, respectively, after the initial diagnosis of BD-IPMN, and 68 and 24 months after surgery. In the first case, a tiny cyst developed in the remnant pancreas 4 years after resection, and 1 year later, it increased in size up to 27 mm and had a solid component. The lesion was resected and final pathology showed pancreatic adenocarcinoma arising from IPMN. The second patient developed jaundice secondary to an unresectable mass in the pancreatic head with liver metastasis. No cystic lesions were recognized in the remaining pancreas before the prior partial resection and no new imaging study was performed postoperatively before the discovery of the new inoperable lesion; this patient died 7 months later.

Analysis of the Risk of Malignancy

Overall, 45 patients (7.8%) developed pancreatic malignancy during the study period; in 36 (80%, and 6% of the entire cohort), malignancy arose within the cyst. In the remaining 9 patients (20%, and 1.6% of the entire cohort), malignancy arose away from the pancreatic cyst, that is, distinct or concomitant pancreatic adenocarcinoma (Figure 3).

Branch duct intraductal papillary mucinous neoplasms with follow-up fewer than 5 years. Of the 214 patients followed fewer than 5 years, 37 (17%) underwent surgery and 22 (59%) had pancreatic malignancy (11 high-grade dysplasia, 8 invasive IPMN, 2 concomitant pancreatic ductal adenocarcinoma [PDAC], and 1 mucinous cystadenocarcinoma). Additionally, 1 patient was resected for a concomitant ampullary adenocarcinoma and 3 others had unresectable pancreatic cancer, of which 2 had invasive IPMN and 1 a concomitant PDAC who underwent palliative surgery. Therefore, overall 4.3% (25 of 577) of the entire cohort developed pancreatic malignancy within 5 years. Of note, 13 of these patients (52%) did not experience any symptoms during the surveillance period. Invasive pancreatic cancer occurred in 2.4% (14 of 577).

Branch duct intraductal papillary mucinous neoplasms with follow-up of 5 years or more. Of the 363 patients followed for more than 5 years, 81 (14%) underwent surgery, and in 15 (19%) either invasive cancer (7 invasive IPMN, 4 distinct PDAC) or high-grade dysplasia (n ¼ 4) was found. Three of the patients with invasive tumors underwent palliative surgery for an advanced concomitant PDAC. In addition, 3 other patients presented inoperable invasive IPMN at the moment of the identification of malignancy and, as described, 2 patients developed malignancy after initial resection of a benign IPMN. Accordingly, malignancy in patients followed for more than 5 years occurred in 5.5% (20 of 363), and invasive cancer in 4.4% (16 of 363) (Figure 4). In 5 patients (5 of 121), malignant transformation occurred after 10 years of follow-up. Thirty-five percent (7 of 20) of the patients who were followed >5 years and developed malignancy had no symptoms at all, and the diagnosis or the decision to operate was done purely by imaging or endoscopy.

Impact of worrisome features/high-risk stigmata on risk of malignancy after 5 years. Twenty-two percent of patients who were followed for more than 5 years (81 of 363) had WF or HRS by the 5-year time point. The risk of malignancy in this subgroup was 10%, and median time elapsed between identification of WF/HRS and the diagnosis of malignancy was 102 months (range, 1À132 months). By comparison, in the 282 patients who had absence of WF/HRS at the 5-year mark, malignancy developed in 12 (4.3%; 2 showed high-grade dysplasia and 10 invasive carcinoma) (P ¼ .09). All 12 of these patients

Table 2. Branch Duct Intraductal Papillary Mucinous Neoplasm Morphologic Features at Diagnosis and During Follow-Up of 577 Patients

Variable	At diagnosis	During follow-up
Multifocality	219 (38)	317 (55)
>3 lesions	119 (21)	206 (36)
Dominant location		
Head	148 (26)	156 (27)
Neck	55 (10)	56 (10)
Uncinate process	98 (17)	96 (17)
Body	167 (29)	162 (28)
Tail	109 (19)	107 (19)
Septations	182 (32)	248 (43)
Nodule	8 (1)	54 (9)
Thick wall	4 (1)	28 (5)
Cyst ≥3 cm	53 (9)	121 (21)
Cyst diameter, mm, median (range)	14 (2À54)	20 (3À90)
Communication with MPD	211 (37)	310 (54)
MPD dilatation ≥5 mm	0 (0)	48 (8)
Positive cytology	2 (0)	32 (6)
Worrisome features	72 (12)	142 (25)
High-risk stigmata	3 (1)	53 (9)

NOTE. Data are n (%) unless otherwise noted.

developed WF/HRS later on during follow-up, at a median of 93 months after diagnosis (range, 60À126 months).

Suspected and Presumed Branch Duct Intraductal Papillary Mucinous Neoplasms

Patients classified at the time of initial diagnosis as having "suspected" IPMN (60%) did not show a higher risk of malignancy or need for surgery when compared to the patients with "presumed" BD-IPMN (risk of malignancy of 8% vs 8%; P ¼ .984, and surgical resection in 18% vs 24%; P ¼ .059, respectively).

Diabetes, Branch Duct Intraductal Papillary Mucinous Neoplasms, and Malignancy

Of the 9 patients who developed a concomitant PDAC, only 2 (22%) experienced diabetes (long-standing diabetes before diagnosis of BD-IPMN in both cases), while 11 (31%) of the 36 patients with pancreatic malignancy arising from IPMN had a history of diabetes during surveillance. Diabetes did not correlate to malignancy in the entire cohort (24% vs 29%; P ¼ .419), or to the type of pancreatic malignancy, associated or distinct from IPMN (31% vs 22%; P ¼ .622).

Low-Risk Branch Duct Intraductal Papillary Mucinous Neoplasms

Among the 363 patients followed for more than 5 years, 108 (30%) had a cyst that was <1.5 cm in size, and only 1 patient in this group (0.9%) developed malignancy, which was a distinct ductal adenocarcinoma 65 months after initial diagnosis of BD-IPMN. In contrast, 19 patients (7.5%) with

Table 3. Operative Procedures, Postoperative Complications, and Pathology Data in 118 Patients Undergoing Surgery for Branch Duct Intraductal Papillary Mucinous Neoplasm

Variable	Data
Age at surgery, y, median (range)	67 (28À89)
Male sex, n (%)	47 (40)
Follow-up before surgery, mo, median (range)	34 (6À241)
Follow-up after surgery, mo, median (range)	54 (0À205)
Type of surgery, n (%)	118 (20)
Pancreaticoduodenectomy	53 (45)
Distal pancreatectomy with splenectomy	27 (23)
Distal pancreatectomy spleen-preservation	15 (13)
Middle pancreatectomy	18 (15)
Total pancreatectomy	1 (1)
Palliative surgery, unresectable tumor	4 (3)
Indication for operation, n (%)	
Main pancreatic duct dilatation	20 (17)
Increased cyst size	72 (61)
Solid component	41 (35)
Positive cytology	26 (22)
Symptoms	25 (21)
Pathology, n (%)	114 (97)
BD-IPMN, n (%)	75 (66)
Low-grade, n	56
High-grade, n	10
Invasive, n	9
MD-IPMN, n (%)	16 (14)
Low-grade, n	6
High-grade, n	5
Invasive, n	5
Concomitant PDAC, n (%)	4 (4)
Other, n (%)	19 (17)
Ampullary adenocarcinoma	1 (1)
MCN, n (%)	8 (7)
Low-/high-grade, n	7
Invasive, n	1
SCA	4 (4)
Other (lymphoepithelial cyst, epithelial cyst, retention cyst, squamoid cyst)	6 (5)
Postoperative complications, n (%)	54 (46)
Abdominal abscess	11 (11)
Pancreatic fistula	18 (15)
Biliary fistula	1 (1)
Delayed gastric emptying	7 (6)
Cardiopulmonary complications	14 (12)
Other	28 (24)
Readmission, n (%)	14 (12)
Postoperative mortality, n (%)	2 (1.7)
Postoperative follow-up, n (%)	
Presence of stable known cyst in the remnant pancreas	12 (11)
New cysts	7 (6)
Significant change of known cysts	1 (1)
Malignant recurrence	11 (10)
After resection of benign IPMN	2 (2)
-Concomitant PDAC	1 (1)
-PDAC arising from IPMN	1 (1)

HGD, high-grade dysplasia; IGD, intermediate-grade dysplasia; LGD, low-grade dysplasia; MCN, mucinous cystic neoplasm; MD-IPMN, main duct intraductal papillary mucinous neoplasm; SCA, serous cystadenoma.

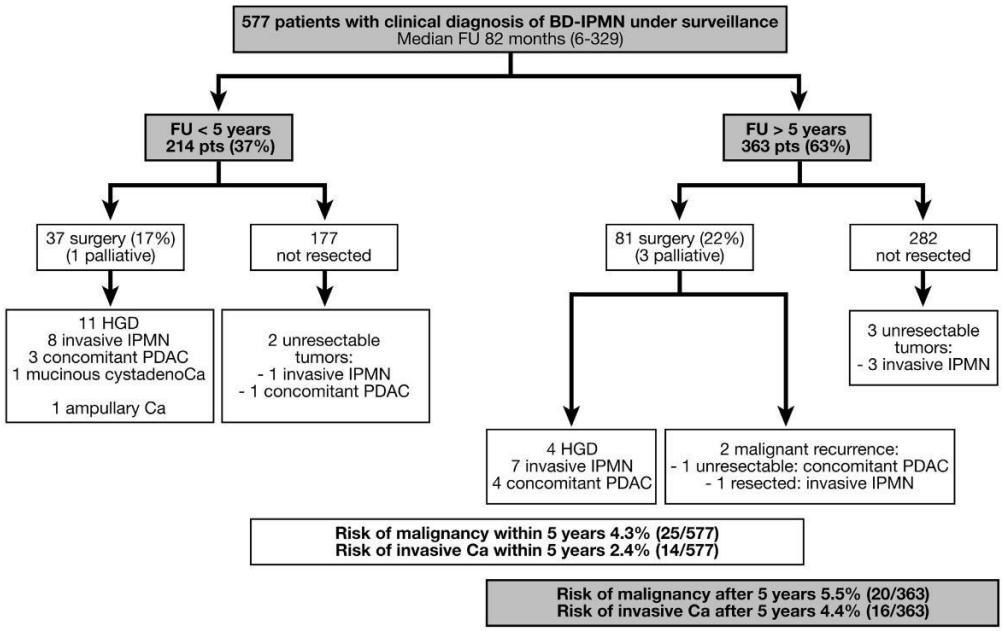

Figure 3. Flow chart. Malignant progression in 577 patients with a clinical diagnosis of BD-IPMNs under surveillance (with follow-up at less than and more than 5 years). FU, follow-up; HGD, high-grade dysplasia.

cysts >1.5 cm developed malignancy (P ¼ .01), and 5 of these occurred after 10 years of follow-up. Ten of these patients had an invasive IPMN, 4 had high-grade dysplasia found in final histopathology, and 3 patients developed a concomitant PDAC. The remaining 2 patients developed malignant recurrence, a concomitant PDAC and a PDAC arising from an IPMN, respectively, as described previously. The 1.5-cm cutoff showed a negative predictive value for malignancy of 99% and a sensitivity of 95% (Supplementary Figure 2).

Standardized Incidence Ratio of Pancreatic Cancer

Patients with BD-IPMN who were followed for more than 5 years without developing WF or HRS (n ¼ 282) had an 18.8 times (95% confidence interval, 9.7À32.8; P < .001) higher age-standardized incidence rate of pancreatic malignancy than expected in the comparable US population. In a sensitivity analysis that considered only invasive carcinomas as malignant cases, the age-standardized incidence rate of invasive carcinoma was 15.6 times (95% confidence interval, 7.5À28.7; P < .001) higher compared with that expected in the US general population.

Discussion

Several series have shown that the prevalence of incidentally discovered pancreatic cysts ranges between 2.1% and 19.6%, depending on the imaging modality, with a median cyst size ranging from 5 to 8 mm.[13–18] In the midst of this abundance of pancreatic cystic lesions, often small and indolent in appearance, the main concern is identifying which are BD-IPMN and which of these are susceptible to malignant progression in order to establish the type and duration of surveillance. The issue is relevant, and will become more so given the increasing frequency of diagnosis of pancreatic cysts, underscoring the need to have more data regarding the natural history of BD-IPMNs.

The main purpose of this study was to evaluate the behavior over time of a large cohort of patients with BD-IPMNs undergoing primary surveillance, and to analyze the risk of pancreatic malignancy after 5 years. It is important to highlight that this cohort may not be representative of the population of incidentally discovered cysts at large, because it was derived from patients referred to gastroenterologists and surgeons. This is reflected in the median cyst size of 14 mm at the time of initial diagnosis, which is, on average, twice as large as the cysts described in radiologic series. With a median follow-up close to 7 years, we found that >50% of patients with either presumed or suspected BD-IPMN had progression of the cysts. This progression was manifested mostly by an increase in median size (from 14 to 20 mm) and multifocality (from 38% to 55%), but also by development of solid components (from 2% to 13%) and development of duct dilation in 8% of patients. In aggregate, 34% of patients had either WFs or

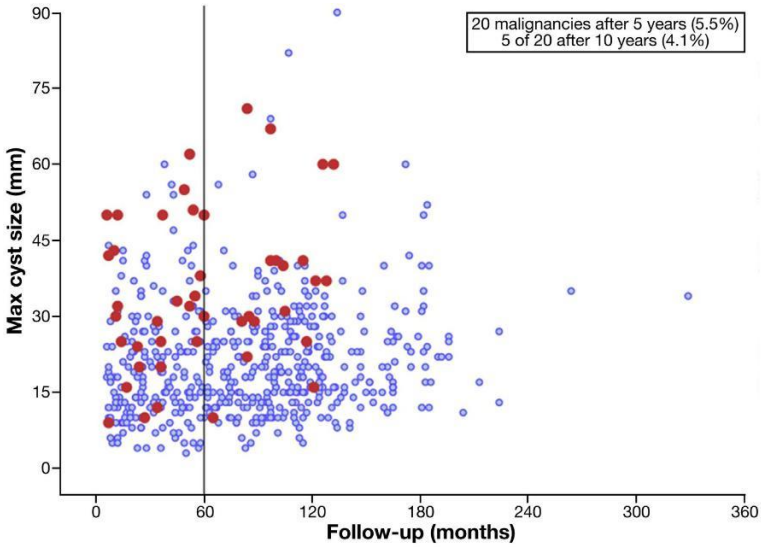

Figure 4. Development of malignancy in relation with maximum cyst size and duration of follow-up. The vertical line indicates the 5 year-time point. The red dots represent patients with malignant progression and the blue dots all of the remaining patients.

high-risk stigmata (as defined by the guidelines of the International Association of Pancreatology) at the end of follow-up, whereas at the time of diagnosis, only 13% had these features. Importantly, progression was not limited to the first 5 years of follow-up.

Regarding the risk of malignancy after 5 years, the data in the literature are controversial, likely due to a difference in the composition of the cohorts and heterogeneity of the inclusion criteria. Handrich et al[19] analyzed the fate of 49 patients with long-term follow-up (more than 5 years) who were diagnosed with small (◌°2 cm) simple pancreatic cysts, showing that none developed pancreatic malignancy, whereas Cadili et al[20] showed that 3 (4.7%) of 64 patients with a median follow-up of 86 months and a median cyst size of 2.5 cm (range, 1.3À4 cm) developed malignancy after 5 years. In another recent series of 95 patients with cysts <3 cm followed for more than 5 years, the rate of malignant progression was similar at 4.2%.[21] In the present study, which involves 577 suspected or presumed BD-IPMNs under surveillance, we found that the development of malignancy not only persists, but in fact may be greater after 5 years of follow-up. In the first 5 years, 4.3% of patients developed malignancy, whereas in the 363 patients followed for more than 5 years, the rate was 5.5%, and is still present (4%) after 10 years of follow-up. Of note, in 2 patients, pancreatic cancer developed after a partial resection for a benign IPMN more than 8 years after the first discovery of the cyst. In contrast to our findings, Kwong et al[22] found a very low-risk of pancreatic malignancy after 5 years of follow-up. In their study, 722 patients with suspected asymptomatic neoplastic pancreatic cysts were selected by EUS evaluation, and 310 of them were followed for more than 5 years (median follow-up of 87 months). Within 5 years, 12% of patients (87 of 722) underwent surgery and 8.7% (63 of 722) developed pancreatic adenocarcinoma. After 5 years, surprisingly, only 1% of the patients (3 of 310) underwent surgery and the same number developed invasive pancreatic cancer; all 3 of these tumors were unresectable. Because the demographic characteristics of these 310 patients are similar to our cohort, the discrepancies with our results are perhaps attributable to the inclusion of all types of neoplastic cysts (not only suspected BD-IPMNs), only cysts without symptoms, and the exclusion of patients with acute pancreatitis, which can be a presenting symptom and a risk factor for malignancy in IPMNs. Even though all of the patients in the study by Kwong et al[22] were identified by EUS, suggesting the capture of cysts with more high-risk features, only 27% (85 of 310) underwent FNA (vs 66% in our series), 2% showed a solid component during the entire evaluation period (vs 15% in our series), and 1% of patients were taken to surgery (vs 22% in our series). It is unclear what accounts for these differences.

Kwong et al[22] also found that no cancers developed after the 5-year mark in cysts lacking all 3 American Gastroenterological Association high-risk features (cyst size >3 cm, dilated MPD, and mural nodule), while in only 1% of those with 1 risk feature was malignant transformation observed. Accordingly, they supported the discontinuation of surveillance after 5 years as proposed in the American Gastroenterological Association guideline.[22] By contrast, our study found that patients are not free from the risk of malignancy, even in the absence of WFs at 5 years. Twelve of 282 patients (4.3%) who had no WF/HRS at the 5-year mark developed malignancy and the risk was not significantly lower than that for cystic lesions with early-onset (ie, within 5 years) WF (P ¼ .09). These numbers represent an

18.8-fold increase in the risk of pancreatic cancer relative to the US population. Even though this result was identified in a population of patients followed at a referral center for pancreatic disease and therefore may not be generalizable to all patients with BD-IPMNs, it does suggest that patients with BD-IPMNs have a significantly higher risk of malignant transformation and, therefore, surveillance should not be discontinued after 5 years. Similarly, Khannoussi et al[23] also found that in 53 patients with a median follow-up of 84 months (range, 60À132 months) and who lacked signs of malignancy after 5 years of follow-up, 4% developed pancreatic cancer at a later time point.

Recently, Mukewar et al[7] analyzed the impact of WFs and HRS on the risk of cancer. The patients in that cohort had a median follow-up of 4.2 years (interquartile range, 1.8À7.1 years) and some had main duct and mixed-type IPMNs. In that study, patients with HRS had a 5-year risk of pancreatic malignancy of 49.7%, whereas in those with WFs, the risk was only 4.1% ($P < .001$).[7] Furthermore, in a multi-institutional study, Crippa et al[4] analyzed the survival outcomes of non-operated IPMNs with WF and, even including main duct and mixed-type IPMNs, they showed a 5-year disease-specific survival of 96%, indicating that WFs have a low impact on short-term survival outcomes.[4] This is very useful information for a clinician taking care of patients with IPMNs who have a short life expectancy because of advanced age or comorbidities, but does not give a forecast regarding the long-term fate of patients with pancreatic cysts that have WFs. In the present study, 22% of patients who were followed for more than 5 years had either WFs or HRS by the 5-year time point, and 10% of them developed pancreatic malignancy subsequently. Importantly, the median time elapsed between diagnosis of a cyst with WF/HRS and diagnosis of malignancy, although highly variable (between 1 and 132 months), was 102 months, indicating that progression can be very slow.

There is increasing awareness that patients with IPMNs, particularly of the branch duct type, are not only at risk of developing malignancy within the IPMN, but also away from the cysts. These tumors, referred to as distinct, concurrent, or concomitant adenocarcinomas, can occur during surveillance of patients being followed for IPMN, and also in patients who have had a resection for IPMN.[24–26] Distinguishing one type of pancreatic malignancy from the other is not straightforward, particularly if the distinct adenocarcinoma occurs in the vicinity of a known IPMN. In the present study, the ratio of malignancy arising within an IPMN vs distinct adenocarcinoma was 4:1. The current guidelines for surveillance of patients with IPMNs recommend repeating imaging at 6 months, and then lengthening the interval to 1 or 2 years, depending on size.[2] This works well to identify the morphologic changes that come hand in hand with malignant transformation of the IPMNs, but is not intended to detect in a timely fashion development of distinct adenocarcinoma, which has a more aggressive biology. In this series, of the 45 malignancies identified, 86% of those arising within the cyst were resected with an intent for cure, whereas only 44% of the distinct adenocarcinomas were operable, and were all stage II or higher.

In the light of these findings, except for patients with short life expectancy or significant comorbidities that would preclude surgery, we strongly support continued surveillance after 5 years from the initial diagnosis. In patients with BD-IPMNs, the 18.8-fold increased incidence rate of developing pancreatic cancer after 5 years of observation (which is almost 3 times greater than that of an individual with familial pancreatic cancer who has 2 first-degree family members with this disease[27]) argues that the frequency of surveillance should be increased, rather than diminished, with the goal of identifying patients with high-grade dysplasia where the disease can be cured. Waiting to see signs of invasive cancer defeats the purpose of surveillance in a disease as aggressive as pancreatic cancer.

The analysis of this cohort identified a subgroup of cysts that appear to have a significantly lower risk of progression or development of malignancy. Of the 108 patients with pancreatic cysts that were ◌°1.5 cm and remained so for more than 5 years, only 1 developed pancreatic cancer, and this was a distinct adenocarcinoma. By contrast, 7.5% of patients whose cysts were >1.5 cm after 5 years of follow-up developed malignancy. The role of cyst size and growth rate as predictors of malignancy is controversial and several studies have concluded that cyst size alone should not be used to determine the treatment strategy.[4,5,7,28–34] However, for small cysts, there are no other parameters that are useful and, in fact, many of them may not even be IPMNs. Recently, Yoen et al[21], in 95 patients followed for more than 5 years, showed an appreciable difference in outcomes according to the initial cyst size: cysts <1 cm were indolent compared to the larger ones. Accordingly, they proposed a new follow-up strategy: cysts <1.5 cm without ductal change might be followed at longer intervals of more than 3 years.[21] In our study, to discriminate low-risk BD-IPMNs, we considered stability of the cyst <1.5 cm for more than 5 years. Because all cysts are initially small, and whenever we discover a new cyst the moment in its natural history is unknown, dictating surveillance strategy at that point may be unsafe. An initial observation time of 5 years to assess the stability of the cyst might be a more appropriate method to discriminate low-risk BD-IPMNs, and from then on, plan longer interval surveillance, or, if other studies confirm these findings, discontinue it.

This study has several limitations. The first is the absence of the histologic confirmation of the diagnosis of BD-IPMN in patients that did not undergo resection. The communication of the cyst with the main duct was considered an essential requisite to distinguish BD-IPMNs from other cyst types in other studies; however, it is visible on cross-sectional imaging only in a small percentage of cases.[7,13] On the basis of the presence of duct communication, Mukewar et al[7] distinguished patients with high and low likelihood of being BD-IPMN and referred to them as suspected and presumed, respectively. They described that suspected BD-IPMNs had a higher risk of pancreatic cancer and of undergoing surgery than presumed IPMNs. In our series, using the same criteria of categorization described by Mukewar et al, we did not confirm this. Another limitation is the retrospective nature of the study; as a result, the management of patients changed over time, the surveillance was

not homogeneous regarding imaging modalities and timing, and some patients were lost to follow-up. Moreover, this work represents the experience of a single pancreatic referral center, which may limit generalizability of our results to the full population of patients with BD-IPMNs.

In conclusion, in patients with pancreatic cysts that are thought to be BD-IPMNs, the risk of malignancy persists after 5 years and even after 10 years of follow-up. The absence of WFs or HRS at a 5-year time point does not exclude the development of pancreatic malignancy, and the risk in these patients is 18.8 times higher than in the general population. Because of this, we strongly support continued surveillance after 5 years from the initial diagnosis. We also show that stability of small cysts ◌°1.5 cm may be a useful parameter to safely discriminate low-risk BD-IPMNs after 5 years of follow-up. This is an important issue for further investigation because it may help reduce costs related to surveillance and improve patients' quality of life.

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.2017.07.019.

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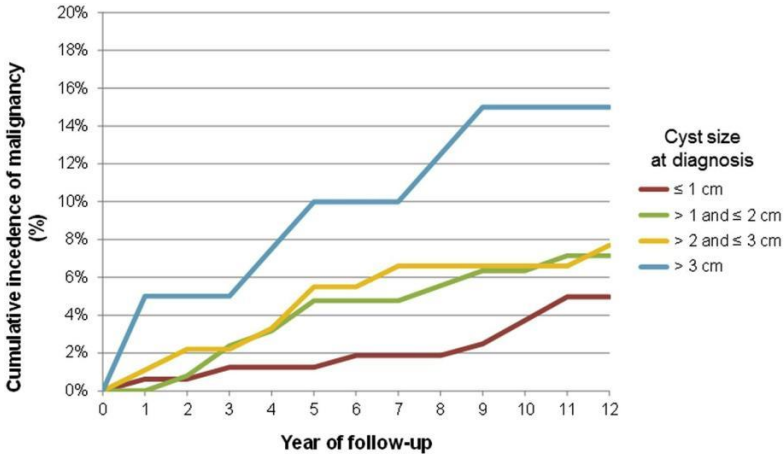
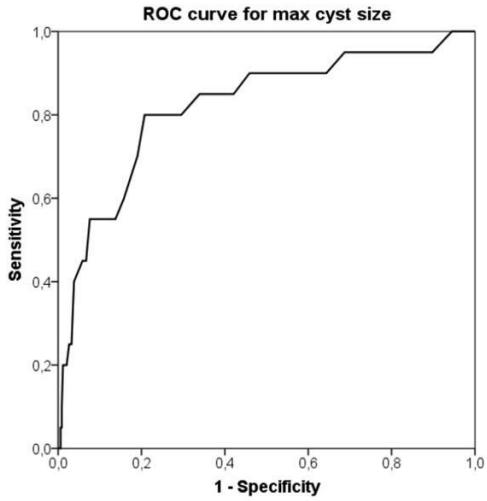
Received April 7, 2017. Accepted July 16, 2017.

Reprint requests
Address requests for reprints to: Carlos Fernández-del Castillo, MD, Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Wang Ambulatory Care Center 460, 15 Parkman Street, Boston, Massachusetts 02114. e-mail: cfernandez@partners.org; fax: (617) 724-3383.

Conflicts of interest
The authors disclose no conflicts.



Supplementary Figure 1. Cumulative incidence of pancreatic malignancy stratified by the time from diagnosis and the cyst size at diagnosis. The lines represent the cumulative incidence of pancreatic malignancy for different cyst size at diagnosis (○°1 cm, >1 and ○°2 cm, >2 and ○°3 cm and >3 cm) stratified by year of follow-up.



Supplementary Figure 2. Receiver operating characteristic (ROC) curve analysis used to select a maximum cyst size cutoff for malignancy. Area under the ROC curve was 0.820 ($P < .001$). ROC curve analysis showed that the value of cyst size 1.5 cm could predict pancreatic malignancy with a sensitivity of 95% and a negative predictive value of 99%.

PLOS ONE

RESEARCH ARTICLE

Tumor engraftment in patient-derived xenografts of pancreatic ductal adenocarcinoma is associated with adverse clinicopathological features and poor survival

Ilaria Pergolini[1,2☯], Vicente Morales-Oyarvide[1☯], Mari Mino-Kenudson[3], Kim C. Honselmann[1], Matthew W. Rosenbaum[3], Sabikun Nahar[1], Marina Kem[3], Cristina Ferrone[1], Keith D. Lillemoe[1], Nabeel Bardeesy[4], David P. Ryan[1], Sarah P. Thayer[1¤], Andrew L. Warshaw[1], Carlos Fernandez-del Castillo[1], Andrew S. Liss[1*]

1 Department of Surgery and the Andrew L. Warshaw, MD Institute for Pancreatic Cancer Research, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, United States of America, **2** Department of Surgery, Universita' Politecnica delle Marche, Ancona, Italy, **3** Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, United States of America, **4** Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, Massachusetts, United States of America

☯ These authors contributed equally to this work.
¤ Current address: Division of Surgical Oncology and the Fred and Pamela Buffett Cancer Center, University of Nebraska Medical Center, Omaha, Nebraska, United States of America.
* aliss@mgh.harvard.edu

OPEN ACCESS

Citation: PergoliniI, Morales-OyarvideV, Mino-KenudsonM, HonselmannKC, RosenbaumMW, NaharS, et al. (2017) Tumorengraftment in patient-derivedxenografts of pancreatic ductal adenocarcinoma is associated withadverse clinicopathologicalfeatures and poor survival. PLoSONE12(8): e0182855. https://doi.org/10.1371/journal.pone.0182855

Editor: Ilse Rooman, VrijeUniversiteitBrussel, BELGIUM

Received: April7, 2017

Accepted: July 24, 2017

Published: August30, 2017

Data AvailabilityStatement: Allrelevant data are availablefrom the HarvardDataverse: Liss, Andrew,2017, "ReplicationData for: Tumor engraftment in patient-derivedxenografts of pancreatic ductal adenocarcinoma is associated withadverse clinicopathologicalfeatures and poor survival",doi:10.7910/DVN/TPHZMU, Harvard Dataverse, V1.

Abstract

Patient-derived xenograft (PDX) tumors are powerful tools to study cancer biology. However, the ability of PDX tumors to model the biological and histological diversity of pancreatic ductal adenocarcinoma (PDAC) is not well known. In this study, we subcutaneously implanted 133 primary and metastatic PDAC tumors into immunodeficient mice. Fifty-seven tumors were successfully engrafted and even after extensive passaging, the histology of poorly-, moderately-, and well-differentiated tumors was maintained in the PDX models. Moreover, the fibroblast and collagen contents in the stroma of patient tumors were recapitulated in the corresponding PDX models. Analysis of the clinicopathological features of patients revealed xenograft tumor engraftment was associated with lymphovascular invasion ($P = 0.001$) and worse recurrence-free (median, 7 vs. 16 months, log-rank $P = 0.047$) and overall survival (median, 13 vs. 21 months, log-rank $P = 0.038$). Among successful engraftments, median time of growth required for reimplantation into new mice was 151 days. Reflective of the inherent biological diversity between PDX tumors with rapid (<151 days) and slow growth, differences in their growth were maintained during extensive passaging. Rapid growth was additionally associated with lymph node metastasis ($P = 0.022$). The association of lymphovascular invasion and lymph node metastasis with PDX formation and rapid growth may reflect an underlying biological mechanism that allows these tumors to adapt and grow in a new environment. While the ability of PDX tumors to mimic the cellular and non-cellular features of the parental tumor stroma provides a valuable model to study the interaction of PDAC cells with the tumor microenvironment, the association of

Funding: Grant number P01CA117969is from the NationalInstitutes of Health(SPT). The funders had no role in study design, data collectionand analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

successful engraftment with adverse clinicopathological features suggests PDX models over represent more aggressive forms of this disease.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the most common pancreatic cancer, with more than 53,000 cases diagnosed per year in the United States. Only 8% of these patients survive beyond five years, making PDAC the fourth leading cause of cancer-related deaths [1]. The ineffectiveness of treatments and the scant improvement of survival outcomes may be ascribed to the fact that PDAC has historically been modeled as a single disease entity. By contrast, recent advances in genomics have revealed the heterogeneity of this disease [2–4]. Although activating mutations in KRASoccur in ~90% of PDAC, there are few additional genes (such as TP53 and SMAD4) commonly mutated or inactivated in pancreatic cancer [5]. Contributing to the challenges associated with treating PDAC is a highly desmoplastic stroma that promotes the aggressive local growth of the tumor and the intrinsic chemo-resistance of the cancer cells [6–9]. Thus, modeling the tumor microenvironment and its crosstalk with the cancer cells is particularly important in developing new therapies for PDAC.

Several in vitro and in vivo preclinical models are available to study the biology of cancer, including cell lines and xenograft tumors derived from them, genetically-engineered mouse models, organoids, and patient-derived xenograft (PDX) tumor models [10,11]. Among these, PDX tumors have the advantage of mimicking the genetic complexity of human PDAC in a platform that has the potential to recapitulate many of the features of the tumor microenvironment. Moreover, a model that faithfully reflects the original tumor biology may predict clinical outcomes and allow for the development of personalized targeted therapies [12–15]. However, the growth of PDX tumors from pancreatic cancer is variable and the determinants of their growth are unknown [16–19].

Understanding the dynamic behind xenograft tumor formation, the features that affect the success of tumor engraftment, and the prognostic implications could be an important key for new perspectives in the knowledge of pancreatic cancer. The aims of our study were to identify clinical and pathological factors associated with successful tumor engraftment and xenograft growth rate, and to evaluate whether tumor engraftment and xenograft rate of growth were prognostic of patient outcomes. We also analyzed PDX tumors to assess the ability of these models to reproduce the histological features of the original pancreatic tumors.

Materials and methods

Patient population and tumor samples

All study participants provided IRB-approved informed consent for their medical records and tissue samples to be used in this study. Patient clinical data was entered into a de-identified clinical database allowing for the anonymous analysis of demographic, clinical and pathological variables. We collected fresh tumor samples from 133 patients with histologically-confirmed stages I-IV PDAC who underwent surgery for curative intent, diagnostic laparoscopy, or palliation of symptoms at the Massachusetts General Hospital between April, 2009 and July, 2012.

Xenograft tumors

Patient tumor samples were mechanically minced into small fragments (1–2 mm^3) and either implanted into mice or cryopreserved in freezing media (10% DMSO, 20% FBS, 70% DMEM/

Ham's F-12 50/50 supplemented with 1% PS) for future implantation. Cryopreserved tumors were rapidly thawed in a 37˚C water bath and washed twice with PBS prior to implantation. For tumor implantation, 6–8 week-old nu/j mice (Jackson Laboratory) were anesthetized with Isoflurane and a small incision made on the dorsal flank. Approximately 70–100 mg of tumor tissue coated in 50–100 of μl of Matrigel (Corning, 354248) was subcutaneously implanted into the flank of each mouse and the incision was closed with a single suture (4–0 Coated Vicryl, Ethicon). Mice were administered buprenorphine as needed as an analgesic. Patient tumors were implanted in a median number of 4 mice (range 1–5). In 10.5% of cases the tumor tissue was enough for only one mouse. Mice were monitored weekly for tumor growth. Mice that lacked a palpable tumor after 6 months were removed from the study and the patient tumors were categorized as no engraftment. Successful tumor engraftment was defined as tumors that grew large enough (1 cm) to be reimplanted into new mice. Mice with tumors < 1 cm after 180 days were retained in the study until tumors reached sufficient size for reimplantation. Tumors implanted in mice that were removed from the study due to health reasons before the tumor reached an appropriate size for reimplantation were categorized as no engraftment. For tumors that successfully formed xenografts, we recorded the time of growth between initial implantation and reimplantation into new mice. The presence of pancreatic adenocarcinoma in xenograft tumors was confirmed by histological analysis of hematoxylin and eosin (H&E) stained sections by pathologists with a special interest in pancreatic cancer (M.M.K. and M.W.R). Maximum tumor size in this study did not exceed 1.5 cm. Mice were euthanized by CO_2 asphyxiation in accordance to the guidelines set forth in the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals.

Immunohistochemistry

Representative sections of primary human tumors were stained with a 1:50 dilution of a goat polyclonal antibody specific to SMAD4 (sc-1909, Santa Cruz Biotechnology, Dallas, TX, USA) using the Bond RX IHC staining platform (Leica Biosystems, Buffalo Grove, IL, USA) with BOND Epitope Retrieval Solution 2 (Leica Biosystems, AR9640). The staining of SMAD4 was scored by a pathologist and SMAD4 expression was considered preserved (positive) when detected in the nucleus and/or cytoplasm of PDAC cells. Staining of stromal cells in each tumor section was used as an internal positive control. For analysis of cancer-associated fibroblasts, deparaffinized sections of patient and PDX tumors were stained with a 1:200 dilution of a rabbit polyclonal antibody specific to alpha smooth muscle actin (ab5694, Abcam, Cambridge, MA, USA) as described previously [20]. The collagen content of tumors was visualized by picrosirius red staining (Picro Sirius Red Stain Kit, Abcam, Cambridge, MA, USA).

Genetic analysis of patient tumors

DNA extracted from formalin-fixed paraffin-embedded tumor samples from 20 patients was analyzed for somatic mutations using SNaPshot multiplex assays (Applied Biosystems) [21]. Common to these assays were the tests for variants in loci found in APC, BRAF, CTNNB1, EGFR, KIT, KRAS, NOTCH1, NRAS, PI3KA, PTEN, and TP53. On average, approximately 5% mutant allele is sufficient for detection in these assays. Only mutations in KRAS and TP53 were detected in our series.

Outcome measures

The primary outcome measures of this study were successful tumor engraftment and xenograft rate of growth. We also evaluated whether tumor engraftment and xenograft growth rate were associated with patient recurrence-free survival (RFS) and overall survival (OS). RFS was

defined as time between surgery and evidence of disease recurrence or death from any cause; analyses of RFS were restricted to patients with resectable primary tumors and no evidence of metastatic disease (n = 112). OS was defined as time between surgery and death from any cause; analyses of OS were performed in the entire study population. Follow-up continued through February, 2017.

Statistical analysis

We evaluated the associations of clinical and pathological features with tumor engraftment and xenograft rate of growth using univariate analyses. Analyses of categorical data were performed using chi-square or Fisher exact tests, where appropriate; continuous data were analyzed with Mann-Whitney U test. Associations of tumor engraftment and xenograft growth rate with patient survival were analyzed using log-rank tests and multivariable-adjusted Cox proportional hazards regression adjusting for potential confounders. Kaplan-Meier survival curves, median survival time, and two- and five-year survival rates were also presented. Cox regression models adjusted for patient age and sex, receipt of neoadjuvant and adjuvant therapy, American Joint Committee on Cancer (7^{th} edition) clinical stage, tumor differentiation grade, presence of lymphovascular invasion, surgical margins, and tumor location. Statistical significance was set at $P<0.05$ and all hypothesis tests were two-sided. Statistical analyses were conducted using SAS software (version 9.4; SAS Institute, Cary, NC).

Results

Primary or metastatic tumor samples were collected from 133 patients with histologically-confirmed diagnosis of PDAC. The baseline characteristics of patients in our study are presented in Tables 1 and 2. The median age of the patients in our study was 68 (range 35–93). Fifty-seven (43%) tumors implanted into immunodeficient mice were successfully engrafted, while 76 (57%) failed to do so. A number of studies have reported different methods of cryopreserving tumors for future growth as xenograft tumors [22,23]. In our study, there was no significant difference in the engraftment rate between freshly implanted (n = 24) and cryopreserved (n = 109) tumors (50% vs 41%, P = 0.498).

Histological analysis of the xenograft tumors revealed that the grade of differentiation of the human tumors was retained in each of the corresponding xenografts. Moreover, the histology of poorly-, moderately-, and well-differentiated tumors was retained through at least 10 generations in mice (Fig 1A). Characteristic of the stroma of PDAC tumors is the presence of cancer associated fibroblasts and abundance of collagen. The corresponding xenograft tumors contain α-smooth muscle actin-expressing fibroblasts similarly to patient tumors (Fig 1B). Furthermore, picrosirius red staining revealed that collagen content of xenograft tumors was largely composed of either organized or disorganized fibers and these collagen subtypes were similarly found within the malignant epithelium of the corresponding patient tumor (Fig 1B). Collectively, these results demonstrate that patient-derived xenograft models of PDAC retain the histological and microenvironmental characteristics of the original tumor.

Clinical and genetic characteristics, pathological features, and tumor engraftment status

To gain insight into the factors that determine tumor engraftment, we evaluated the demographic, clinical, and pathological characteristics of patients based on tumor engraftment status (Tables 1 and 2). We found no significant associations between demographic or clinical features and tumor engraftment. Neoadjuvant chemotherapy has become increasingly common in patients with PDAC and is associated with increased fibrosis and a reduction in viable

Table 1. Baseline demographic and clinical characteristics of 133 patients with resected pancreatic ductal adenocarcinoma by tumor engraftment status.

	Overall	Tumor engraftment		
		Yes	No	P value
No. Patients	133	57 (43%)	76 (57%)	
Men, n (%)	70 (53%)	34 (60%)	36 (47%)	0.160
Age, median (IQR)	68 (18)	66 (16)	70 (18.5)	0.730
Serum CA19-9, median (IQR)	128 (376.6)	118 (347.0)	128.5 (436.5)	0.916
Body mass index, median (IQR)	25.9 (6.0)	26.8 (6.3)	25.2 (6.0)	0.390
Diabetes, n (%)				
No	101 (76%)	41 (72%)	60 (79%)	0.345
Yes	32 (24%)	16 (28%)	16 (21%)	
New-onset or worsening diabetes, n (%)*				
No	11 (34%)	6 (37%)	5 (31%)	0.809
Yes	20 (63%)	10 (63%)	10 (63%)	
Unknown	1 (3%)	-	1 (6%)	
Neoadjuvant therapy, n (%)				
No	91 (68%)	42 (74%)	49 (64%)	0.258
Yes	42 (32%)	15 (26%)	27 (36%)	
Resection, n (%)				
No	17 (13%)	9 (16%)	8 (11%)	0.368
Yes	116 (87%)	48 (84%)	68 (89%)	
Metastatic disease, n (%)				
No	112 (84%)	45 (79%)	67 (88%)	0.149
Yes	21 (16%)	12 (21%)	9 (12%)	
Type of resection, n (%)				
Whipple	91 (68%)	34 (59%)	57 (75%)	0.131
Middle	4 (3%)	1 (2%)	3 (4%)	
Distal	20 (15%)	13 (23%)	7 (9%)	
Total	1 (1%)	-	1 (1%)	
No resection	17 (13%)	9 (16%)	8 (11%)	
Adjuvant therapy, n (%)**				
No	30 (27%)	10 (22%)	20 (30%)	0.421
Yes	79 (70%)	33 (73%)	46 (69%)	
Unknown	3 (3%)	2 (5%)	1 (1%)	
Recurrence, n (%)**				
No	28 (25%)	9 (20%)	19 (28%)	0.317
Yes	84 (75%)	36 (80%)	48 (72%)	
Site of recurrence, n (%)***				
Locoregional	21 (25%)	9 (25%)	12 (25%)	0.488
Distant	54 (64%)	24 (67%)	30 (63%)	
Both locoregional and distant	8 (10%)	2 (5%)	6 (12%)	
Unknown	1 (1%)	1 (3%)	-	

Abbreviations: IQR, interquartile range.

*Among patients with diabetes mellitus (n = 32).

** Among patients undergoing resection with curative intent and absence of metastatic disease (n = 112).

*** Among patients with evidence of recurrence following resection with curative intent (n = 84).

https://doi.org/10.1371/journal.pone.0182855.t001

Table 2. Pathological characteristics of 133 patients with resected pancreatic ductal adenocarcinoma by tumor engraftment status.

	Overall	Tumor engraftment		
		Yes	No	P value
No. Patients	133	57 (43%)	76 (57%)	
Tumor differentiation grade, n (%)				
Well differentiated	7 (5%)	3 (5%)	4 (5%)	0.846
Moderately differentiated	65 (49%)	26 (46%)	39 (52%)	
Poorly differentiated	53 (40%)	24 (42%)	29 (38%)	
Unknown	8 (6%)	4 (7%)	4 (5%)	
AJCC 7th ed. stage, n (%)				
IA	1 (1%)	1 (2%)	-	0.333
IB	6 (4%)	2 (3%)	4 (5%)	
IIA	17 (13%)	5 (9%)	12 (16%)	
IIB	88 (66%)	37 (65%)	51 (67%)	
III	-	-	-	
IV	21 (16%)	12 (21%)	9 (12%)	
AJCC 7th ed. pT, n (%)*				
pT1	2 (2%)	1 (2%)	1 (2%)	0.928
pT2	11 (10%)	4 (9%)	7 (10%)	
pT3	99 (88%)	40 (89%)	59 (88%)	
pT4	-	-	-	
AJCC 7th ed. pN, n (%)*				
pN0	24 (21%)	8 (18%)	16 (24%)	0.440
pN1	88 (79%)	37 (82%)	51 (76%)	
Tumor location, n (%)				
Body/Tail	30 (23%)	18 (32%)	12 (16%)	0.031
Head/Uncinante	103 (77%)	39 (68%)	64 (84%)	
Size cm, median (IQR)*	3.0 (1.5)	3.5 (1.7)	2.8 (1.6)	0.051
Lymphovascular invasion, n (%)				
Absent	40 (30%)	8 (14%)	32 (42%)	0.001
Present	81 (61%)	43 (75%)	38 (50%)	
Unknown	12 (9%)	6 (11%)	6 (8%)	
Perineural invasion, n (%)				
Absent	6 (4%)	2 (3%)	4 (5%)	0.681
Present	110 (83%)	46 (81%)	64 (84%)	
Unknown	17 (13%)	9 (16%)	8 (11%)	
Surgical margins, n (%)*				
R0	98 (87%)	41 (91%)	57 (85%)	0.532
R1	13 (12%)	4 (9%)	9 (13%)	
R2	1 (1%)	-	1 (2%)	
SMAD4, n (%)**				
Retained	40 (62%)	20 (69%)	20 (57%)	0.331
Lost	24 (38%)	9 (31%)	15 (43%)	

Abbreviations: IQR, interquartile range.

*Among patients undergoing resection with curative intent and absence of metastatic disease (n = 112).

**Among tumors with available SMAD4 immunohistochemistry (n = 64).

https://doi.org/10.1371/journal.pone.0182855.t002

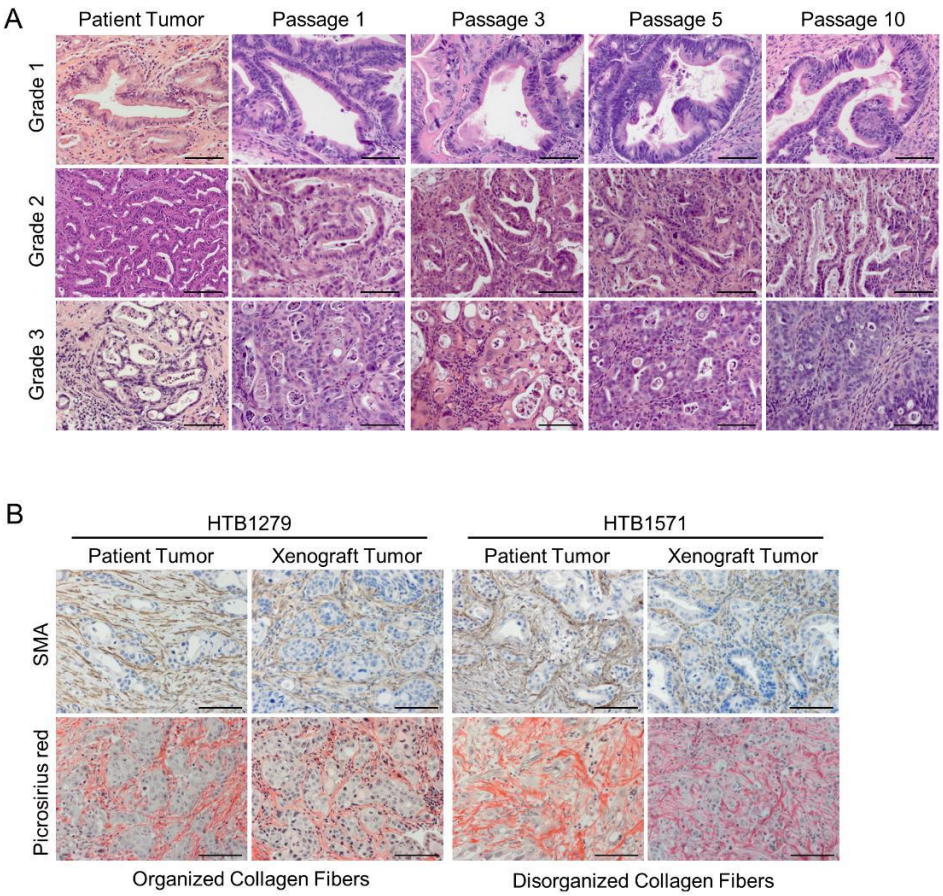


Fig 1. PDX models of PDAC retain the histological and stromal features of the parental tumor. (A) H&E staining of well-differentiated (grade 1), moderately-differentiated (grade 2) and poorly-differentiated (grade 3) tumors. The primary patient tumor and corresponding passages of the PDX models are shown. Scale bars = 100 μm. (B) Immunohistochemistry for α-smooth muscle actin (SMA; top panels) and picrosirius red staining for collagen (bottom panels) was performed on representative PDX models and corresponding patient tumors. Scale bars = 100 μm.

https://doi.org/10.1371/journal.pone.0182855.g001

cancer cells, which could influence the ability of a tumor to successfully establish a xenograft [24]. Notably, 42 (32%) patients in our series received neoadjuvant chemotherapy and this did not adversely affect tumor engraftment (Table 1).

Analysis of pathological features of patient tumors demonstrated that lymphovascular invasion and location of the primary tumor within the pancreas were significantly associated with tumor engraftment. Tumors with successful engraftment had higher frequency of lymphovascular invasion (43/57, 75%) compared to tumors that did not form xenografts (38/76, 50%; P = 0.001). Among patients whose tumors successfully engrafted, primary tumors were located

in the body and tail of the pancreas in 32% (18/57) of cases; in contrast, only 16% (12/76) of primary tumors that did not engraft were located in the pancreatic body and tail (P = 0.031). Features such as tumor grade, nodal metastases, or type of implanted tumor tissue (i.e., primary vs. metastatic) were not significantly associated with engraftment status.

To evaluate whether common molecular alterations found in PDAC are associated with tumor engraftment, we performed genetic analysis of KRAS and TP53, and immunohistochemistry for SMAD4. Genetic analysis of engrafted and non-engrafted tumors revealed 18/20 (90%) tumors contained activating mutations in codon 12 or 61 of KRAS, while hot spot mutations in TP53 were identified in three (15%) tumors (Fig 2A). However, there was no association between these mutations and the success of tumor engraftment. SMAD4 is frequently inactivated in PDAC through both genetic and epigenetic mechanisms [25]. Therefore, we employed immunohistochemistry to evaluate the expression of SMAD4 in 64 patient tumors, 45% of which were successfully engrafted (Fig 2B). The expression of SMAD4 was lost in 38% of tumors, and there was no significant difference in the loss of SMAD4 expression between engrafted and non-engrafted tumors (31% vs 43%, P = 0.331, Fig 2C). Taken together, these results suggest that alterations in these core PDAC pathways are not predictive of tumor engraftment.

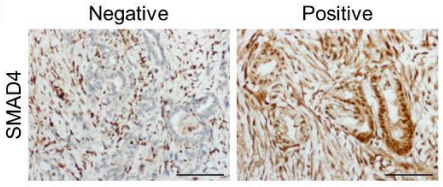
Clinical characteristics, pathological features, and xenograft growth rate

Among successful engraftments, the median time of growth required for reimplantation into new mice was 151 days (range 39–346 days; Fig 3A). Xenograft tumors that were reimplanted in less than 151 days were defined as having a rapid growth rate. Analysis of representative rapid and slow (◌° 151 days) xenograft tumor lines revealed that the relative differences in the growth rate of these tumors was maintained for at least 10 generations (Fig 3B). Since these differences in the time to tumor engraftment may be related to the inherent characteristics of the

A

	Patient ID	KRAS	TP53
Successful Tumor Engraftments	HTB1279	Gly12Asp	-
	HTB1294	Gly12Asp	-
	HTB1312	-	-
	HTB1335	Gly12Asp	Arg273His
	HTB1398	Gly12Val	-
	HTB1413	Gly12Asp	-
	HTB1555	Gly12Arg	-
	HTB1568	-	-
	HTB1571	Gly12Arg	Cys176Ser
Unsuccessful Tumor Engraftments	HTB1351	Gly12Asp	-
	HTB1354	Gly12Val	Arg248Trp
	HTB1404	Gly12Asp	-
	HTB1420	Gln61His	-
	HTB1424	Gly12Asp	-
	HTB1443	Gly12Val	-
	HTB1446	Gly12Arg	-
	HTB1526	Gly12Val	-
	HTB1539	Gly12Asp	-
	HTB1579	Gly12Ala	-
	HTB1602	Gly12Val	-

B



C

	Overall	SMAD4 Expression		
		Negative	Positive	*P*
Tumor Engraftment (n=64)				
Yes	29	9 (38%)	20 (50%)	0.438
No	35	15 (62%)	20 (50%)	
Xenograft Growth				
Rapid	18	6 (67%)	12 (60%)	1.000
Slow	11	3 (33%)	8 (40%)	

Fig 2. Molecular characteristics of patient tumors do not predict tumor engraftment. (A) Genetic analysis of *KRAS* and *TP53* in patient tumors. Amino acid changes resulting from mutations are listed. Samples for which mutations were not detected are indicated by a dash. (B) Representative positive and negative immunohistochemical staining of SMAD4 in primary patient tumors. Staining of stromal cells on each slide served as a positive control. Scale bars = 100 μm. (C) Summary of SMAD4 expression in patient tumors that were successfully or unsuccessfully engrafted in mice.

https://doi.org/10.1371/journal.pone.0182855.g002

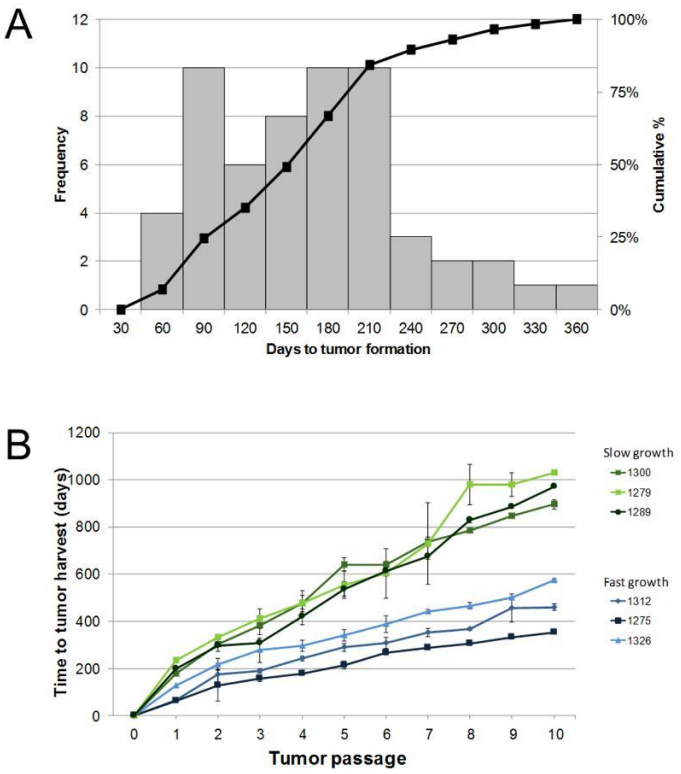


Fig 3. The time to tumor engraftment correlates with the rate of PDX tumor growth. (A) Time to tumor engraftment was grouped in 30-day intervals and the frequency of their occurrence is shown on the left y-axis. The cumulative percentage of tumors engrafted over time is shown on the right y-axis. (B) The growth of PDX tumors that exhibited rapid and slow engraftment. The median and standard deviation of tumors for passages 2–10 are shown. Passage 1 tumors represent the founding PDX tumor from which subsequent PDX passages were derived.

https://doi.org/10.1371/journal.pone.0182855.g003

tumor biology, we performed a comparison of the clinical (Table 3) and pathological (Table 4) features between tumors with rapid and slow growth. Rapid growth was significantly associated with patients of male gender (P = 0.020), primary tumors located in the head of the pancreas (P = 0.029), and lymph node metastases (P = 0.022). Collectively, our results suggest that both tumor engraftment and xenograft growth rate are associated with adverse pathological features (e.g. lymphovascular for tumor engraftment and lymph node metastases for xenograft growth rate).

Tumor engraftment, rate of growth, and survival outcomes

To determine the prognostic value of tumor engraftment, we analyzed the relationship between patient survival outcomes and PDX formation and growth rate. Overall, 30 (22.6%)

Table 3. Baseline demographic and clinical characteristics of 57 patients with patient-derived PDAC xenografts based on xenograft growth rate.

	Overall	Xenograft Growth		
		Rapid	Slow	P value
No. patients	57	28 (49%)	29 (51%)	
Men, n (%)	34 (60%)	21 (75%)	13 (45%)	0.020
Age, median (IQR)	66 (16.0)	66 (11.5)	71 (24)	0.193
Serum CA19-9, median (IQR)	118 (347.0)	145 (376.5)	94 (195.0)	0.429
Body mass index, median (IQR)	26.8 (6.3)	27.3 (7.9)	26.3 (5.3)	0.334
Diabetes, n (%)				
No	41 (72%)	20 (71%)	21 (72%)	0.934
Yes	16 (28%)	8 (29%)	8 (28%)	
New-onset or worsening diabetes, n (%)*				
No	6 (37%)	2 (25%)	4 (50%)	0.608
Yes	10 (63%)	6 (75%)	4 (50%)	
Neoadjuvant therapy, n (%)				
No	42 (74%)	20 (71%)	22 (76%)	0.704
Yes	15 (26%)	8 (29%)	7 (24%)	
Resection, n (%)				
No	9 (16%)	4 (14%)	5 (17%)	1.00
Yes	48 (84%)	24 (86%)	24 (83%)	
Metastatic disease, n (%)				
No	45 (79%)	23 (82%)	22 (76%)	0.561
Yes	12 (21%)	5 (18%)	7 (24%)	
Type of resection, n (%)				
Whipple	34 (59%)	20 (72%)	14 (48%)	0.253
Middle	1 (2%)	-	1 (4%)	
Distal	13 (23%)	4 (14%)	9 (31%)	
Total	-	-	-	
No resection	9 (16%)	4 (14%)	5 (17%)	
Adjuvant therapy, n (%)**				
No	10 (22%)	3 (13%)	7 (32%)	0.281
Yes	33 (73%)	18 (78%)	15 (68%)	
Unknown	2 (5%)	2 (9%)	-	
Recurrence, n (%)**				
No	9 (20%)	3 (13%)	6 (27%)	0.284
Yes	36 (80%)	20 (87%)	16 (73%)	
Site of recurrence, n (%)***				
Locoregional	9 (25%)	5 (25%)	4 (25%)	0.412
Distant	24 (67%)	13 (65%)	11 (69%)	
Both locoregional and distant	2 (5%)	2 (10%)	-	
Unknown	1 (3%)	-	1 (6%)	

Abbreviations: IQR, interquartile range.

*Among patients with diabetes mellitus (n = 16).

** Among patients undergoing resection with curative intent and absence of metastatic disease (n = 45).

*** Among patients with evidence of recurrence following resection with curative intent (n = 36).

https://doi.org/10.1371/journal.pone.0182855.t003

patients of our cohort were alive at the end of follow-up period, and among them the median follow up time was 45 months. In patients who underwent resection with curative intent without evidence of metastatic disease, the median, 2-year, and 5-year RFS was 10 months (95% CI

Table 4. Baseline pathological characteristics of 57 patients with patient-derived PDAC xenografts based on xenograft growth rate.

	Overall	Xenograft Growth		
		Rapid	Slow	P value
No. patients	57	28 (49%)	29 (51%)	
Tumor differentiation grade, n (%)				
Well differentiated	3 (5%)	1 (4%)	2 (7%)	0.728
Moderately differentiated	26 (46%)	14 (50%)	12 (41%)	
Poorly differentiated	24 (42%)	11 (39%)	13 (45%)	
Unknown	4 (7%)	2 (7%)	2 (7%)	
AJCC 7th ed. stage, n (%)				
IA	1 (2%)	-	1 (3.5%)	0.106
IB	2 (3%)	1 (3%)	1 (3.5%)	
IIA	5 (9%)	-	5 (17%)	
IIB	37 (65%)	22 (79%)	15 (52%)	
III	-	-	-	
IV	12 (21%)	5 (18%)	7 (24%)	
AJCC 7th ed. pT, n (%)*				
pT1	1 (2%)	-	1 (4%)	0.304
pT2	4 (9%)	1 (4%)	3 (14%)	
pT3	40 (89%)	22 (96%)	18 (82%)	
pT4	-	-	-	
AJCC 7th ed. pN, n (%)*				
pN0	8 (18%)	1 (4%)	7 (32%)	0.022
pN1	37 (82%)	22 (96%)	15 (68%)	
Tumor location, n (%)				
Body/Tail	18 (32%)	5 (18%)	13 (45%)	0.029
Head/Uncinante	39 (68%)	23 (82%)	16 (55%)	
Size cm, median (IQR)*	3.5 (1.7)	3.3 (1.7)	3.7 (1.5)	0.910
Lymphovascular invasion, n (%)				
Absent	8 (14%)	2 (7%)	6 (21%)	0.140
Present	43 (75%)	24 (86%)	19 (65%)	
Unknown	6 (11%)	2 (7%)	4 (14%)	
Perineural invasion, n (%)				
Absent	2 (3%)	-	2 (7%)	0.489
Present	46 (81%)	24 (86%)	22 (76%)	
Unknown	9 (16%)	4 (14%)	5 (17%)	
Surgical margins, n (%)*				
R0	41 (91%)	22 (96%)	19 (86%)	0.346
R1	4 (9%)	1 (4%)	3 (14%)	
R2	-	-	-	
SMAD4, n (%)**				
Retained	20 (69%)	12 (67%)	8 (73%)	1.000
Lost	9 (31%)	6 (33%)	3 (27%)	

Abbreviations: IQR, interquartile range.

*Among patients undergoing resection with curative intent and absence of metastatic disease (n = 45).

**Among tumors with available SMAD4 immunohistochemistry (n = 29).

https://doi.org/10.1371/journal.pone.0182855.t004

7–15), 25.3%, and 12.7%, respectively. In the entire study population, the median, 2-year, and 5-year OS was 16 months (95% CI 13–19), 38.0%, and 14.7%, respectively.

Patients with tumors that successfully engrafted had significantly shorter RFS (median, 7 vs. 16 months, log-rank P = 0.047; Fig 4A) and OS (median, 13 vs. 21 months, log-rank P = 0.038; Fig 4B) compared to patients with tumors that failed to engraft. Moreover, the multivariable-adjusted survival analyses revealed tumor engraftment as an independent predictor of worse RFS (HR 2.05, 95% CI 1.20–3.50, P = 0.009) (Table 5). Survival analyses based on xenograft growth rate did not show significant associations with patient survival outcomes, although analyses were limited by sample size (Table 5, Fig 4C and 4D). Patients with rapid-growing xenografts had shorter RFS (median, 6 vs. 10 months, log-rank P = 0.189) and OS (median, 8 vs. 14, log-rank P = 0.328) than those with slow-growing xenografts, although these differences were not statistically significant.

Discussion

The genetic heterogeneity of PDAC and the intense desmoplastic reaction of the tumor stroma have made improvements in survival outcomes difficult to achieve. Preclinical models that

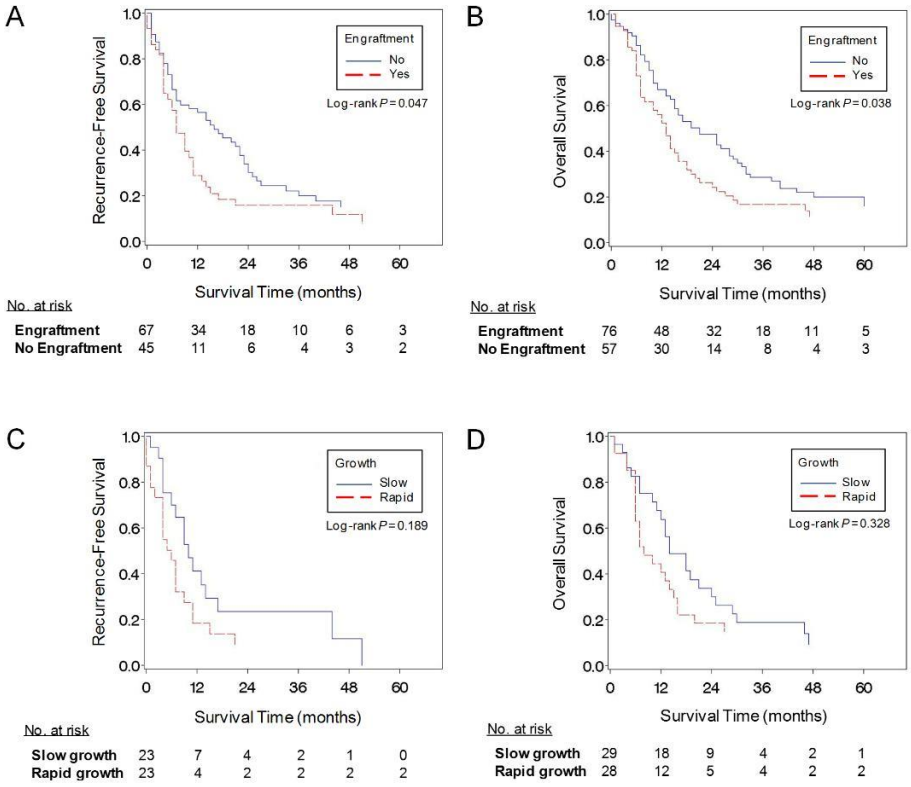


Fig 4. PDAC tumor engraftment in mice is associated with poor patient outcomes. (A) Kaplan-Meier curves of recurrence-free survival and tumor engraftment for patients with resectable primary tumors and no evidence of metastatic disease. (B) Kaplan-Meier curves of overall survival and tumor engraftment for the entire study population. (C) Kaplan-Meier curves of recurrence-free survival and rate of tumor engraftment for patients with resectable primary tumors and no evidence of metastatic disease. (D) Kaplan-Meier curves of overall survival and rate of tumor engraftment for the entire study population.

https://doi.org/10.1371/journal.pone.0182855.g004

Table 5. Recurrence-free and overall survival by tumor engraftment and xenograft growht rate.

	Recurrence-Free Survival					
	No. patients	**Median (Months)**	**2-year**	**5-year**	**HR* (95% CI)**	**P**
Tumor engraftment						
No engraftment	67	16.0	30.3%	15.2%	1.00 (reference)	
Engraftment	45	7.0	16.9%	8.5%	2.05 (1.20–3.50)	0.009
Xenograft growth rate						
Slow growth	22	10.0	23.5%	0.0%	1.00 (reference)	
Rapid growth	23	6.0	10.5%	10.5%	2.37 (0.90–6.25)	0.082
	Overall Survival					
	No. patients	**Median (Months)**	**2-year**	**5-year**	**HR* (95% CI)**	**P**
Tumor engraftment						
No engraftment	76	21.0	48.7%	16.5%	1.00 (reference)	
Engraftment	57	13.0	24.3%	11.2%	1.44 (0.90–2.31)	0.125
Xenograft growth rate						
Slow growth	29	14.0	30.0%	9.4%	1.00 (reference)	
Rapid growth	28	8.0	18.5%	14.8%	1.63 (0.79–3.38)	0.190

Abbreviations: HR, hazard ratio.

*Cox proportional hazards regression adjusting for patient age, sex, neoadjuvant therapy, adjuvant therapy, American Joint Committee on Cancer stage, tumor location, tumor grade of differentiation, lymphovascular invasion, and resection margins.

https://doi.org/10.1371/journal.pone.0182855.t005

closely recapitulate the complexity of human pancreatic cancer are indispensable to study the biology of this disease and assess novel therapeutic agents. In this setting, PDX tumor models of PDAC allow for the faithful propagation of the human neoplastic cells. We showed that the histological architecture of the original tumors is maintained in the PDX models for different grades of differentiation, even after extensive passages. Moreover, although the non-malignant stroma of the human tumor is replaced with cells from the host mouse, these components seem to mimic several features of the original tumor stroma, including cancer-associated fibroblasts (CAFs) and tumor vasculature [26–28]. In PDAC, the CAFs are largely responsible for the production of the extracellular matrix found in the tumor stroma, and the production of collagen by CAFs is mediated by their close association with PDAC cells [29,30]. Despite the replacement of human CAFs by murine fibroblasts, we demonstrated that the collagen structures found in PDX models of PDAC closely resemble those found in the original tumor. Therefore, PDX tumors provide a system to investigate the cellular and non-cellular components of the stroma and their interactions with PDAC cells.

The pathological factors associated with PDX formation have been described for a variety of cancers [31,32]. However, their identification in PDAC has largely remained elusive, with a single study correlating tumor size (> 3.5 cm) with successful xenograft generation [16,18,19]. In our series, we identified lymphovascular invasion and lymph node metastasis as potential determinants for PDX formation and rapid growth, respectively. An important role for lymphovascular invasion is highlighted by the fact that only 14% of tumors without lymphovascular invasion successfully engrafted. These results suggest that engraftment may reflect an underlying biological mechanism that allows these tumors to adapt and grow in a new environment. Interestingly, previous studies employing NOD/SCID mice have failed to make similar correlations with these pathological features and PDAC engraftment and growth [16–18]. While the more immunocompromised background of these mice likely allows for more efficient tumor engraftment, it is possible that the reduced selective pressure of NOD/SCID mice masks the inherent biological differences of patient tumors.

The association of molecular alterations with PDAC PDX formation has been controversial. The focus of this analysis has been on the loss of SMAD4 expression, which has been implicated in metastatic spread of PDAC [33]. Previously, Garrido-Laguna et al. found that engrafted tumors were more frequently associated with SMAD4 inactivation [17]. In contrast, in a study by Jun et al. there was no association between loss of SMAD4 and success of tumor engraftment [16]. Similarly, our analysis of SMAD4 expression in patient tumors did not find any correlation with the success or timing of tumor engraftment. Moreover, our study and others have demonstrated that successfully and unsuccessfully engrafted tumors exhibit a similar distribution of KRAS mutations and comparable expression or mutation of TP53. Collectively, these results suggest that that single genetic alterations may not be determinant of tumor engraftment, allowing PDX models of PDAC to recapitulate the broad genetic diversity of patient tumors.

As seen in PDX models of other cancers, successful engraftment of PDAC tumors correlates with shorter recurrence-free and overall survival. In the present study, tumors successfully engrafted in mice showed significantly poorer overall survival and xenograft formation was an independent predictor of poor survival. Similarly, Garrido-Laguna et al. showed that patients whose pancreatic tumors failed to engraft had an 81% reduced risk of death, and Thomas et al. demonstrated that patients whose tumors successfully engrafted experienced recurrence significantly earlier than those whose did not grow [17,18]. Despite the shorter survival of patients with PDAC that form PDX tumors, most tumors are established in mice 4–5 months before recurrence in patients undergoing surgical resection. In our cohort of patients with recurrent disease, xenograft tumors developed a median of 166 days before diagnosis of recurrence. Similarly, Thomas et al. detected first palpable signs of tumor formation a median of 134.5 days before radiographic recurrence identification [18]. Given the limited number of therapeutic options and the variable rate of successful growth, the systematic use of PDX tumors for real-time chemo-sensitivity testing is not practical at this time. However, by predicting recurrence months before current surveillance modalities, these models might provide a window of opportunity for increased surveillance and differentiation of treatment, especially in patients at higher risk of recurrent disease.

Our study had limitations. The patient population derives from a single referral center, which may result in variations relative to the general population. For instance, nearly a third of patients underwent neoadjuvant treatment. However, this was not associated with the rate of tumor engraftment; moreover, multivariable-adjusted survival models adjusted for different peri-operative treatment status, minimizing any potential biases. A small proportion (16%) of patients in our study had metastatic disease at the time of surgery, introducing heterogeneity in analyses of OS. To address this issue, we conducted a separate analysis of RFS among the more homogeneous subset of patients with resectable primary tumors and no evidence of metastatic disease.

In conclusion, successful establishment of PDAC PDX predicts an increased risk of disease recurrence and mortality in the original patients. Lymphovascular invasion and lymph node positivity might reflect an underlying biological mechanism that allows these tumors to establish and thrive in a new host environment. These models are able to faithfully reproduce the cancer and stromal architecture from the original tumor, and may therefore be valuable tools to test new therapeutic alternatives and identify patients who are at very high risk of disease recurrence following resection.

Acknowledgments

We would like to thank Yuto Suyama, Annika Reczek, and Kate Von Alt for their technical assistance.

Author Contributions

Conceptualization: Ilaria Pergolini, Vicente Morales-Oyarvide, Carlos Ferna´ndez-del Castillo, Andrew S. Liss.

Data curation: Ilaria Pergolini, Vicente Morales-Oyarvide, Andrew S. Liss.

Formal analysis: Ilaria Pergolini, Vicente Morales-Oyarvide, Mari Mino-Kenudson, Kim C. Honselmann, Cristina R. Ferrone, Keith D. Lillemoe, Nabeel Bardeesy, David P. Ryan, Sarah P. Thayer, Andrew L. Warshaw, Carlos Ferna´ndez-del Castillo, Andrew S. Liss.

Funding acquisition: Keith D. Lillemoe, David P. Ryan, Sarah P. Thayer, Andrew L. Warshaw, Carlos Ferna´ndez-del Castillo, Andrew S. Liss.

Investigation: Ilaria Pergolini, Vicente Morales-Oyarvide, Mari Mino-Kenudson, Kim C. Honselmann, Matthew W. Rosenbaum, Sabikun Nahar, Marina Kem, Andrew S. Liss.

Methodology: Ilaria Pergolini, Vicente Morales-Oyarvide, Mari Mino-Kenudson, Kim C. Honselmann, Sabikun Nahar, Marina Kem, Andrew S. Liss.

Project administration: Sarah P. Thayer, Andrew L. Warshaw, Carlos Ferna´ndez-del Castillo, Andrew S. Liss.

Resources: Mari Mino-Kenudson, Cristina R. Ferrone, Keith D. Lillemoe, Nabeel Bardeesy, Sarah P. Thayer, Andrew L. Warshaw, Carlos Ferna´ndez-del Castillo.

Supervision: Keith D. Lillemoe, Nabeel Bardeesy, David P. Ryan, Andrew L. Warshaw, Carlos Ferna´ndez-del Castillo, Andrew S. Liss.

Validation: Andrew S. Liss.

Visualization: Andrew S. Liss.

Writing – original draft: Ilaria Pergolini, Vicente Morales-Oyarvide, Andrew S. Liss.

Writing – review & editing: Ilaria Pergolini, Vicente Morales-Oyarvide, Mari Mino-Kenudson, Kim C. Honselmann, Matthew W. Rosenbaum, Sabikun Nahar, Marina Kem, Cristina R. Ferrone, Keith D. Lillemoe, Nabeel Bardeesy, David P. Ryan, Sarah P. Thayer, Andrew L. Warshaw, Carlos Ferna´ndez-del Castillo, Andrew S. Liss.

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Risk of misdiagnosis and overtreatment in patients with main pancreatic duct dilatation and suspected combined/main-duct intraductal papillary mucinous neoplasms

Stefano Crippa, MD, PhD,[a] Ilaria Pergolini, MD,[a] Corrado Rubini, MD,[b] Paola Castelli,[c] Stefano Partelli, MD, PhD,[a] Claudio Zardini, MD,[d] Giorgia Marchesini, MD,[b] Giuseppe Zamboni, MD,[c,e] and Massimo Falconi, MD,[a] Ancona, Negrar, and Verona, Italy

Background. Segmental/diffuse dilatation of the main pancreatic duct (MPD) is the typical feature of combined/main-duct intraductal papillary mucinous neoplasms (CMD-IPMNs). MPD dilation in IPMNs may be also expression of mucus hypersecretion/obstructive chronic pancreatitis (OCP). The aim of this study was to evaluate the presence and extension of MPD involvement by tumor/OCP and assess the risk of overtreatment.

Methods. Retrospective analysis of suspected CMD-IPMNs resected between January 2009 and October 2014 were included. Pathologic correlations among MPD dilatation, IPMN, and OCP was searched.

Results. Overall, 93 patients were resected for suspected CMD-IPMNs. At pathology, CMD-IPMNs were found in 69 patients (74%). Branch-duct IPMNs (BD-IPMNs) were found in 8 cases (9%), pancreatic ductal adenocarcinoma (PDAC) in absence of IPMN in 9 (10%), cystic neuroendocrine tumor (NET G2) in 1 (1%), serous cystadenoma in 2 (2%), and OCP alone/mucinous metaplasia in 4 patients (4%). Overall, 18 patients (19%) underwent an overtreatment because unnecessary (2 BD-IPMNs, 2 serous cystadenomas, and 4 OCPs only) or too extensive resections (9 CMD-IPMNs and 1 PDAC with associated OCP). In these, total pancreatectomy was the most common procedure (67%). Median size of MPD in IPMN-involved area was 12 mm compared with 7 mm when only OCP was found ($P < .05$).

Conclusion. There is a considerable risk of overtreatment in patients with a preoperative morphologic diagnosis of CMD-IPMNs. Partial pancreatectomy with margin examination should be performed instead of upfront total pancreatectomy. Radiologic observation can be considered in asymptomatic patients with "worrisome" MPD dilatation (5–9 mm) and lacking other high-risk stigmata. (Surgery 2016;159:1041-9.)

From the Departments of Surgery[a] and Biomedical Sciences and Public Health,[b] Universita' Politecnica delle Marche, Ospedali Riuniti, Ancona; the Division of Pathology[c] and General Surgery,[d] Sacro Cuore-Don Calabria Hospital, Negrar; and Department of Pathology,[e] Universita° di Verona, Verona, Italy

Stefano Crippa and Ilaria Pergolini contributed equally to this paper.

Stefano Crippa, Stefano Partelli, and Massimo Falconi are now with the Division of Pancreatic Surgery, Vita e Salute University, San Raffaele Hospital, Milan, Italy.

Source of funding: None.

Accepted for publication November 4, 2015.

Reprint requests: Massimo Falconi, MD, Department of Surgery, Division of Pancreatic Surgery, Vita e Salute University, San Raffaele Hospital, Via Olgettina 60, Milan 20132, Italy. E-mail: falconi.massimo@hsr.it.

0039-6060/$ - see front matter

http://dx.doi.org/10.1016/j.surg.2015.11.003

THE PRESENCE AND EXTENT of main pancreatic duct (MPD) involvement in intraductal papillary mucinous neoplasms (IPMNs) are crucial for the appropriate management of these patients.[1,2]

Our understanding of IPMN has evolved significantly in the last 2 decades, and it has become evident that IPMNs can originate from the MPD (MD-IPMNs), from its secondary branches (BD-IPMNs) or from both (combined IPMNs). This distinction is not only of morphologic significance because IPMNs involving the main duct, either MD-IPMNs or combined IPMNs, have a high rate of malignancy, whereas BD-IPMNs are more often benign.[3-7] For these reasons, resection of all

combined/main-duct (CMD)-IPMNs is warranted.[1-6]

Segmental or diffuse dilation of MPD, in the absence of other causes of duct obstruction, is the typical imaging feature of CMD-IPMNs. According to recent international consensus guidelines, a threshold for MPD dilation of >5 mm can be adopted for CMD-IPMNs, thereby increasing the sensitivity for radiologic diagnosis without losing specificity.[1] The extent of operative resection is planned based on MPD features, although frozen section margins are of paramount importance for deciding the resection line in partial pancreatectomies. However, in CMD-IPMNs, MPD can be dilated for reasons other than direct tumor involvement, including ductal hypertension caused by mucin hypersecretion/mucin plugs, and obstructive chronic pancreatitis (OCP). In this light, pancreatic resections planned for presumed CMD-IPMNs based on MPD dilation may result in possible overtreatment with exposure of patients to short- and long-term morbidity and possible mortality as a consequence of pancreatic surgery. Of note, some authors recently showed that diagnostic errors are fairly common in the preoperative assessment of pancreatic cystic neoplasms (PCNs).[8-11] Although most of these erroneous diagnoses involved serous cystadenomas and branch duct IPMNs, the rate of incorrect diagnosis was not negligible, even for CMD-IPMNs.[8-11]

In this study, we compared the preoperative and postoperative pathologic diagnoses in a series of patients who underwent pancreatic resection for presumed CMD-IPMNs. We aimed to determine the presence and extension of MPD dilation owing to IPMN or to other causes, to evaluate the clinical relevance of diagnostic errors, and to identify new strategies to improve the diagnostic accuracy in these patients.

METHODS

A retrospective analysis identified patients with a preoperative clinical-radiologic diagnosis of CMD-IPMNs who underwent pancreatic resection between January 2009 and October 2014 in the Division of General Surgery of the Sacro Cuore-Don Calabria Hospital, Negrar and in the Department of Surgery of Ospedali Riuniti, Universita Politecnica delle Marche, Ancona, Italy.

Demographic information, clinical history, diagnostic workup, type of surgery, postoperative course, and pathology were recorded. Perioperative mortality was defined as in-hospital or 60-day death.

The clinical-radiologic diagnosis of CMD-IPMNs was defined by the presence of segmental or diffuse dilation of MPD >5 mm without other causes of duct obstruction, possibly associated to $1 dilated cyst communicating with the MPD (combined IPMNs). Preoperative workup for suspected CMD-IPMNs included multidetector computed tomography with contrast medium and/or MRI with MR cholangiopancreatography (MRCP). Because the presence of CMD-IPMNs on multidetector computed tomography/MRCP represents an indication for surgical resection according to international consensus guidelines,[1] further testing including endoscopic ultrasound with fine-needle aspiration (EUS/FNA) or endoscopic retrograde cholangiopancreatography (ERCP) were not performed routinely. Peroral and/or intraoperative pancreatoscopy are not performed at either institution.

A review of preoperative imaging was not performed, and preoperative diagnosis was recorded at the time of initial diagnosis before surgical operation. We specifically avoided re-reviewing of radiologic images to describe our actual experience.

The type of resection was planned according to the site of the tumor, as indicated by preoperative imaging findings. Because of the significant risk of harboring malignancy, all CMD-IPMNs are treated with typical pancreatectomies with lymph node dissection. The extent of resection was determined based on preoperative workup, intraoperative findings, and the results of frozen section analysis of the resection margin. Pancreatic resection margin was referred as "positive" for the presence of either intermediate- or high-grade dysplasia and invasive carcinoma, and "negative" for either the presence of a normal ductal epithelium or low-grade dysplasia. If a "positive" margin was found at intraoperative examination, the resection was extended up to a total pancreatectomy. The presence of a dilated MPD lacking any epithelial lining (denudation) at the margin was an indication to extent the resection as well. In fact, some studies showed that denudation represents one of the factors associated with IPMN recurrence.[12] Also International Association of Pancreatology (IAP) guidelines highlighted that denudation may prove the presence of an adjacent tumor.[1] For these reasons, the presence of denudation at the resection margin is an indication for further resection.

However, the decision to perform a total pancreatectomy was individualized considering also patient's age and comorbidities. Preoperative

clinical-radiologic diagnosis was compared with the final pathologic diagnosis based on the 2010 World Health Organization classification system for the tumors of the pancreas.[7] The method for routine systematic pathologic evaluation was based on the recommendation by the World Health Organization 2010 Classification of IPMNs and by Capella et al.[7,13] The IPMNs were divided into noninvasive and invasive neoplasms. Noninvasive IPMNs were graded on the basis of the greatest degree of dysplasia into low-grade dysplasia, moderate dysplasia, and high-grade dysplasia or carcinoma in situ. In patients with pathologically confirmed IPMNs, we focused our attention to the presence in the specimen of OCP, to the extension of IPMN-involved areas and to the size of MPD in IPMN-involved area and in areas when only OCP was found. The assessment of duct diameter in relation to areas of IPMN and area with only OCP was acquired from the pathology reports. All pathologic examinations were performed or supervised by 2 senior pathologists (C.R., G.Z.).

Data are presented as median values and ranges. Categorical variables are presented as numbers and percentage and were compared using a Chi-square test and Fisher's exact test as appropriate. When comparing 2 groups, normally distributed continuous variables were analyzed using a 2-sample Student t test, and the Mann–Whitney U test was used for nonnormally distributed variables.

RESULTS

In the study period, 93 patients (60 males, 33 females; median age, 67 years; range, 31–80) with a preoperative clinical-radiologic diagnosis of CMD-IPMNs underwent surgical resection.

Demographics, clinical presentation, and preoperative diagnosis. Demographic, preoperative, and surgical details are outlined in Table I. Overall, 41 patients (44%) were asymptomatic. All patients underwent cross-sectional high-resolution imaging. MRI/MRCP was performed in 89 patients (96%) and multidetector computed tomography in 45 (48%). Twenty patients (22%) had an EUS/FNA and 13 (14%) underwent ERCP. ERCP was performed as a diagnostic procedure in 4 patients, and in the remaining 9 it was required to achieve a preoperative biliary drainage in jaundiced patients. Results of FNA are also shown in Table I. In 27 patients (29%), a diffuse involvement of the entire gland by IPMN was found at imaging, whereas CMD-IPMN occurred in the pancreatic head in 40 patients (43%) and in the body/tail in 26 (28%; Fig 1). Among these 93 patients, a malignant CMD-IPMN was suspected after preoperative workup in 22 cases (24%) because of the presence of solid components, atypical/malignant cytology, or jaundice. Table II summarizes the criteria for resection in the 93 patients.

Table I. Demographics and clinical-radiologic characteristics, operative procedures, postoperative complications, and mortality of the 93 resected patients with preoperative diagnosis of combined/main-duct intraductal papillary mucinous neoplasms (CMD-IPMNs)

Characteristic	n (%)
Median age, y (range)	67 (31–80)
Gender	
Male	60 (65)
Female	33 (35)
Median CA 19.9, U/L (range)	16.5 (0.8–1,043)
Symptoms at the diagnosis	
Yes	52 (56)
No	41 (44)
Pre-operative radiologic workup	
MRI/MRCP	89 (96)
Multidetector computed tomography	45 (48)
ERCP	13 (14)
EUS-FNA	20 (22)
Undetermined cytology	5 (25)
Malignant cells	5 (25)
Atypical cells	1 (5)
Benign cells	4 (20)
Mucinous cells	5 (25)
Preoperative CMD-IPMN location	
Head	40 (43)
Body-tail	26 (28)
Entire pancreas	27 (29)
Operative procedures	
Total pancreatectomy	31 (33)
Pancreaticoduodenectomy	37 (40)
Distal pancreatectomy	25 (27)
Postoperative complications	37 (40)
Pancreatic fistula	16 (26)*
Biliary fistula	4 (6)y
Delayed gastric emptying	6 (6)
Intraabdominal abscess	24 (26)
Bleeding	11 (12)
Reoperation	4 (4)
Mortality	4 (4)

*The rate of pancreatic fistula was calculated considering 62 patients who underwent partial pancreatectomy.

yThe rate of biliary fistula was calculated considering 68 patients who underwent pancreaticoduodenectomy or total pancreatectomy.

CA 19-9, Carbohydrate antigen 19-9; CMD-IPMN, combined/main-duct intraductal papillary mucinous neoplasms; ERCP, endoscopic retrograde cholangiopancreatography; EUS-FNA,endoscopic ultrasound with fine-needle aspiration; MDTC, contrast-medium computed tomography; MRCP, MRI with cholangiopancreatography.

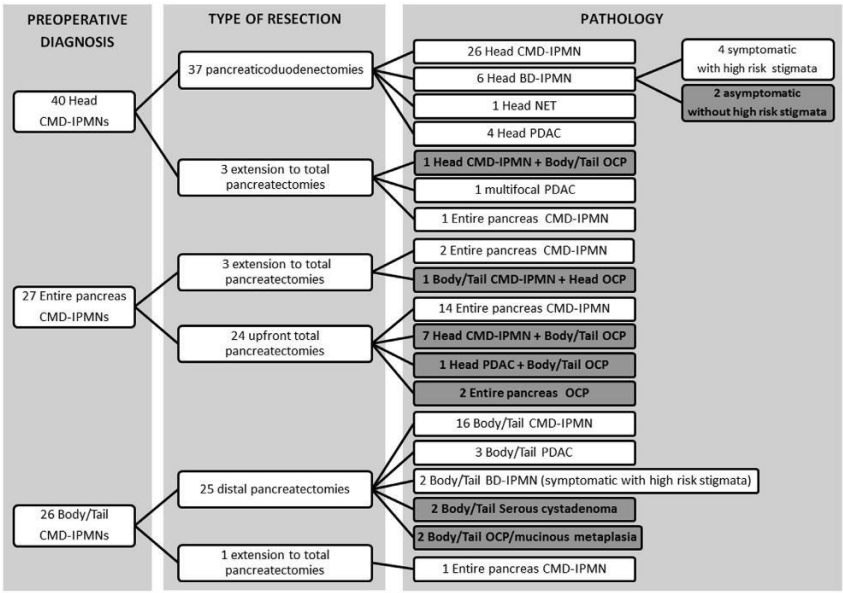

Fig 1. Course of 93 patients with suspected combined/main-duct intraductal papillary mucinous neoplasms (CMD-IPMNs) from the preoperative diagnosis to pathology. Cases of overtreatment are highlighted. BD-IPMN, Branch-duct intraductal papillary mucinous neoplasm; NET, G2 neuroendocrine tumor grading 2; OCP, obstructive chronic pancreatitis; PDAC, pancreatic ductal adenocarcinoma.

Surgical treatment, frozen section analysis, and postoperative course. We performed 37 pancreaticoduodenectomies (PD; 40%), 31 total pancreatectomies (33%) and 25 distal pancreatectomies (27%; Table I). Among 31 total pancreatectomies, 7 patients initially underwent a partial pancreatectomy that was extended subsequently to total pancreatectomy because of positive resection margins. In all patients who underwent partial pancreatectomy, 69 patients (74%) frozen section analysis of the resection margins was carried out. Positive resection margins were found intraoperatively in 17 patients (25%), including 7 patients with invasive carcinoma (41%), 5 (29%) with high grade dysplasia, 3 (18%) with intermediate dysplasia, and 2 (12%) with epithelial denudation. These 17 patients underwent an extension of resection. Six patients---3 with resection margins positive for invasive carcinoma, 2 for high-grade dysplasia, and 1 for intermediate dysplasia---underwent total pancreatectomy immediately. In the remaining 11 patients, an extension of surgical resection was performed, up to total pancreatectomy in 1 case (positive for high-grade dysplasia).

Therefore, 58 of 69 patients (84%) had a single frozen section analysis, 10 (15%) had 2, and only 1 patient (1%) underwent 2 extensions of surgical resection with 3 frozen section analysis, for an overall number of 81 intraoperative examinations of transection margins. Definitive examination corroborated frozen section in 79 cases (98%). In the remaining 2 cases, the diagnosis changed from negative to positive (1 for high-grade dysplasia and 1 for intermediate-grade dysplasia), but none underwent reoperation.

Overall, major postoperative complications occurred in 37 patients (40%), including clinically relevant pancreatic fistula (26%), biliary fistula (6%), delayed gastric emptying (6%), intraabdominal abscess (26%), and bleeding (12%). Four patients required reoperation (4%) because of bleeding in 3 patients and intraabdominal abscess in 1. The 60-day postoperative mortality rate was 4% (1 patient for cardiac arrhythmia, 2 for sepsis and multiorgan failure, and 1 for postoperative bleeding complicated by acute myocardial infarction).

Table II. Indications for surgery of the 93 patients with a preoperative suspected combined/main duct intraductal papillary mucinous neoplasm (CMD-IPMN)

Preoperativesuspecteddiagnosis	N (%)
MD-IPMN (MPD dilation >5 mm)	23 (25)
Median MPD diameter (mm)	9 (5–30)
MPD diameter $1 cm	12 (52)
Presence of nodule	4 (17)
Symptoms at the diagnosis	10 (43)
Jaundice	1 (4)
Abdominal pain	5 (22)
Acute pancreatitis	3 (13)
Weight loss	2 (9)
Diabetes	4 (17)
C-IPMN (MPD dilation > 5 mm and cyst > 5 mm)	70 (75)
Median MPD diameter (mm)	7.5 (5–30)
MPD diameter $ 1 cm	27 (39)
Presence of nodule	11 (16)
Symptoms at the diagnosis	42 (60)
Jaundice	7 (10)
Abdominal pain	18 (26)
Acute pancreatitis	13 (19)
Weight loss	5 (7)
Diabetes	7 (10)

C-IPMN, Combined intraductal papillary mucinous neoplasm; MPD, main pancreatic duct; MD-IPMN, main-duct intraductal papillary mucinous neoplasm.

Table III. Pathologic data

Pathology	n (%)
Final histologic diagnosis	93 (100)
CMD-IPMN	69 (74)
Low-grade dysplasia	7 (10)
Intermediate-grade dysplasia	21 (31)
High-grade dysplasia	14 (20)
Invasive carcinoma	27 (39)
BD-IPMN	8 (9)
Low-grade dysplasia	2 (25)
Intermediate-grade dysplasia	6 (75)
High-grade dysplasia	/
Invasive carcinoma	/
PDAC	9 (10)
NET	1 (1)
SCA	2 (2)
OCP/mucinous metaplasia	4 (4)
Histologic subtype of IPMN	
CMD-IPMN	69 (74)
Gastric	15 (22)
Intestinal	20 (29)
Mixed (gastric/intestinal)	12 (17)
Pancreatobiliary	6 (9)
Colloid	12 (17)
Tubular	4 (6)
BD-IPMN	8 (9)
Gastric	6 (75)
Intestinal	2 (25)
Concomitant OCP in the surgical specimen	
Yes	75 (81)
No	18 (19)
CMD-IPMN location	69 (74)
Entire pancreas	18 (26)
Head	34 (49)
Body/tail	17 (25)

BD-IPMN, Branch-duct intraductal papillary mucinous neoplasm; CMD-IPMN, combined/main-duct intraductal papillary mucinous neoplasm; NET, G2 neuroendocrine tumor grading 2; OCP, obstructive chronic pancreatitis; PDAC, pancreatic ductal adenocarcinoma; SCA, serous cystadenoma.

Pathology. Table III shows the final pathologic data. The presence of CMD-IPMNs was confirmed in 69 of 93 (74%) patients. In the remaining 24 patients (26%), BD-IPMNs were found in 8 cases (9%), pancreatic ductal adenocarcinoma (PDAC) in the absence of IPMN in 9 (10%), cystic neuroendocrine tumor (NET G2) in 1 (1%), serous cystadenoma (SCA) in 2 (2%), and OCP alone/mucinous metaplasia in 4 patients (4.5%; Fig 1). In 81% of patients, OCP was recognized histologically in the specimens, either in the tissue around the IPMN, or concomitant with IPMN but in a different area of the specimen.

Comparison between preoperative clinical–radiologic diagnosis and final pathologic diagnosis. Considering 69 pathologically confirmed CMD-IPMNs, a diffuse involvement of entire pancreas was recognized in 18 cases (26%), whereas head and body/tail were involved in 34 (49%) and 17 (25%) patients, respectively (Table II). Of these 69 patients (Fig 2), 26 underwent pancreaticoduodenectomy (38%)---16 distal pancreatectomy (23%) and 27 total pancreatectomy (39%)---21 (78%) as an upfront procedure and 6 (22%) after a partial resection. In all 42 patients (61%) who underwent a partial resection, a segmental IPMN localization was observed at pathology. In 21 patients who underwent upfront total pancreatectomy, a diffuse MPD involvement by IPMN was recognized only in 14 cases (67%). In the remaining 7 cases (33%), CMD-IPMN was located only in the head of the pancreas, being OCP present in the remaining portion.

Considering 6 patients who underwent an extension of resection up to total pancreatectomy, a diffuse MPD involvement by CMD-IPMN was found in 4 cases (67%). In the remaining 2 patients (33%), IPMN was present only in a portion of the pancreas.

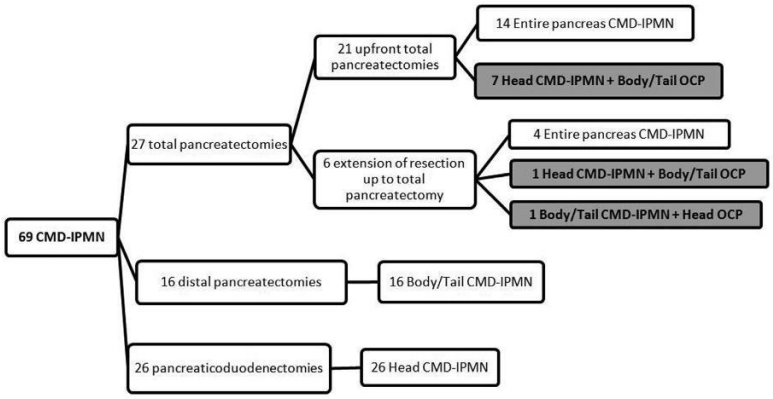

Fig 2. The flow chart of 69 pathologically confirmed combined/main-duct intraductal papillary mucinous neoplasms (CMD-IPMNs). Operative procedures and final pathology with localization of CMD-IPMN in the surgical specimen. Cases of too extensive resection are highlighted. OCP, Obstructive chronic pancreatitis.

The median size of MPD in IPMN-involved areas was 12 mm, compared with 7 mm in areas where only OCP was found ($P < .05$). Considering 69 patients with a confirmed diagnosis of CMD-IPMN at pathology, we have found a "worrisome" MPD dilation of 5-7 mm and of 5-9 mm in 26 (38%) and in 35 patients (51%), respectively. Supplementary Figure 1 shows an example of total pancreatectomy performed for a suspected CMD-IPMN with dilation of the entire MPD at preoperative imaging but with evidence of IPMN only in the distal pancreas. Twenty-four patients (26%) had a pathologic diagnosis other than CMD-IPMN, but surgery was still indicated in 6 of 8 patients with BD-IPMNs because they were symptomatic and/or with high-risk stigmata, and in patients with NET G2 (n = 1) and PDAC (n = 9). In the PDAC group, 1 patient underwent total pancreatectomy after initial PD because of positive resection margin for high-grade dysplasia but at final pathology no tumor was found in the distal pancreas. In these 9 patients, preoperative imaging showed the typical features of CMD-IPMN, with a high suspicion of malignant because of the presence of a solid component. The carbohydrate antigen 19-9 value did not differ between patients with malignant IPMN and/or PDAC and those with benign lesions (median carbohydrate antigen 19-9, 43 vs 12; $P = .83$).

Therefore, considering the entire cohort of 93 patients, overtreatment was observed in 18 cases (19%), because of unnecessary resection (2 BD-IPMNs, 2 serous cystadenomas, and 4 OCPs only) or too extensive resections (9 CMD-IPMNs and 1 PDAC; Fig 1). Of these 18 patients, 12 had no symptoms or mild symptoms such as vague abdominal pain. Three patients with OCP had mild symptoms and 1 was asymptomatic. Conservative or endoscopic management could be considered in these cases. Preoperative workup did not differ between patients who underwent overtreatment and the remaining ones, although only 2 of 18 patients (11%) underwent EUS compared with 18 of 75 (24%; $P = .194$). Total pancreatectomy was the most common procedure (67%) performed in these 18 patients (Fig 1). Five of these 12 patients had severe postoperative complications including biliary fistula followed by sepsis and multiorgan failure in 1 case, postoperative bleeding (n = 2), myocardial infarction in 1 case, and intraabdominal abscess in another. Two of these patients required reoperation and overall 2 patients died of postoperative complications. No patient died of insulin-related coma in the long-term follow-up.

DISCUSSION

International consensus guidelines recommend surgical resection in all surgically fit patients with CMD-IPMNs.[1] The preoperative localization of CMD-IPMN is of crucial importance for the appropriate planning of surgical resection, and it is based on the extension and size of MPD dilation. However, pancreatic tumors other than CMD-IPMNs and OCP can affect the presence of MPD dilation.[1] It is well-known that the preoperative diagnostic accuracy in the

setting of PCNs is quite low, ranging from 47 to 78%, although improved results are reported for CMD-IPMNs.[8,11,14,15]

In this study, we analyzed retrospectively data from patients who underwent pancreatic resection for a presumed CMD-IPMNs. We found that the rate of correct diagnosis for CMD-IPMNs was 74%. These results are in keeping with Salvia et al[15] and Barron et al,[9] who reported a diagnostic accuracy for CMD-IPMNs of 80.7 and 74.9%, respectively. In contrast, Correa-Gallego et al[8] have shown an accuracy as high as 94% for 16 patients with CMD-IPMNs, but of only 68% for all 125 patients with pancreatic cystic lesions included in their experience. In our experience, 10% of errors in preoperative diagnosis were PDAC with retrograde MPD dilatation. These data are in line with other large series of qualified centers. Del Chiaro et al[11] reported a 9.5% rate of misdiagnosis between IPMN and PDAC in patients resected with a preoperative diagnosis of IPMN. Salvia et al[15] showed an incidence of ductal adenocarcinoma of 4.8% (23/497) in a cohort of population resected for presumed PCNs, but it increased to 22% in those 103 with an incorrect preoperative diagnosis. Small PDAC may, therefore, present with a cystic phenotype because of pancreatic duct obstruction and differential diagnosis with CMD-IPMNs cannot be obvious. Unfortunately, in the 9 patients with PDAC, EUS with FNA was not performed. The restricted use of EUS/FNA is a limit of our study. As suggested by IAP guidelines, EUS with FNA can be used for detecting mural nodules and invasion, and it can be of help in identifying PDAC as well.[1] However, despite the misdiagnosis, it must be underlined that these 9 patients with a final diagnosis of PDAC deserved a surgical resection. In fact, another important issue that should be considered is the clinical relevance of the diagnostic error. In this setting, Del Chiaro et al[11] found a rate of incorrect preoperative diagnosis of 39.1% in a cohort of 141 PCNs that underwent resection but with a rate of clinically relevant errors of only 8.5%. On the contrary, our study showed a rate of overtreatment of 19% owing to too extensive surgical resections or to erroneous preoperative diagnosis. In these patients, a different therapeutic management should have been performed. Specifically, in 9 CMD-IPMNs and 1 PDAC, we performed total pancreatectomy, but final pathology showed the presence of tumor only in a segment of the specimen, with OCP in the remaining pancreas. Then, another 8 patients---2 with asymptomatic BD-IPMNs without high-risk stigmata, 2 with serous cystadenomas, and 4 with OCP alone/mucinous metaplasia---were misguided to an aggressive management; in these cases, pancreatic resection could have avoided. IPMN are commonly associated with OCP,[16] and an OCP was histologically recognized in >80% of specimens in our study. How these 2 entities occur coincidentally remains unclear. It is likely that OCP may be the result of partial ductal obstruction by mucin plugs in patients with IPMNs. Then, the concomitant presence of pancreatic cystic lesions, duct dilation, and calcification should raise the suspicious of an IPMN, especially when patients do not present typical clinical and demographics characteristics of those with chronic pancreatitis (ie, male sex, age <50 years, history of smoking and/or alcohol intake).[16,17] Two possible areas of errors can be identified, namely, the preoperative workup and the intraoperative decision making. The overall diagnostic accuracy for CMD-IPMNs is reported between 75 and 94%.[8,9,15] Consensus guidelines recommend CT and MRI with MRCP as the preferred diagnostic modalities for the workup of pancreatic cystic lesions including IPMNs.[1,18] When "high-risk stigmata" (ie, MPD >10 mm, enhancing solid components, jaundice) are identified, surgery is mandatory. Whereas, when IPMNs with "worrisome features" (ie, MPD 5–9 mm, nonenhancing solid components, abrupt change of MPD caliber, branch duct size >30 mm) are found, further workup with EUS/FNA is required. However, several authors showed that preoperative imaging procedures are poorly reliable to predict the extent of CMD-IPMN.[8,11,19] In this light, Barron et al[9] recently suggest that cross-sectional imaging studies and EUS should not be the only methods used for the classification of IPMN type and the choice of surgical management. In this work, we performed a limited number of EUS (22%) and this represents a limitation of the study, because an increased number of EUS may have lessened the rate of overtreatments. In fact, when a radiologic diagnosis of CMD-IPMNs was done after CT and/or MRI/MRCP, surgical treatment was planned immediately , especially in patients with symptoms or high-risk stigmata, without further workup. EUS was performed in only 11% of patients with unnecessary surgery compared with 24% of the remaining ones; therefore, one could argue that EUS could improve the diagnostic yield particularly in the overtreatment group and it could be used more frequently, especially to evaluate suspected multifocal disease and to better analyze "worrisome features." However, EUS with FNA did not improve the diagnostic accuracy in PCNs in several recent studies, and its role and

clinical effectiveness in CMD-IPMNs remains unclear.[11,14,15]

For these reasons, some authors have stressed the importance of frozen sectioning of the pancreatic resection margin during pancreatectomy for CMD-IPMNs, showing that frozen sectioning was better than preoperative morphologic assessment to accurately adapt the extent of pancreatectomy.[5,19] In our series, in accordance with previous literature,[6,10,18-21] definitive pathologic examination of resection margins confirmed frozen section analysis in 98%. These results underline that frozen section analysis would allow determining the resection line, and it is of paramount importance to choose the optimal surgical strategy. Of note, in our experience upfront total pancreatectomy was the most common procedure performed in case of overtreatment. Overall, 24 patients underwent upfront total pancreatectomy because of a "strong" preoperative suspect of a CMD-IPMN involving the entire pancreatic gland (ie, a diffuse MPD dilation). The decision was always individualized for each patient, considering age, comorbidities, and presence of preoperative diabetes. Ten of 24 upfront total pancreatectomies were considered as an overtreatment because of too extensive or unnecessary surgery. Therefore, in the setting of diffuse dilation without clear high-risk stigmata, more careful evaluation is warranted, because some of these patients may not have an IPMN involving the entire pancreas, but rather chronic pancreatitis.[1] In such patients, partial pancreatectomy should be planned instead of upfront total pancreatectomy, and operative resection should be eventually extended based on frozen sectioning results. On the contrary, when MPD dilation is diffuse with high-risk stigmata (ie, MPD >10 mm and/or multiple mural nodules) an upfront total pancreatectomy could be considered.

On pathology, we found a not surprising, significant difference in the median size of MPD between areas with and without CMD-IPMN (12 vs 7 mm; $P < .05$). In keeping with our results, Barron et al[9] demonstrated that the diameter of MPD in CMD-IPMN is significantly greater than in BD-IPMN with dilated MPD. They also suggested that the dilation of MPD in cases of MPD-involved IPMN is likely greater than that resulting from chronic pancreatitis.[9] These results support the IAP recommendations that MPD dilation of 5–9 mm should be considered as a "worrisome feature," suggesting further workup and possibly no immediate resection but strict follow-up.[1] A further help in planning the extent of resection may be provided by pancreatoscopy, especially in the setting of worrisome features. Pancreatoscopy can be performed both preoperatively and intraoperatively. Peroral pancreatoscopy and its associated investigations (ie, intraductal ultrasonography), can give additional data to support radiologic findings and may improve differential diagnosis between malignant and benign IPMNs.[22-25] It allows tissue sampling under direct vision and irrigation cytology, for a determination of the presence and extent of MPD involvement.[25-28] Yelamali et al suggested that intraoperative pancreatoscopy with narrow band imaging, through the cut end of the duct at the surgical margin during a partial resection, seems to be more accurate than preoperative pancreatoscopy and it does not carry the risk of pancreatitis associated with peroral pancreatoscopy.[23,29] In this study, pancreatoscopy was not performed at all, and this represents another limitation of the study because pancreatoscopy could improve the diagnostic yield, thereby decreasing the rate of possible overtreatment.

In conclusion, there is a considerable risk of misdiagnosis and possible overtreatment in patients with preoperative diagnosis of CMD-IPMNs. To improve their diagnostic accuracy, EUS with FNA should be used more frequently, especially to evaluate suspected multifocal disease and to find "worrisome features" and new diagnostic strategies should be considered, including advanced endoscopic techniques such as pancreatoscopy. Partial pancreatectomy with frozen section analysis of pancreatic margin should be performed instead of upfront total pancreatectomy. Radiologic observation can be considered in asymptomatic patients with "worrisome" MPD dilatation (5–9 mm) and lacking other high-risk stigmata as suggested by IAP guidelines.[1]

SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.surg.2015.11.003.

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Implications of Perineural Invasion on Disease Recurrence and Survival After Pancreatectomy for Pancreatic Head Ductal Adenocarcinoma

Stefano Crippa, MD, PhD,* Ilaria Pergolini, MD,† Ammar A. Javed, MD,‡ Kim C. Honselmann, MD,† Matthew J. Weiss, MD,‡ Francesca Di Salvo, PhD,* Richard Burkhart, MD,‡ Giuseppe Zamboni, MD,§ Giulio Belfiori, MD,* Cristina R. Ferrone, MD,† Corrado Rubini, MD,‖ Jun Yu, MD, PhD,‡ Giulia Gasparini, MD,* Motaz Qadan, MD, PhD,† Jin He, MD,‡ Keith D. Lillemoe, MD,† Carlos Fernandez-del Castillo, MD,† Christopher L. Wolfgang, MD,‡ and Massimo Falconi, MD*✉

Objective: To describe PNI and to evaluate its impact on disease-free (DFS) and overall survival (OS) in patients with resected pancreatic ductal adenocarcinoma (PDAC).

Summary of Background Data: Although PNI is a prognostic factor for survival in many GI cancers, there is limited knowledge regarding its impact on tumor recurrence, especially in "early stage disease" (PDAC ≤20 mm, R0/ N0 PDAC).

Methods: This multicenter retrospective study included patients undergoing PDAC resection between 2009 and 2014. The association of PNI with DFS and OS was analyzed using Cox proportional-hazards models.

Results: PNI was found in 87% of 778 patients included in the study, with lower rates in PDAC ≤20 mm (78.7%) and in R0/N0 tumors (70.6%). PNI rate did not differ between patients who underwent neoadjuvant therapy and upfront surgery (88% vs 84%, P = 0.08). Although not significant at multivariate analysis (P = 0.07), patients with PNI had worse DFS at univariate analysis (median DFS: 20 vs 15 months, P < 0.01). PNI was the only independent predictor of DFS in R0/N0 tumors (hazard ratio [HR]: 2.2) and in PDAC ≤ 20 mm (HR: 1.8). PNI was an independent predictor of OS in the entire cohort (27 vs 50 months, P = 0.01), together with G3 tumors, pN1 status, carbohydrate antigen (CA) 19.9 > 37 and pain.

Conclusions: PNI represents a major determinant of tumor recurrence and patients' survival in pancreatic cancer. The role of PNI is particularly relevant in early stages, supporting the hypothesis that invasion of nerves by cancer cells has a driving role in pancreatic cancer progression.

Keywords: pancreatic cancer, pancreaticoduodenectomy, perineural invasion, recurrence, surgery, survival

(Ann Surg 2022;276:378–385)

Perineural invasion (PNI) is the infiltration of nerves by cancer cells.[1] Neumann described it for the first time in 1862 in head and neck cancers spreading along nerves into the skull.[2] The role of PNI in cancer biology has been underestimated for many years because it was considered as a "passive process," with less relevance compared with hematogenous and lymphatic spread.[3] Subsequently PNI was found in several cancers, including prostate,[4,5] breast, colorectal and gastric neoplasms.[4–8] Remarkably, the incidence of PNI in pancreatic ductal adenocarcinoma (PDAC) is the highest among all solid tumors, representing a distinctive hallmark.[8] The pancreas is highly innervated by the autonomic nervous system through plexi from ganglia of the celiac and superior mesenteric artery.[9] Due to a strong neurotropism of PDAC cells, these plexi may represent the route for cancer spread, and possible sources for recurrence after resection of PDAC and possibly for lymph node metastases.[10,11] Of note, recent studies suggest that PNI can have a driving role in cancer progression, even in early phases of tumorigenesis.[12] As a matter of fact, the denervation of adrenergic nerves and the ablation of sensory neurons in an animal model of PDAC resulted in the inhibition of cancer progression.[13,14] From a clinical standpoint, PNI has been associated with the presence of neuropathic pain in PDAC, a debilitating symptom associated with impairment of patients' quality of life and decreased survival.[8,15] Although different studies showed that PNI is a dismal prognostic factor for survival, there is limited knowledge regarding its impact on disease-free survival (DFS) after pancreatic resection.[16] Moreover, no study has evaluated specifically the prognostic role of PNI in PDAC at its earliest stages.

In this context, we aimed to describe PNI features and to evaluate the impact of PNI on DFS and OS in a large cohort of patients undergoing surgical resection for PDAC. Because PNI may act as an early event in carcinogenesis,[12] we investigated also specific sub-groups of patients with less advanced PDAC including patients with R0/N0 disease or with size smaller than 20 mm.

From the *School of Medicine, Vita-Salute San Raffaele University, Division of Pancreatic Surgery, Pancreas Translational & Clinical Research Center, IRCCS San Raffaele Scientific Institute, Milan, Italy; †Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts; ‡Department of Surgery, The Sol Goldman Pancreatic Cancer Research Center, The Johns Hopkins University School of Medicine, Baltimore, Maryland; §Department of Pathology, Ospedale Sacro Cuore-Don Calabria, Negrar, Italy; and ‖Department of Pathology, Universita` Politecnica delle Marche, Ospedali Riuniti, Ancona, Italy.

✉falconi.massimo@hsr.it.

Carlos Fernandez del Castillo, Christopher L Wolfgang, and Massimo Falconi share the senior authorship of this work.

Supported was provided by the Gioja Bianca Costanza Fund for the PhD Scholarship of Giulia Gasparini, MD and the Research Fellowship of Giulio Belfiori, MD.

The authors declare no conflicts of interest.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.annalsofsurgery.com).

ISSN: 0003-4932/22/27602-0378

DOI: 10.1097/SLA.0000000000004464

METHODS

Study Design and Endpoints

This multicenter retrospective study includes patients with histologically-proven PDAC of the head of the pancreas who underwent surgical resection between January 2009 and December 2014. Data were collected from prospective databases maintained at the Departments of Surgery of the Johns Hopkins University, Baltimore, USA, at of the Massachusetts General Hospital, Harvard Medical School Boston, USA and at the Divisions of Pancreatic Surgery of Ospedale Sacro Cuore-Don Calabria Negrar, Italy and of Ospedali Riuniti, Ancona, Italy.

The primary end-point of the study was DFS whereas the secondary end-point was overall survival (OS).

Inclusion and Exclusion Criteria

Only patients with carcinoma of the pancreatic head were included to consider a homogenous cohort of patients with similar lymphatic and perineural spread, including the plexi of the celiac and superior mesenteric arteries. Patients with a solid lesion in the pancreatic head who underwent total pancreatectomy for positive pancreatic margin at intraoperative examination were also included in the analysis. Exclusion criteria were: PDAC in the pancreatic body-tail, synchronous distant metastases at the time of resection, macroscopically positive surgical margins (R2 resection), in-hospital or 90-day postoperative mortality. Patients with incomplete follow-up data and/or with lack of data regarding tumor recurrence were also excluded from the analysis. A minimum follow-up of 12 months was required unless patient developed tumor recurrence and eventually died before this time.

Data Collection

Demographic, clinical, operative, and postoperative data were collected including adjuvant/neoadjuvant treatment. Specific details of neoadjuvant and adjuvant therapy were not analyzed because they were considered beyond the scope of this study. Pathologic details included size of the primary tumor, grade, TNM staging (according to American Joint Committee on Cancer Staging Manual, seventh ed)[17] microvascular and PNI, R-status (0/1), N-status (0/1). Resection margin (R) status was defined as R0 when distance of tumor cells to the closest resection margin was >1 mm or R1 when distance was less than 1 mm.[18] PNI was considered as present/absent based on the initial pathological evaluation.

Follow-up

Patient follow-up included the evaluation CA 19.9 levels and of chest and abdominal computed tomography every 6 months for the first 5 years, and yearly thereafter. Recurrence was considered as the first site of recurrence and it was classified as local or systemic. Local recurrence was defined as recurrence in the pancreatic remnant or as soft tissue along the portal or superior mesenteric vein, the hepatic, celiac, or superior mesenteric artery, or in the retroperitoneum. Systemic recurrence was defined as recurrence in the liver, lungs, peritoneum or in other distant locations. Biopsy was not required to confirm tumor recurrence when there was a high likelihood based on clinical and radiological findings. Recurrence was analyzed considering local versus systemic versus local + systemic recurrence for patients who experienced both at the moment of recurrence diagnosis. We also grouped together patients with local and systemic recurrence with those with local recurrence alone. This choice was based on the hypothesis that PNI may be a source for retroperi-toneal/local recurrence, with the need of evaluating specifically the characteristics of local recurrence.

Statistical Analysis

Descriptive statistics were calculated to summarize patients' demographic and prognostic factors. Comparison between groups were performed using the Chi-squared t-test for categorical variables. DFS and OS by PNI presence, both overall and in specific "early stage" subgroups, were estimated by the Kaplan-Meier method for cumulative probability. Every local/systemic relapse or distant metastasis was considered an event for the purpose of calculating the DFS. The time between surgery and a relapse event or last follow up was the survival time for DFS and the time between surgery and death or last follow up was the survival time for OS. Log-rank tests were used to identify differences by PNI groups. Multivariable Cox proportional hazard models were used to investigate the impact of various factors on the risk of disease progression. In the univariate analysis, we considered age (stratified into 4 groups: $\leq$55 years, >55 and $\leq$65 years, >65 and $\leq$75 years, >75), adjuvant and neoadjuvant therapy administration, diabetes, pain, jaundice, weight loss, vascular resection, grading, pT status grouped in 3 classes (T1, T2, T3/4), pN status (N0, N1), CA19.9 grouped in: Ca19.9 $\leq$37 and CA19.9 >37, R status (R0, R1), presence of PNI, microvascular invasion, and postoperative variables as complications, grade of complication,[19] and pancreatic fistula. The variables that were found to be statistically significant on univariate analysis or that are known to predict the risk of disease progression were included in the multivariable analysis. P-values were considered significant when less than 0.05. All statistical analyses were performed by Stata 14 software (Stata-Corp, College Station, TX).

RESULTS

Patient Cohort

In the study period a total of 1118 patients underwent PD or total pancreatectomy for a histologically confirmed PDAC of the head of the pancreas at the participating Institutions. Of these, 340 (30.4%) were excluded because they did not meet the inclusion criteria (distant metastases: 66 patients; in-hospital or 90-day surgery-related mortality: 70 patients; lack of complete pathology data: 38 patients; R2 resection: 35 patients; uncomplete or uncertain recurrence data: 131 patients). Seven hundred seventy-eight patients (376 female, 48%; median age 66 years, range 35–92) were included in the final analysis. Demographics, clinical-pathological, operative, and postoperative data are summarized in Table 1.

PNI was found in 87% of patients. The rates of R1 and N1 were 34.5% and 70%, respectively. Adjuvant treatment was given to 67.4% of patients whereas 37.1% of patients received neoadjuvant therapy. Overall, 181 patients (23.3%) received neither adjuvant nor neoadjuvant treatment.

PNI Features

The rate of PNI did not differ between patients who underwent neoadjuvant therapy and upfront surgery (84.1% vs 88.6%, $P = 0.08$). Table 2 shows the association between PNI and clinico-pathological characteristics of the entire cohort and in patients undergoing neoadjuvant therapy and upfront surgery. In the entire cohort, there was a significant increase in PNI rate consistently with larger tumor size (53.9% in tumors $\leq$10 mm, 84.2% in tumors between 10 and 20 mm, 88% in tumors between 20 and 30 mm and 92.5% in those >30 mm, $P < 0.01$), with lymph node metastases (72.5% in pN0 and 93% in pN1, $P < 0.01$), R status (84.1% in R0 vs 92.2% in R1, $P < 0.01$) and

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TABLE1. Clinical and Pathological Characteristics of the Entire Cohort of 778 Patients Undergoing Pancreatectomy for Pancreatic Head Adenocarcinoma

Variables	All Patients (n = 778)
	N (%)
Sex	
Male	402 (52)
Female	376 (48)
Age (yrs)	
Median (range)	66 (35–92)
< 55	123 (15.8)
> 55 and < 65	234 (30.1)
> 56 and < 75	279 (35.9)
> 75	142 (18.2)
Tumor size (mm)	
< 10	39 (5.0)
> 10 and < 20	177 (22.8)
> 20 and < 30	308 (39.6)
> 30	254 (32.7)
Grading	
0/1	70 (9)
2	425 (54.6)
3	283 (36.4)
pT status	
T0/1	73 (9.4)
T2	120 (15.4)
T3/4	585 (75.2)
pN status	
N0	233 (30.0)
N1	545 (70.0)
CA19.9	
< 37	193 (24.8)
> 37	383 (49.2)
Unknown	202 (26.0)
R status[18]	
R0	510 (65.6)
R1	268 (34.5)
Perineural Invasion	
No	102 (13)
Yes	676 (87)
Microvascular Invasion	
No	284 (36.5)
Yes	494 (63.5)
Surgery type	
Pylorus preserving pancreaticoduodenectomy	279 (35.9)
Whipple resection	473 (60.8)
Total pancreatectomy	26 (3.3)
Adjuvant treatment	
No treatment	254 (32.6)
Chemotherapy	389 (50.0)
Chemo-radiation/radiotherapy	135 (17.4)
Neoadjuvant treatment	
No	489 (62.9)
Yes	289 (37.1)
Diabetes	
No	615 (79.1)
Yes	163 (20.9)
Pain	
No	531 (68.3)
Yes	247 (31.6)
Jaundice	
No	326 (41.9)
Yes	452 (58.1)
Weight loss	
No	621 (79.8)
Yes	157 (20.2)
Vascular resection	
No	611 (78.5)
Yes	167 (21.5)
Postoperative complications	
No	366 (47.0)
Yes	412 (53.0)
Clavien Dindo grade	
0–2	643 (82.7)
3–4	135 (17.3)
Pancreatic fistula	
No	723 (92.9)
Yes	55 (7.1)

microvascular invasion (94.7% and 73.2% in patients with and without microvascular invasion, P < 0.01). No significant association was found between the PNI rates and the grading (82.9% in the G1 group, 86.3% in the G2 group, and 88.7% in the G3 group, P = 0.26). These differences were seen both in patients undergoing upfront surgery and in those who received neoadjuvant treatment.

One hundred eighty patients (23.1%) had R0/N0 tumors, and 127 of them had evidence of PNI (70.6%). Another 216 (27.7%) had tumor size less or equal to 20 mm. In this latter group, 170 patients (78.7%) had PNI, 114 (52.8%) had pN1 disease and 65 (30.1%) had R1 resection. When these 2 categories are matched, the rate of PNI was 36% in 25 patients with a R0/N0 tumor less than 10 mm and it increased up to 58.8% in the 80 patients with R0/N0 tumor that were between 10 and 20 mm in size. Of the 150 patients with tumor size less or equal to 20 mm with R0 resection, 110 (72.8%) had PNI.

Influence of PNI on DFS

Median DFS for the entire cohort was 16 months (Supplementary Fig. 1A, http://links.lww.com/SLA/C593). 30.4% of patients had local recurrence, 12.2% had both a local and systemic recurrence, whereas 57.4% had only systemic recurrence. Overall, 42.6% of patients had local recurrence in the study. Table 3 shows the influence of factors on DFS in multivariable analysis in the entire cohort and in the 2 "early stage" groups (please refer to Supplementary Table 1, http://links.lww.com/SLA/C594 for the whole univariate and multi-variate analysis). N1 status (HR: 1.7), CA 19.9 > 37 (HR: 1.4), R1 status (HR: 1.2), and weight loss (HR: 1.3) were independent predictors of tumor-recurrence. Although not statistically significant at multivariate analysis (P = 0.07), patients with PNI had a significant higher risk of a worse DFS at univariate analysis (HR = 1.5, P < 0.01), (median DFS: 20 vs 15 months, log-rank test P-value < 0.01, Fig. 1A). The administration of both adjuvant chemotherapy and chemoradia-tion were also associated with improved DFS at univariate analysis. The rate of PNI did not differ among patients with local, systemic, and local plus systemic recurrence (87.7% vs 90.9% vs 90.3%, P = 0.522). When patients with any local recurrence (local alone and local associated with systemic recurrence) are considered, the rate of PNI was not significantly different when compared with those with systemic recurrence alone (88.4% vs 90.9%, P = 0.329).

Considering patients with R0/N0 disease (n = 180), univariate analysis identified PNI, neoadjuvant treatment, weight loss, and diabetes as factors influencing DFS but the only independent predictor of DFS was PNI (HR: 2.2, P < 0.01). Median DFS was 20 and 34 months for patients with and without PNI, respectively, with a significant difference between the 2 curves (log rank test P-value = 0.03, Fig. 1B). In this subgroup of patients, the rate of PNI was significantly higher in

TABLE2. Association Between Perineural Invasion (PNI) and Patients Characteristics, in the Entire Cohort and by Treatment (Upfront Surgery Versus Neoadjuvant Treatment)

	All (n = 778)		Patients Receiving Neoadjuvant Treatment (n = 289)		Patients Receiving Upfront Surgery (n = 489)	
Variables	Patients With PNI Presence, n (%)	P	Patients With PNI Presence, n (%)	P	Patients With PNI Presence, n (%)	P
Tumor size						
≤ 10	21 (53.9)	< 0.01	11 (50)	< 0.01	10 (58.8)	< 0.01
> 10 and ≤20	149 (84.2)		49 (76.6)		100 (88.5)	
> 20 and ≤30	271 (88.0)		83 (86.5)		188 (88.7)	
> 30	235 (92.5)		100 (93.5)		135 (91.8)	
Grading						
0/1	58 (82.9)	0.26	22 (78.6)	0.35	36 (85.7)	0.72
2	367 (86.3)		136 (82.9)		231 (88.5)	
3	251 (88.7)		85 (87.6)		166 (89.3)	
pT status						
T0/1	45 (61.6)	< 0.01	17 (48.6)	< 0.01	28 (73.7)	< 0.01
T2	104 (86.7)		41 (87.2)		63 (86.3)	
T3/4	527 (90.1)		185 (89.4)		342 (90.5)	
pN status						
N0	169 (72.5)	< 0.01	86 (70.5)	< 0.01	83 (74.7)	0.01
pN1	507 (93.0)		157 (94.1)		350 (92.6)	
R[18]						
R0	429 (84.1)	< 0.01	169 (81.3)	0.03	260 (86.1)	0.03
R1	247 (92.2)		74 (91.4)		173 (92.5)	
CA 19.9*						
≤ 37	166 (86.0)	0.34	53 (85.5)	0.11	113 (86.3)	0.15
> 37	336 (87.7)		149 (83.7)		187 (91.2)	
Neoadjuvant treatment						
No	433 (88.6)	0.08				
Yes	243 (84.1)					
Microvascular invasion						
No	208 (73.2)	< 0.01	93 (71.5)	< 0.01	115 (74.7)	< 0.01
Yes	468 (94.7)		150 (94.3)		318 (94.9)	
Diabetes						
No	534 (86.8)	0.93	183 (84.3)	0.84	351 (88.2)	0.60
Yes	142 (87.1)		60 (83.3)		82 (90.1)	
Pain						
No	467 (88.0)	0.39	154 (88.5)	< 0.01	313 (87.7)	0.58
Yes	209 (84.6)		89 (77.4)		120 (90.8)	
Jaundice						
No	271 (83.1)	< 0.01	128 (82.6)	0.45	143 (83.6)	0.01
Yes	405 (89.6)		115 (85.8)		290 (91.2)	
Weight Loss						
No	538 (86.6)	0.70	186 (83.0)	0.37	352 (88.7)	0.84
Yes	138 (87.9)		57 (87.7)		81 (88.0)	

*P-value was calculated excluding 202 patients (174 with PNI) were excluded from this analysis because of unknown values of CA 19.9 or because CA 19.9 was measured during jaundice

patients who had exclusively systemic recurrence compared to those with local recurrence alone or local plus systemic recurrence (84.3 vs 65.8%, P = 0.039).

In patients with tumors ≤ 20 mm (n = 216), both univariate and multivariate analysis identified CA 19.9 > 37 (HR: 1.5, P = 0.02) and PNI (HR: 1.8, P = 0.02) as the only predictors of lower DFS. Median DFS was 18 months for patients with PNI compared with 24 months in those who did not (log rank test P-value = 0.02, Fig. 1C). The rate of PNI did not change based on the pattern of recurrence (83.6% and 86.3% in patients with local/local plus systemic recurrence and systemic recurrence alone, respectively, P = 0.663). In patients with tumors ≤20 mm and R0 resection (n = 150) univariate analysis identified only PNI as a predictor of DFS (HR = 1.8, P = 0.02). Median DFS was 15.5 months in patients with PNI and 21 months in those without PNI.

In 289 patients who underwent neoadjuvant therapy, univariate analysis identified PNI (HR = 1.5, P = 0.035), T-status (HR = 1.6, P = 0.02), N status (HR = 1.5, P < 0.01), microvascular invasion (HR = 1.4, P = 0.01), and diabetes (HR = 1.54, P < 0.01) as significant features affecting DFS, but only diabetes resulted as independent (HR = 1.6, P < 0.01) predictor of DFS.

In 524 patients who underwent adjuvant treatment, N status (HR = 1.65, P < 0.01), R status (HR = 1.3, P = 0.012), Ca 19.9 > 37 (HR = 1.4, P < 0.01), PNI (HR = 1.6, P < 0.01), microvascular invasion (HR = 1.3, P = 0.03), pain (HR = 1.2, P = 0.04), and postoperative complications (HR = 1.2, P = 0.04) were predictors of DFS at univariate analysis whereas N status (HR = 1.7, P < 0.01), CA19.9 > 37 (HR = 1.4, P < 0.01), PNI (HR = 1.5, P = 0.03), and pain (HR = 1.3, P = 0.02), were independent predictors of DFS at multivariate analysis.

TABLE3. Factors Influencing DFS for All Patients and for Early Stage Disease Patients: Univariate and Multivariate Analysis

	All n = 778				R0 (Werbecke) and N0 n = 180				Tumor size ≤20 mm n = 216			
	Univariate Analysis		Multivariate Analysis		Univariate Analysis		Multivariate Analysis		Univariate Analysis		Multivariate Analysis	
Variables	HR	P	HR	P	HR	P	HR	P	HR	P	HR	P
pT status												
0/1	1		1		1		1					
2	0.9 (0.7–1.5)	0.93	1.3 (0.8–2.0)	0.35	1.0 (0.6–1.8)	0.99	0.8 (0.3–1.7)	0.52				
3	1.4 (1.1–1.9)	0.02	1.2 (0.9–1.8)	0.24	1.1 (0.7–1.7)	0.69	0.8 (0.5–1.5)	0.53				
pN status												
0	1		1		—	—	—	—	1		1	
1	1.6 (1.3–1.9)	< 0.01	1.7 (1.3–2.1)	< 0.01	—	—	—	—	1.3 (0.9–1.8)	0.13	1.3 (0.9–1.8)	0.19
R[18] *												
0	1		1		—	—	—	—	1			
1	1.5	< 0.01	1.2 (1.0–1.5)	0.04	—	—	—	—	1.4 (1.0–1.9)	0.08		
CA19.9												
< 37	1		1		1		1		1		1	
> 37	1.5 (1.2–1.8)	< 0.01	1.4 (1.1–1.7)	< 0.01	1.2 (0.8–1.8)	0.42	1.1 (0.6–1.7)	0.12	1.4 (1.0–2.0)	0.05	1.5 (1.1–2.2)	0.02
PNI												
No	1		1		1		1		1		1	
Yes	1.5 (1.2–2.0)	< 0.01	1.3 (0.9–1.5)	0.07	1.6 (1.0–2.5)	0.04	2.2 (1.2–3.9)	< 0.01	1.7 (1.1–2.5)	0.02	1.8 (1.1–2.9)	0.02
Adjuvant treatment												
No treat	1		1		1				1			
Cht	1.2 (1.0–1.5)	0.04	0.9 (0.7–1.2)	0.69	1.2 (0.8–1.8)	0.50			1.1 (0.7–1.5)	0.80		
Cht-Rt	1.4 (1.1–1.8)	0.01	0.9 (0.7–1.1)	0.70	1.3 (0.7–2.3)	0.42			1.3 (0.8–2.3)	0.27		
Diabetes												
No	1.1				1		1		1			
Yes	1.2 (0.9–1.4)	0.19			1.8 (1.2–2.7)	< 0.01	1.4 (0.8–2.4)	0.26	1.1 (0.8–1.8)	0.38		
Pain												
No	1		1		1				1			
Yes	1.2 (1.0–1.4)	0.04	1.2 (0.9–1.3)	0.08	1.4 (0.9–2.0)	0.11			1.1 (0.8–1.6)	0.45		
Weight loss												
No	1		1		1		1		1			
Yes	1.3 (1.1–1.6)	0.02	1.3 (1.0–1.7)	0.04	1.9 (1.3–2.9)	< 0.01	1.8 (0.9–3.3)	0.09	1.3 (0.9–2.0)	0.30		

*R status was defined according to Verbecke.[18]
◦CD indicates Clavien-Dindo.[19]

Influence of PNI on OS

Median OS was 30 months for the 778 patients included in the study (Supplementary Fig. 1B, http://links.lww.com/SLA/C593). The results of multivariate analysis of OS are shown in Table 4 (please refer to Supplementary Table 2, http://links.lww.com/SLA/C595 for the whole univariate and multivariate analysis). G3 tumors (HR: 1.7), pN1 status (HR: 1.8), CA 19.9 >37 (HR: 1.4), PNI (HR: 1.6), and pain (HR: 1.3) were found to be independent predictors of OS. Specifically, median OS was 50 months in patients without PNI compared with 27 months in those with PNI (Fig. 2A). Pain was the only independent predictor of OS (HR: 1.9) in 180 patients with R0/N0 disease. In this group, although there was a trend toward improved survival in patients without PNI, the difference did not reach significance (median OS: 54 vs 42 months, P = 0.23, Fig. 2B). In the subgroup of patients with tumor size smaller or equal to 20 mm, PNI (HR: 1.8, P = 0.05) was the only independent predictor of OS. Median OS was 55 months in patients without PNI compared with 34 months in those with PNI with a significant difference between the 2 curves (log rank test P-value < 0.01, Fig. 2C).

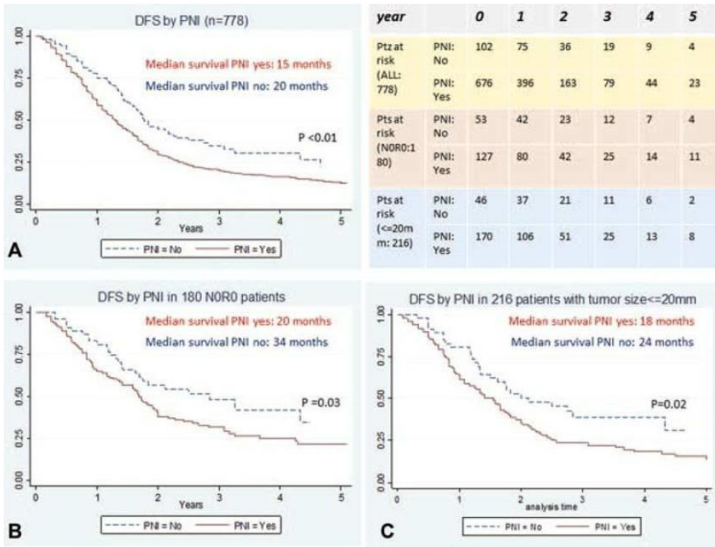
In patients with tumors ≤20 mm and R0 resection (n = 150) univariate analysis identified only PNI as a predictor of OS (HR = 1.9, P = 0.03). Median OS was 28 months in patients with PNI and 50 months in those without PNI.

In 289 patients who underwent neoadjuvant therapy, univari-ate analysis identified T-status (HR = 1.8, P = 0.02), N-status (HR = 1.7, P < 0.01), PNI (HR = 1.8, P = 0.02), microvascular invasion (HR = 1.3, P = 0.05) as predictors of OS, but only N status was found as the only independent (HR = 1.5, P = 0.02) predictor of OS.

In 524 patients who underwent adjuvant treatment, univariate analysis identified grading (HR G2 vs G1 = 1.6, P = 0.04; HR G3 vs G1 = 2.0, P < 0.01), N status (HR = 1.8, P < 0.01), CA 19.9 > 37 (HR = 1.4, P < 0.01), PNI (HR = 1.8, P < 0.01), microvascular invasion (HR = 1.3, P = 0.04) as predictors of OS, but only grading (HR G3 vs G1 = 1.9, P = 0.01), N status (HR = 1.8, P < 0.01), CA19.9 > 37 (HR = 1.4, P = 0.03), and PNI (HR = 1.6, P < 0.03) were independent prognostic factors of OS.

DISCUSSION

This is the largest study to date that describes the characteristics and the prognostic role of PNI in patients with resected ductal adenocarcinoma of the head of the pancreas. In this paper we specifically analyzed the relationship between PNI and tumor-recurrence, an outcome that has been incompletely evaluated in previous studies, and for the first time we provide specific data focusing on early-stage PDAC. In the entire cohort we found a PNI rate of 87%, and PNI was identified as a relevant prognostic factor for both tumor recurrence and survival after pancreatoduodenectomy. We also demonstrated that PNI is a hallmark of pancreatic cancer even in the early stages of the disease, and in this setting PNI significantly affects recurrence



year		0	1	2	3	4	5
Ptz at risk (ALL: 778)	PNI: No	102	75	36	19	9	4
	PNI: Yes	676	396	163	79	44	23
Pts at risk (N0R0:180)	PNI: No	53	42	23	12	7	4
	PNI: Yes	127	80	42	25	14	11
Pts at risk (<=20mm: 216)	PNI: No	46	37	21	11	6	2
	PNI: Yes	170	106	51	25	13	8

FIGURE 1. Disease-free survival (DFS) stratified by the presence/absence of PNI. In the entire cohort, 5-yr DFS rates were 22.1% and 12.5% in patients without and with PNI, respectively (A). In the subgroup with N0/R0 disease, 5-yr DFS rates were 34.9% and 21.7% in patients without and with PNI, respectively (B). In the subgroup with tumor size < 20 mm, 5-yr DFS rates were 30.4% and 12.3% in patients without and with PNI, respectively (C). PNI indicates perineural invasion.

and survival. In fact, based on the hypothesis that PNI plays a pivotal role in the early progression of PDAC development,[12] we specifically investigated 180 patients (23.1% of the cohort) with R0 and N0 disease and 216 individuals (27.7%) with tumor size smaller or equal to 20 mm, showing that PNI is an independent predictor of recurrence in this setting.

In our study we found several interesting features related to PNI. First, the rate of PNI was much higher in patients with lymph node metastases (93% vs 72.5% in pN0), microvascular invasion (94.7% vs 73.2%) and R1 resection (92.2% vs 84.1% in R0), thus confirming that PNI is associated with more aggressive disease. The relationship between PNI and lymph node metastases is complex and deserves further attention.[20] Kayahara et al[21] described cancer cells growing along nerves in contact with lymph nodes, suggesting that PNI may constitute a "mechanical" route for the development of lymph node metastases. Second, PNI was analyzed based on different tumor size. Tumors smaller or equal to 10 mm had still a remarkable 53.9% rate of PNI, which increased to 92.5% for PDAC larger than 30 mm. Considering all 216 tumors ≤20 mm, the rate of PNI was as high as 78.7%. Likewise, PNI was described in 70.6% of patients with R0/N0 disease. The lowest rate of PNI (30.1%) was found in the 25 patients (3.1%) with a R0/N0 tumor less than 10 mm, but even at this "very early stage" 1 out of 3 patients already had nerves infiltrated by cancer cells. All these findings support the hypothesis that PNI is an early event in pancreatic carcinogenesis. Third, we did not find significant differences in the rate of PNI between patients who underwent neoadjuvant therapy and upfront surgery (84% vs 8 %, P = 0.08). This data differs from the current literature. Different authors found a significantly lower rate of PNI in patients receiving preoperative chemotherapy/chemoradiation (50%–70%) compared with those undergoing immediate resection (80%–0%).[22–25] A possible explanation, besides the limitations of a retrospective analysis, is related to the study period that encompasses a "pre-FOLFIRINOX era" where most patients underwent gemcitabine alone or gemcitabine/oxaliplatin chemotherapy.

The primary endpoint of the study was DFS. This outcome has been evaluated by few studies, but it may have a significant clinical impact given the possible role of PNI as a route of tumor dissemination beyond vascular and nodal invasion.[10,11] Takahashi et al and Fouquet et al identified PNI as an independent predictor of DFS in 2 studies with 110 and 166 patients with resected PDAC.[26,27] A meta-analysis of 4 studies with a clear definition of DFS identified PNI as a poor prognostic factor for recurrence.[16] When we considered the entire cohort of 778 patients, patients with PNI had a higher risk of an earlier tumor recurrence at univariate analysis (median DFS 20 vs 15 months, Fig. 1A) but we failed to identify PNI among the independent predictors of DFS (pN1 and R1 status, CA 1. > 37, weight loss). However, PNI was described as an independent predictor of DFS in patients with PDAC ≤20mm and in those with R0/N0 disease - in this subgroup PNI was the only predictor of recurrence. Surprisingly, in R0/N0 subgroup, the rate of PNI was significantly higher in patients who had exclusively systemic recurrence compared to those with local or local/systemic recurrence. Taken together these observations underline the striking role of PNI in increasing the risk of tumor recurrence after surgery, especially at earlier stages of PDAC progression, where PNI represents the determinant factor of recurrence. Takahashi correlated the presence of an intra-pancreatic PNI with the infiltration of extra-pancreatic, retroperitoneal nerve plexi by cancer cells,[10] thus justifying local recurrence in the setting of R0 resection. However, our data suggest that in early stage PDAC (ie, R0/N0 tumors), the presence of PNI is also correlated with a higher risk of recurrence even at systemic level. This data should be confirmed, but PNI may represent the stigmata of a more aggressive disease. When the disease is more

TABLE4. Factors Influencing OS for All Patients and for Early Stage Disease Patients: Univariate and Multivariate Analysis

	All n = 778				R0 (Werbecke) and N0 n = 180				Tumor Size < 20 mm n = 216			
	Univariate Analysis		Multivariate Analysis		Univariate Analysis		Multivariate Analysis		Univariate Analysis		Multivariate Analysis	
Variables	HR	P	HR	P	HR	P	HR	P	HR	P	HR	P
pT status												
0/1	1		1		1		1					
2	1.2 (0.7–1.8)	0.93	1.3 (0.8–2.2)	0.32	0.8 (0.4–1.7)	0.54	0.9 (0.4–2.1)	0.82				
3	1.7 (1.1–2.5)	< 0.01	1.2 (0.8–1.8)	0.48	1.0 (0.6–1.8)	0.86	0.8 (0.4–1.5)	0.53				
pN status												
0	1		1						1		1	
1	1.8 (1.5–2.3)	< 0.01	1.8 (1.4–2.4)	< 0.01					1.3 (0.9–2.1)	0.31	1.2 (0.8–1.8)	0.26
R[18]												
0	1		1						1		1	
1	1.3 (1.1–1.6)	< 0.01	1.1 (0.9–1.3)	0.43					1.4 (1.0–2.1)	0.10	1.1 (0.8–1.8)	0.61
Grading												
0/1	1		1		1				1			
2	1.7 (1.2–2.6)	0.01	1.5 (0.9–2.4)	0.09	1.8 (0.7 – 4.1)	0.24	—	—	1.8 (0.8– 4.5)	0.21		
3	1.9 (1.2–2.9)	< 0.01	1.7 (1.1–2.8)	0.03	1.6 (0.7–4.0)	0.34	—	—	2.3 (1.0–6.6)	0.08		
CA19.9												
≤ 37	1		1		1		1		1			
> 37	1.4 (1.1–1.8)	< 0.01	1.4 (1.1–1.7)	0.03	1.0 (0.6–1.7)	0.97	1.0 (0.6–1.7)	0.93	1.2 (0.8–1.8)	0.30		
PNI												
No	1		1		1		1		1		1	
Yes	1.8 (1.3–2.5)	< 0.01	1.6 (1.1–2.3)	0.01	1.4 (0.8–2.5)	0.26	1.6 (0.8–3.1)	0.14	2.0 (1.2–3.4)	0.02	1.8 (1.0–3.1)	0.05
Microvascular invasion												
No	1		1		1				1			
Yes	1.3 (1.1–1.6)	< 0.01	0.9 (0.7–1.1)	0.38	0.9 (0.5–1.4)	0.55			1.2 (0.9–1.8)	0.25		
Pain												
No	1		1		1		1		1			
Yes	1.2 (1.0–1.6)	0.02	1.3 (1.0–1.6)	0.04	1.6 (1.0–2.5)	0.05	1.9 (1.1–3.2)	0.02	1.0 (0.7–1.5)	0.92		

*R status was defined according to Verbecke.[18]

CD indicates Clavien-Dindo.[19]

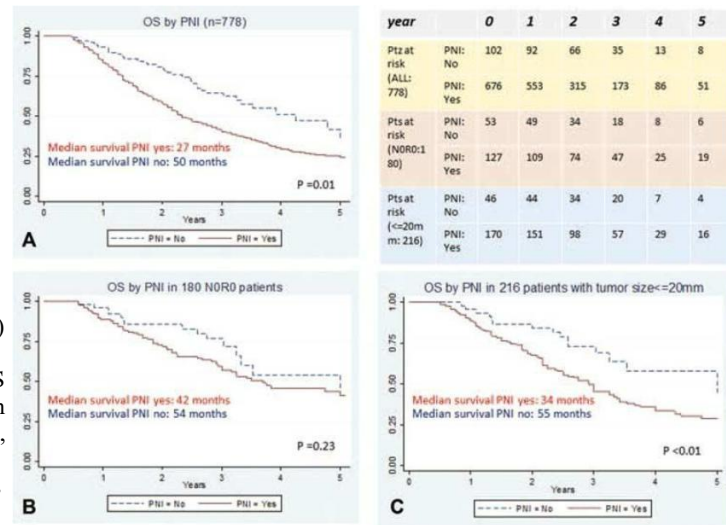


FIGURE 2. Overall survival (OS) stratified by the presence/absence of PNI. In the entire cohort, 5-yr OS rates were 36.5% and 24.4% in patients without and with PNI, respectively (A). In the subgroup with N0/R0 disease, 5-yr DFS rates were 45% and 41.3% in patients without and with PNI, respectively (B). In the subgroup with tumor size < 20 mm, 5-yr DFS rates were 54.7% and 27.9% in patients without and with PNI, respectively (C). PNI indicates perineural invasion.

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advanced, other prognostic factors including N+ and R1 status had a more relevant role in determining recurrence compared with PNI, as showed in the DFS analysis of 778 patients.

Of note, the evidence of poor DFS and OS associated with PNI in early-stage pancreatic cancer can have a clinical impact. In fact, in this setting, the presence of PNI itself should be considered an indication for adjuvant treatment, despite the absence of other prognostic factors such as lymph node metastases or R1 resection.

Several studies have demonstrated the association between presence of PNI and decreased survival in PDAC. A recent meta-analysis of 36 studies identified PNI as an independent negative prognostic factor of OS in patients undergoing resection for PDAC.[16] Our study confirms this data. Median OS was almost double in patients without PNI (50 vs 27 months in those with PNI), and PNI was an independent predictor of OS in the entire cohort together with pain, G3 tumors, CA 19.9 > 37 and N+ status. PNI and pain were the only independent predictors of OS in PDAC smaller than 20 mm and in patients with R0/N0 disease, respectively. Pain is a negative prognostic factor for survival, and it is strongly linked with the presence and the severity of PNI.[15,28] In keeping with the findings observed for tumor-recurrence, PNI itself or pain - a clinical manifestation of PNI-acted as the only factors that influenced OS in early stages PDAC, and these 2 parameters significantly impacted OS also in the entire cohort.

Our study has some limitations. This is a multicenter retrospective study and recurrences were identified radiologically and only occasionally by biopsy. Follow-up intervals were on a 6-month basis, but it is possible that some recurrences were detected in advance if the patient complained of symptoms, thus requiring an anticipation of surveillance schedule. The relationship between PNI and pain is intriguing but we could not analyze it in detail due to the retrospective design of the study. Finally, the impact of neoadjuvant treatment on PNI status deserves further studies, with a specific evaluation in a prospective fashion. The strengths of the study do include the large number of patients with detailed data on timing and site of recurrence, the analysis of DFS beyond OS, and the specific analysis of subgroups of patients with early-stage PDAC, who have never been studied.

In conclusion, PNI is a distinctive feature of PDAC where it represents a major determinant of tumor recurrence and survival. The role of PNI seems to be particularly relevant at early stages, supporting the hypothesis that nerve invasion by cancer cells has a driving role in pancreatic cancer progression. A better understanding of the molecular mechanisms of PNI and of nerve-cancer cell interactions carries the opportunity for the development of new possible therapeutic strategies that may target this process to inhibit cancer dissemination.

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Timing But Not Patterns of Recurrence Is Different Between Node-negative and Node-positive Resected Pancreatic Cancer

Kim C. Honselmann, MD,[ã,y] Ilaria Pergolini, MD,[ã] Carlos Fernandez-del Castillo, MD,[ã] Vikram Deshpande, MD,[ã] David Ting, MD, Martin S. Taylor, MD, PhD, [ã] Louisa Bolm, MD, Motaz Qadan, MD,[ã] Ulrich Wellner, MD, Marta Sandini, MD,[z] Dirk Bausch, MD,[y] Andrew L. Warshaw, MD,[ã] Keith D. Lillemoe, MD,[ã] Tobias Keck, MD,[y] and Cristina R. Ferrone, MD[ã]

Objective: Our aim was to evaluate recurrence patterns of surgically resected PDAC patients with negative (pN0) or positive (pN1) lymph nodes.

Summary Background Data: Pancreatic ductal adenocarcinoma (PDAC) is predicted to become the second leading cause of cancer death by 2030. This is mostly due to early local and distant metastasis, even after surgical resection. Knowledge about patterns of recurrence in different patient populations could offer new therapeutic avenues.

Methods: Clinicopathologic data were collected for 546 patients who underwent resection of their PDAC between 2005 and 2016 from 2 tertiary university centers. Patients were divided into an upfront resection group (n ¼ 394) and a neoadjuvant group (n ¼ 152).

Results: Tumor recurrence was significantly less common in pN0 patients as compared with pN1 patients, (upfront surgery: 55% vs. 77%, P < 0.001 and 64% vs. 78%, P ¼ 0.040 in the neoadjuvant group). In addition, time to recurrence was significantly longer in pN0 versus pN1 patients in the upfront resected patients (median 16 mo pN0 vs. 10 mo pN1 P < 0.001), and the neoadjuvant group (pN0 21 mo vs. 11 mo pN1, P < 0.001). Of the patients who recurred, 62% presented with distant metastases (63% of pN0 and 62% of pN1, P ¼ 0.553), 24% with local disease (27% of pN0 and 23% of pN1, P ¼ 0.672) and 14% with synchronous local and distant disease (10% of pN0 and 15% of pN1, P ¼ 0.292). Similarly, there was no difference in recurrence patterns between pN0 and pN1 in the neoadjuvant group, in which 68% recurred with distant metastases (76% of pN0 and 64% of pN1, P ¼ 0.326) and 18% recurred with local disease (pN0: 22% and pN1: 15%, P ¼ 0.435).

Conclusion: Time to recurrence was significantly longer for pN0 patients. However, patterns of recurrence for pN0 vs. pN1 patients were identical. Lymph node status was predictive of time to recurrence, but not location of recurrence.

Keywords: lymph node status, PDAC, recurrence, survival

(Ann Surg 2020;272:357–365)

Pancreatic ductal adenocarcinoma (PDAC) is currently the fourth most common cause of cancer death and is predicted to become the second leading cause of cancer death by 2030.[1] Although only surgical resection offers a potential cure, just 10% to 15% of patients are surgical candidates at presentation. Of resected patients, 19% to 22% of patients survive 5 years[2,3] due to local or distant recurrence. While many patients recur at distant sites, 12% to 40% of patients demonstrate a destructive local recurrence pattern.[4–6]

To date, various prognostic factors for recurrence and overall survival have been identified including lymph node/ lympho-vascular- and perineural involvement, tumor grade, portal vein and mesenteric artery involvement, positive resection margins, and genomic characteristics. Similar to other cancers, lymph node metastasis is a strong indicator of more advanced disease.[7–10] Several studies have demonstrated that cancers with lymph node-positive disease are more likely to recur.[8] However, patterns of recurrence in lymph node positive versus lymph node-negative patients have not been studied.

Neoadjuvant therapy in locally advanced and borderline resectable PDAC has shown both prolonged survival and a significant decrease in the rate of lymph node-positivity on resected specimens compared with the rate in the absence of neoadjuvant therapy.[11–13] However, the effect on the recurrence pattern (local versus distant) has also not been studied.

The aim of this study was to explore the timing and nature of recurrence of node-negative compared with node-positive PDAC. The study was stratified for patients who underwent upfront resection versus those who received neoadjuvant treatment followed by resection. We hypothesized that patients with lymph node-negative disease would recur locally first, while lymph node-positive patients would recur distantly first, suggesting that lymph node negative patients may benefit from more aggressive local therapy including adjuvant radiation.

METHODS

Consecutive patients undergoing surgical resection of their pancreatic ductal adenocarcinoma (PDAC) between January 1, 2005 and December 31, 2016 in the Department of Gastrointestinal Surgery at the Massachusetts General Hospital and between January 1, 2013 and December 31, 2016 in the Department of Surgery of the University Medical Center Schleswig-Holstein, Campus Luebeck, Germany were included. Patients with either a minimum of 6 months follow-up or evidence of recurrence were included in the study. Patients who died of perioperative complications within 90 days were excluded. The study was approved by the institutional IRBs.

Data were collected from prospectively maintained databases at the respective institutions. Clinicopathologic variables collected included age at operation, gender, preoperative chemotherapy, CA19-9 levels, tumor size, nodal status, number of resected and positive lymph nodes, tumor grade, lymphovascular and perineural invasion (according to the 7th and 8th editions of the AJCC), R-status according to the "1 mm rule" by the British Royal college of Pathologists,[14] and type of operation (pancreatoduodenectomy,

From the [ã]Department of Gastrointestinal Surgery and Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, MA; [y]Department of Surgery, University Medical Center Schleswig-Holstein, Campus Luebeck, Luebeck, Germany; and [z]MGH Cancer Center, Massachusetts General Hospital and Harvard Medical School, Boston, MA.

KCH was supported by the German Research Foundation (DFG: HO 5737/1-1).

Parts of this manuscript were presented at the American Pancreatic Association 2016 and the German Pancreasclub Meeting 2017.

TK and CF shared last authorship.

The authors report no conflicts of interest.

Reprints: Cristina R. Ferrone, MD, Associate Professor of Surgery, Department of Surgery, Massachusetts General Hospital/Harvard Medical School, 55 Fruit Street, Boston, MA 02114. E-mail: Cferrone@partners.org.

ISSN: 0003-4932/19/27202-0357

DOI: 10.1097/SLA.0000000000003123

distal, or total pancreatectomy). Standard lymphadenectomy was performed in both institutions. Postoperative outcomes and treatments included hospital length of stay (days), postoperative chemotherapy and radiation therapy, date of first recurrence, site of first recurrence, and overall survival.

Patients were staged according to the AHPBA/SSO/SSAT consensus guidelines.[15] Resectable patients were included in the upfront resection group. They did not receive preoperative chemotherapy or radiation. Patients in the neoadjuvant therapy group were only treated at MGH and included resectable, borderline resectable, and locally advanced patients. Short-course proton radiation (5 Gy Â 5 d) with Capecitabine ÆHydroxychloroquine was administered neoadjuvantly to resectable patients (These patients were all part of phase I and II clinical trials published elsewhere[16,17]).

Borderline resectable and locally advanced patients received 8 cycles of FOLFIRINOX or Gemcitabine plus 50.4 Gy radiation and 5-FU.

Locoregional recurrence was defined by radiographic or pathological evidence of recurrent disease in the remnant pancreas, pancreatic bed, retroperitoneum, along the SMA/SMV, porta hepatis, or celiac axis. Distant recurrence was tumor spread outside of the loco-regional area (extra-regional lymph nodes, peritoneum, lungs, and liver). We separately listed "hematogenous metastasis" (liver and lung) from "Other" (extraregional lymph nodes and peritoneum and other rare distant locations such as small intestine). Disease-free survival (DFS) and overall survival (OS) were calculated from the date of diagnosis to the date of recurrence or death, respectively (event), or to the date of last follow-up (censored). Date of death was obtained from the medical records or from the Social Security Death Index.

Patients with pancreatic cancer arising in an IPMN, mucinous adenocarcinoma, adenosquamous carcinoma, chronic pancreatitis, acinar cell carcinoma and acinar cystadenocarcinoma were excluded due to differing tumor biology.

Disease-free survival and overall survival (DFS and OS) were calculated as the median of actual survival from the time of diagnosis. Differences in survival were tested by the log-rank test. Multivariate analyses for local and distant recurrence were calculated with a logistic regression model using the forward conditional approach. Criteria for inclusion were significance on univariate analysis and clinical relevance (age, sex). Propensity score matching was performed with the R-add-on for SPSS Version 21 for all patients without neoadjuvant chemo-radiation. Pathologic node-positive (pN1) and pathologic node-negative (pN0) patients were matched for age, gender, tumor size, and T-stage. P values less than 0.05 were considered statistically significant. All tests used were 2-tailed. Statistical analysis was performed with the IBM SPSS Statistics software for Mac, Version 21.0 (IBM Corp, Armonk, NY).

RESULTS

Demographics and Clinical Data

Of the 715 patients who underwent surgical resection of their PDAC at the 2 academic centers within the study period, 546 patients met the inclusion criteria (Fig. 1). Upfront resection was performed in 394 patients and 152 patients underwent neoadjuvant therapy followed by resection. Median follow-up of the entire cohort was 18 (10–34) months. For the upfront resected group, follow-up was 18 months (10–34) and 21 months (9–35) for the neoadjuvant group. Separate analyses of these 2 cohorts, as well as a propensity score matching, were performed (n ¼ 188).

Upfront Resection Group

For the upfront resection group, in both the pN0 (n ¼ 109, 28%) and pN1 (n ¼ 285, 72%) cohorts, 47% were female and the median age was 68 years with 21% of patients older than 75 years (Table 1). Median preoperative CA19-9 levels were significantly lower in lymph node-negative patients (pN0 65 vs. pN1 140 U/mL, P ¼ 0.045).

Surgical and Pathological Outcomes

The most common operation was pancreaticoduodenectomy (75%)(Table 1). Patientsundergoing distal pancreatectomywere more likely to have node-negative disease. No patient in this group had combined arterial and venous resection. Median length of operation and hospital stay (330 min and 8 d) did not differ between the lymph node cohorts. Ninety-day postoperative mortality was 1.7%.

In the upfront resection group, 109 patients were pN0 and 285 were pN1 (Table 1). The majority (63%) of tumors were grade 2 (moderately differentiated), but pN0 patients displayed lower tumor grading than pN1 patients. Patients with pN1 disease had slightly larger cancers (median 32 mm vs. 29 mm, P ¼ 0.012) and were more likely to have a positive resection margin than patients with pN0 disease (59% vs. 31%, P < 0.001). Perineural invasion and lympho-vascular invasion were also more frequent in pN1 cancers (pN1 93% vs. pN0 73%, P < 0.001 and pN1 73% vs. pN0 37%, respectively P < 0.001). In both cohorts, the majority of patients received adjuvant treatment (pN0 72% and pN1 75%, P ¼ 0.328). Chemotherapy alone and chemoradiation were the most common type of adjuvant therapy (48% and 51%), whereasradiation alone was only given to 2 patients(1%) total (Table 1).

Survival

Median OS for all patients was 18 months (10–34). Median OS for the pN0 patients was significantly longer (median 25 mo, range 11–45) than the pN1 patients (median 16 mo, range 10–29) (P < 0.001). Figure 2 illustrates the significant difference in overall survival.

Recurrence

Overall median DFS was 11 months (6–23). Patients with pN0 disease had a significantly longer DFS of 16 months (7–36), as compared with 10 months (5–20) in patients with pN1 disease (P < 0.0001). Tumor recurrence was significantly less common in pN0 patients as compared with pN1 patients, (55% vs. 77%, P < 0.001). On multivariate analysis, both lymph-node ratio and lymph-node status were significantly associated with recurrence (OR: 1.415–16.398, P ¼ 0.012 and OR: 1.349–4.496, P ¼ 0.003).

Patterns of recurrence between pN0 and pN1 patients were similar. Locoregional recurrence occurred in 27% of pN0 and 23% of pN1 patients (P ¼ 0.672). Distant recurrence occurred in 63% of pN0 and 62% of pN1 patients (P ¼ 0.553) (Fig. 2 and Table 2). This was also true for the new lymph node staging system (8th AJCC), where local recurrence occurred in 27% of pN0 patients, in 21% in pN1 and in 26% in pN2, P ¼ 0.594. Distant recurrence was found in 63% of pN0, 60% of pN1 and 64% of pN2, P ¼ 0.795. Specific sites of distant recurrence were also similar, with 49% of distant metastases to the liver and 22% to the lung in pN0 patients, whereas pN1 patients metastasized in 36% to the liver and in 25% to the lung. Multivariate logistic regression analysis revealed R1 resection as the only independent risk factor for local recurrence (OR: 1.040–3.307, P ¼ 0.033). An R1 resection was independently associated with a decreased prevalence of distant recurrence (OR: 0.311–0.963, P ¼ 0.037) (Table 2). Importantly, lymph node status and lymph node ratio were not independently associated with local versus distant recurrence (Table 2).

Propensity-score Matching

Due to the differences between pN0 and pN1 patients, propensity-score matching was performed (see Methods)

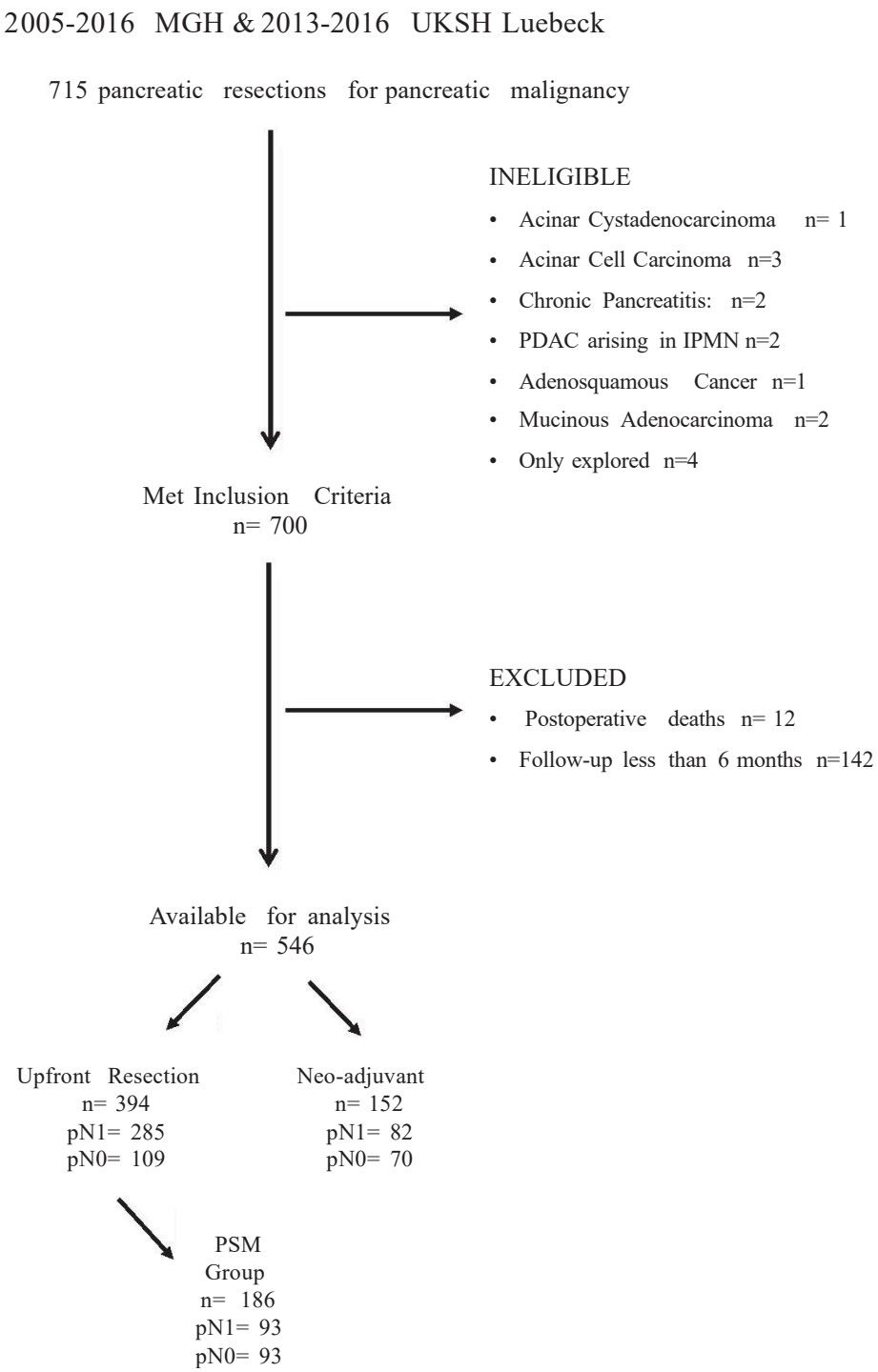

FIGURE 1. Flowchart of study population. IPMN indicates intraductal papillary mucinous neoplasm; MGH, Massachusetts General Hospital; PSM, Propensioty Score Matched; UKSH, University Clinic Schleswig-Holstein.

(Table 3). Clinical characteristics such as age, diabetes, CCI, and pathologic factors such as tumor size and tumor grade did not differ between the groups in propensity-score matching. Despite similar clinicopathologic characteristics, patients with pN0 disease had a longer actual median disease-free survival than patients with pN1 disease (14 vs. 10 mo, P ¼ 0.001). Patterns of recurrence, however, were still similar: 14% to 23% recurred locally and 60% to 72% recurred distantly (P ¼ 0.214 and P ¼ 0.329) (Table 3 and Fig. 2). In multivariate analysis, a preoperative CA-19–9 score more than 37 U/L was the only independent

TABLE 1. Baseline Characteristics of the Upfront Surgery Group

	Total n ¼ 394		pN0 n ¼ 109 (28%)		pN1 n ¼ 285 (72%)		Univariate
	Upfront Surgery Group						
	Median/n	IQR/%	Median/n	IQR/%	Median/n	IQR/%	P
Age in years	68	(61–75)	70	(62–76)	68	(60–75)	0.300
Sex							
Male	208	(53%)	59	(54%)	149	(52%)	0.742
Female	186	(47%)	50	(46%)	136	(48%)	
Preoperative CA 19-9	125	(27–456)	65	(19–363)	140	(39–588)	0.045
Type of surgery							0.063
Whipple	294	(75%)	71	(65%)	223	(78%)	
Distal pancreatectomy	81	(21%)	32	(29%)	49	(17%)	
Total pancreatectomy	13	(3%)	4	(4%)	9	(3%)	
Other	4	(1%)	2	(2%)	2	(1%)	
OR duration, min	330	(253–410)	314	(242–404)	333	(263–411)	0.105
LOS, d	8	(6–13)	8	(6–13)	8	(6–13)	0.326
Complications	255	(65%)	67	(62%)	188	(66%)	0.403
Tumor grade							0.302
G1	25	(6%)	7	(6%)	18	(6%)	
G2	249	(63%)	76	(70%)	173	(61%)	
G3	118	(30%)	26	(24%)	92	(32%)	
G4	2	(1%)	0	(0%)	2	(1%)	
T-stage							0.000
0	0	(0%)	0	(0%)	0	(0%)	
T1	10	(3%)	9	(8%)	1	(1%)	
T2	32	(8%)	20	(18%)	12	(4%)	
T3	338	(86%)	78	(72%)	260	(91%)	
T4	14	(4%)	2	(2%)	12	(4%)	
Tumor size (mm)	30	(24–40)	29	(20–40)	32	(25–40)	0.012
Positive lymph nodes	2	(0–4)	0	(0–0)	3	(2–6)	0.000
Total lymph nodes	18	(13–24)	15	(10–21)	20	(14–25)	0.000
Lymphatic invasion	249	(63%)	40	(37%)	209	(73%)	0.000
Perineural invasion	345	(87%)	80	(73%)	265	(93%)	0.000
Positive margins (○ß1 mm)	202	(51%)	34	(31%)	169	(59%)	0.000
Adjuvant therapy	292	(74%)	78	(72%)	214	(75%)	0.328
Chemotherapy alone	136	(48%)	40	(54%)	96	(46%)	0.473
Chemoradiation	145	(51%)	34	(46%)	111	(53%)	
Radiation alone	2	(1%)	0	(0%)	2	(1%)	

predictor for the occurrence of local or distant metastasis (OR 1.620–10.892, P ¼ 0.003).

Neoadjuvant Patient Group

There were 152 patients who received neoadjuvant treatment at the MGH from January 1, 2009 until December 31, 2014. The most common neoadjuvant regimen consisted of chemotherapy plus radiation (90%), 12 patients received chemotherapy alone (Table 4). Adjuvant therapy was less common than in the upfront resection group with 65% of neoadjuvant patients receiving any type of regimen. Here chemotherapy alone was the most frequent administered therapy (50%), 75 of the 137 (55%) patients who received neoadjuvant chemoradiation were administered adjuvant chemotherapy alone. This cohort was 55% female, but patients were significantly younger (median 64 yrs, P < 0.001) and had lower preresection CA19–9 levels (median 51 U/mL) than those in the upfront resection group. Pathologically patients receiving neoadjuvant therapy had smaller cancers, a lower pN1 rate, and a lower rate of PNI and LVI, than the upfront resectable group. There was 1 patient who had a combined arterial and venous resection.

Interestingly, these trends remained true when comparing patients with pN0 and pN1 disease in the neoadjuvantly treated cohort. Patients with pN0 cancers after receiving neoadjuvant therapy had smaller tumors (median 22 vs. 30 mm, P < 0.001), less lympho-vascular invasion (24% vs. 65% P < 0.001), and perineural invasion (73% vs. 95% P < 0.001) (Table 4) than pN1 patients.

Actual median OS for this cohort from the time of diagnosis was 24 months (13–41). Median OS for the pN0 patients was significantly longer than for the pN1 patients (33 mo (range 20–4
4) vs. 17 mo (range 10–30), P ¼ 0.003). DFS was also significantly longer for pN0 patients than pN1 patients (21 mo (range 11–38) vs. 11 mo (range 8–20), respectively).

Similar to patients who did not receive neoadjuvant therapy patterns of recurrence were similar between pN0 and pN1 patients. Distant recurrence continued to be the most common first site of disease progression (pN0 76% and pN1 64% (P ¼ 0.326)). There was also no difference in specific site of distant recurrence with 38% to the liver and 24% to the lung in pN0 patients and 44% to the liver and 27% to the lung in pN1 patients, respectively. Local recurrence as first site of disease progression occurred in 22% of pN0 patients

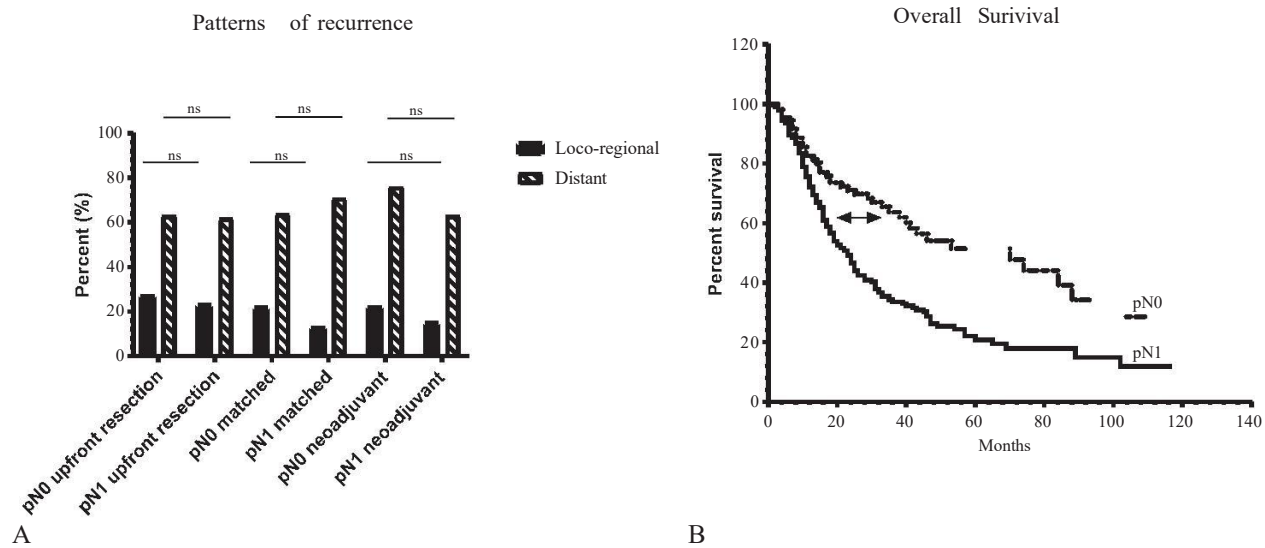

FIGURE 2. A, Patterns of recurrence of all study populations. Ns indicates nonsignificant ($P > 0.05$). B, Overall survival of upfront surgery group divided into pN1 and pN0.

versus 15% of pN1 patients (P ¼ 0.435). Interestingly, clinicopathological parameters were not different between patients who recurred and who did not, as was true for local versus distant metastasis in the neoadjuvant group, therefore no multivariate analysis was performed (Table 3).

DISCUSSION

This is the first report to demonstrate recurrence patterns in resected pN0 versus pN1 PDAC patients who were treatment naive or received neoadjuvant therapy. Unadjusted and adjusted, patterns of recurrence between either upfront resection and those receiving neoadjuvant treatment were strikingly similar, irrespective of nodal involvement, with 60% to 76% presenting with distant metastasis and 14% to 27% with local recurrence (not statistically significant).

Even when pN0 and pN1 patients were matched for clinicopathologic factors, time to recurrence and overall survival continued to be significant between pN0 and pN1 patients, but patterns of recurrence were similar. The basis of this observation is not certain. One possibility is that tumor characteristics (smaller tumor size, lower CA19–9 levels, less perineural invasion, and less lymphovascular invasion) in the pN0 patients may reflect earlier detection. This could represent merely a shift to the right in the curves for recurrence and survival (Fig. 2B). Our neoadjuvant data may support this hypothesis, in that those tumors were significantly downstaged (48% Stage IIa vs. 28% Stage IIa or I), but again the patterns of recurrence continued to be similar. While pN0 patients may have been resected earlier in the biology of the disease, the same proportion of patients presented with symptomatic disease in both the pN0 and pN1 groups. This may highlight that the clinical course does not always represent the true biology of the disease.

An alternate explanation is that patients with pN0 cancers have a different tumor biology than patients with pN1 cancers. Although pN0 tumors were only slightly smaller, they exhibited less aggressive properties such as perineural and lympho-vascular invasion, lower Ca19-9 levels as well as a longer time to metastasis. However, since patients with lung metastases tend to have a more indolent course one would expect that pN0 patients would more commonly present with lung metastases, which they did not.

The results of our upfront resection group are similar to the 692 PDAC patient cohort from Johns Hopkins Hospital who had not received neoadjuvant treatment.[6] Patients presented with distant metastases (58%) more often than isolated local (24%) recurrences as their first site of disease recurrence.

A Japanese study investigated the impact of a positive resection margin (1mm-rule) on recurrence in 117 PDAC patients. Similar to our data, resection margin and nodal status were independent risk factors for a shorter DFS.[18] The authors also reported a significant difference in distant recurrence rates for patients with a positive resection margin. However, the data were not adjusted for confounding variables, and percentages of recurrence were calculated differently.

While nodal disease did not seem to be a good surrogate marker for patterns of recurrence in our patient cohort, our data clearly demonstrate that distant recurrence is the most frequent site of recurrence irrespective of the patient's tumor stage. Even in patients with node negative disease more than half of the patients recur at a median of 16 months. This suggests that PDAC is a systemic disease, which needs to be controlled with effective chemotherapy, and that local modalities such as an operation or radiation therapy are not sufficient to control the disease. While positive resection margins were an independent risk factor for local recurrence in our upfront resected group, it failed to have any impact in our neoadjuvant group. However, the neoadjuvant group did receive upfront radiation therapy. Based on these results, as well as other published data, our group has moved to administering effective neoadjuvant chemotherapy, followed by radiation therapy for borderline resectable disease, followed by surgical resection of the PDAC.

Our study has several limitations. This is a retrospective study and recurrences were identified radiologically, without pathological verification. Since the patients were not phase III clinical trial patients, there was some provider variability regarding postoperative

TABLE2. Outcome Data and Multivariate Analysis of Upfront Surgery Group

	Total n ¼ 394		pN0 n ¼ 109		pN1 n ¼ 285		Univariate
Follow-up Data-upfront Surgery Group							
	Median/n	IQR/%	Median/n	IQR/%	Median/n	IQR/%	P
Overall survival	18	(10–34)	25	(11–45)	16	(10–29)	0.000
Disease-free survival	11	(6–23)	16	(7–36)	10	(5–20)	0.000
Recurrence	278	(71%)	60	(55%)	218	(77%)	0.000
Local	66	(24%)	16	(27%)	50	(23%)	0.672
Distant	172	(62%)	37	(63%)	135	(62%)	0.553
Lung only	42	(24%)	8	(22%)	34	(25%)	0.718
Liver only	67	(39%)	18	(49%)	49	(36%)	
Lung and Liver	11	(6%)	1	(3%)	10	(7%)	
Other/multiple distant sites	52	(30%)	10	(27%)	42	(32%)	
Both	38	(14%)	6	(10%)	32	(15%)	0.292

Recurrence

Univariate (Chi-square)		Multivariate (Logistic Regression)	
Factor	P	OR	P
Age	0.136		0.730
Sex	0.103		0.203
Type of Pancreatectomy	0.976		not included
pN1	0.000	1.349–4.496	0.003
Lymph-node ratio <0.3	0.004	1.415–16.398	0.012
Preoperative CA 19-9 <37 U/mL	0.043		0.061
T-status	0.022		0.464
R0	0.014		0.203
Adjuvant Chemoradiation	0.143		not included
Adjuvant Chemotherapy alone	0.759		not included

Local Recurrence

Univariate (Chi-square)		Multivariate (Logistic Regression)	
Factor	P	OR	P
Age	0.372		0.221
Sex	0.259		0.225
Type of Pancreatectomy	0.649		Not included
pN1	0.548		Not included
Lymph node rate <0.3	0.503		Not included
Preoperative CA 19-9 <37 U/mL	0.795		Not included
T-status	0.456		not included
R1	0.035	1.040–3.307	0.033
Adjuvant Chemoradiation	0.810		not included
Adjuvant Chemotherapy alone	0.742		not included

Distant Recurrence

Univariate (Chi-square)		Multivariate (Logistic Regression)	
Factor	P	OR	P
Age	0.863		0.912
Sex	0.459		0.853
Type of Pancreatectomy	0.646		Not included
pN1	0.971		Not included
Lymph node rate <0.3	0.793		Not included
Preoperative CA 19-9 <37 U/mL	0.548		Not included
T-status	0.436		Not included
R1	0.005	0.306–0.958	0.005
Adjuvant Chemoradiation	0.626		Not included
Adjuvant Chemotherapy alone	0.467		Not included

imaging. Most patients were evaluated and had a CA19-9 level every 3 months, and underwent imaging every 6 months. A change in Ca19-9 value or symptoms, depending on the provider, did increase the frequency of imaging. The timing of postoperative imaging does affect the detection of recurrence. This should be taken into consideration, when evaluating the results.

Our study is the largest multi-institutional study to examine patterns of recurrence after potentially curative resection of PDAC in

TABLE3. Baseline Characteristics and Outcome Parameters of the Propensity Matched Group

	Total n ¼ 186		pN0 n ¼ 93		pN1 n ¼ 93		Univariate
Baseline Characteristics			of Upfront Resection Propensity Matched Group				
	Median/n	IQR/%	Median/n	IQR/%	Median/n	IQR/%	P
Age at operation, yrs	70	(62–77)	71	(63–76)	68	(62–78)	0.537
Sex (male)	102	(55%)	49	(53%)	53	(57%)	0.556
Type of surgery							0.086
Whipple	133	(72%)	60	(65%)	73	(78%)	
Distal pancreatectomy	42	(23%)	28	(30%)	14	(15%)	
Total pancreatectomy	6	(3%)	2	(2%)	4	(4%)	
Other	4	(2%)	2	(2%)	2	(2%)	
Tumor pathology							
Grade							0.262
1	9	(5%)	5	(5%)	4	(5%)	
2	125	(66%)	67	(72%)	58	(62%)	
3	52	(29%)	21	(23%)	32	(34%)	
4	0	(0%)	0	(0%)	0	(0%)	
T-stage							0.850
1	3	(2%)	2	(2%)	1	(1%)	
2	25	(13%)	14	(15%)	11	(12%)	
3	154	(83%)	75	(81%)	79	(85%)	
4	4	(2%)	2	(2%)	2	(2%)	
Tumor size (mm)	30	(20–40)	26	(20–40)	30	(21–40)	0.266
Lymphatic invasion	94	(51%)	34	(37%)	60	(65%)	0.000
Perineural invasion	160	(86%)	73	(79%)	87	(94%)	0.003
Positive margins (○≤1 mm)	62	(33%)	23	(24%)	39	(44%)	0.013
Adjuvant therapy	127	(68%)	66	(71%)	61	(66%)	0.387
Chemotherapy alone	70	(55%)	39	(59%)	31	(51%)	0.634
Chemoradiation	57	(45%)	27	(41%)	30	(49%)	
Radiation alone	0	(0%)	0	(0%)	0	(0%)	

	Total n ¼ 186		pN0 n ¼ 93		pN1 n ¼ 93		Log-rank Test
Follow-up							
Factor	Median/N	95%KI/%	Median/N	95%KI /%	Median/N	95%KI/%	P
Overall survival	17	(10–34)	25	(11–45)	15	(10–26)	0.000
Disease-free survival	12	(7–27)	14	(7–34)	10	(6–18)	0.001
Recurrence	119	(64%)	53	(57%)	66	(71%)	0.077
Local	21	(18%)	12	(23%)	9	(14%)	0.214
Distant	79	(66%)	33	(60%)	46	(72%)	0.329
Both	17	(14%)	7	(8%)	9	(10%)	0.920

Recurrence			
Univariate Analysis (Chi-square)		Multivariate Analysis (Logistic Regression)	
Factor	P	OR	P
Age	0.503		0.337
Sex	0.093		0.223
Type of pancreatectomy	0.909		Not included
pN1	0.016		0.142
Lymph node rate <0.3	0.068		Not included
Preoperative CA 19–9 >37 U/ml	0.002	1.620–10.892	0.003
T-status	0.528		Not included
R1	0.305		Not included
Adjuvant chemoradiation	0.566		Not included
Adjuvant chemotherapy alone	0.420		Not included

lymph node-positive and node-negative patients. Although the time to recurrence and overall survival were significantly longer for pN0 as compared with pN1 cancers, there was no corresponding difference in site of first recurrence. The implications for clinical practice are 2-fold. Irrespective of nodal involvement, an R1 resection was independently associated with an increased likelihood of local recurrence, suggesting a possible benefit of local tumor therapy in addition to systemic therapy. Second, both pN0 and pN1 patients are more likely to present with distant metastatic disease. These findings emphasize the need for potent systemic therapy in the perioperative setting. Pancreatic adenocarcinoma continues to be best treated with multimodality therapy.

TABLE4. Baseline Characteristics and Outcome Parameters of the Neoadjuvant Group

	Total n ¼ 152		pN0 n ¼ 70 (46%)		pN1 n ¼ 82 (54%)		Univariate
	Neoadjuvant Group						
	Median/n	IQR/%	Median/n	IQR/%	Median/n	IQR/%	P
Age, yrs	64	(57–72)	64	(58–71)	64	(57–73)	0.996
Sex							0.465
Male	70	(46%)	30	(43%)	40	(49%)	
Female	82	(54%)	40	(57%)	42	(51%)	
Preop. CA 19-9	51	(14–120)	36	(14–96)	69	(13–208)	0.219
OR duration, min	353	(296–423)	357	(296–431)	346	(295–420)	0.426
LOS, d	7	(6–9)	6	(5–9)	7	(6–8)	0.670
Grade							0.005
0	2	(1%)	2	(3%)	0	(0%)	
G1	8	(5%)	5	(7%)	3	(4%)	
G2	89	(59%)	45	(64%)	44	(54%)	
G3	45	(30%)	10	(14%)	35	(43%)	
X	8	(5%)	8	(11%)	0	(0%)	
T-stage							0.004
0	3	(2%)	3	(4%)	0	(0%)	
T1	10	(6%)	9	(13%)	1	(1%)	
T2	21	(14%)	11	(16%)	10	(12%)	
T3	118	(78%)	47	(67%)	71	(87%)	
T4	0	(0%)	0	(0%)	0	(0%)	
Tumor size (mm)	25	(20–35)	22	(15–31)	30	(23–38)	0.000
Positive lymphn.	1	(0–3)	0	(0–0)	2	(1–4)	0.000
Total lymph nodes	19	(15–23)	18	(14–22)	19	(15–25)	0.174
Lymphatic invasion	71	(46%)	17	(24%)	54	(65%)	0.000
Perineural invasion	131	(85%)	52	(73%)	79	(95%)	0.000
Positive margins	53	(35%)	24	(34%)	29	(35%)	0.889
Adjuvant therapy	100	(65%)	40	(40%)	60	(60%)	0.056
Chemo alone	77	(78%)	33	(83%)	45	(75%)	0.077
Chemoradiation	19	(19%)	5	(13%)	14	(23%)	
Radiation alone	1	(1%)	1	(3%)	0	(0%)	
Neoadjuvant regimen	152	(100%)	70	(100%)	82	(100%)	0.075
Chemotherapy alone	12	(8%)	6	(10%)	6	(4%)	
Chemoradiation	137	(90%)	63	(88%)	74	(89%)	
Radiation alone	0	(0%)	0	(2%)	0	(0%)	

	Total n ¼ 152		pN0 n ¼ 70		pN1 n ¼ 82		Univariate
	Follow-up Data						
	Median/n	IQR/%	Median/n	IQR/%	Median/n	IQR/%	P
Overall survival	24	(13–41)	33	(20–44)	17	(10–30)	0.003
Disease-free survival	15	(9–27)	21	(11–38)	11	(5–20)	0.000
Recurrence	110	(72%)	45	(64%)	64	(78%)	0.040
Local	20	(18%)	10	(22%)	10	(15%)	0.435
Distant	75	(68%)	34	(76%)	41	(64%)	0.326
Lung only	19	(25%)	8	(24%)	11	(27%)	0.568
Liver only	31	(41%)	13	(38%)	18	(44%)	
Lung and liver	3	(4%)	2	(6%)	1	(2%)	
Other/multiple sites	22	(29%)	11	(32%)	11	(27%)	
Local and distant	13	(12%)	1	(2%)	12	(18%)	0.014

	Recurrence	Local Recurrence	Distant Recurrence
	Univariate (Chi-square)		
Factor	P	P	P
Age	0.339	0.653	0.727
Sex	0.943	0.115	0.508
Type of pancreatectomy	0.913	0.873	0.624
pN1	0.060	0.381	0.202
Lymph-node ratio <0.3	0.443	0.335	0.884
Preoperative CA 19-9 <37 U/mL	0.572	0.381	0.391
T-status	0.199	0.932	0.771
R0	0.451	0.172	0.279
Adjuvant Chemoradiation	0.808	0.381	0.444
Adjuvant Chemotherapy alone	0.454	0.205	0.222

Preop. indicates preoperative; OR, operation; LOS, length of stay.

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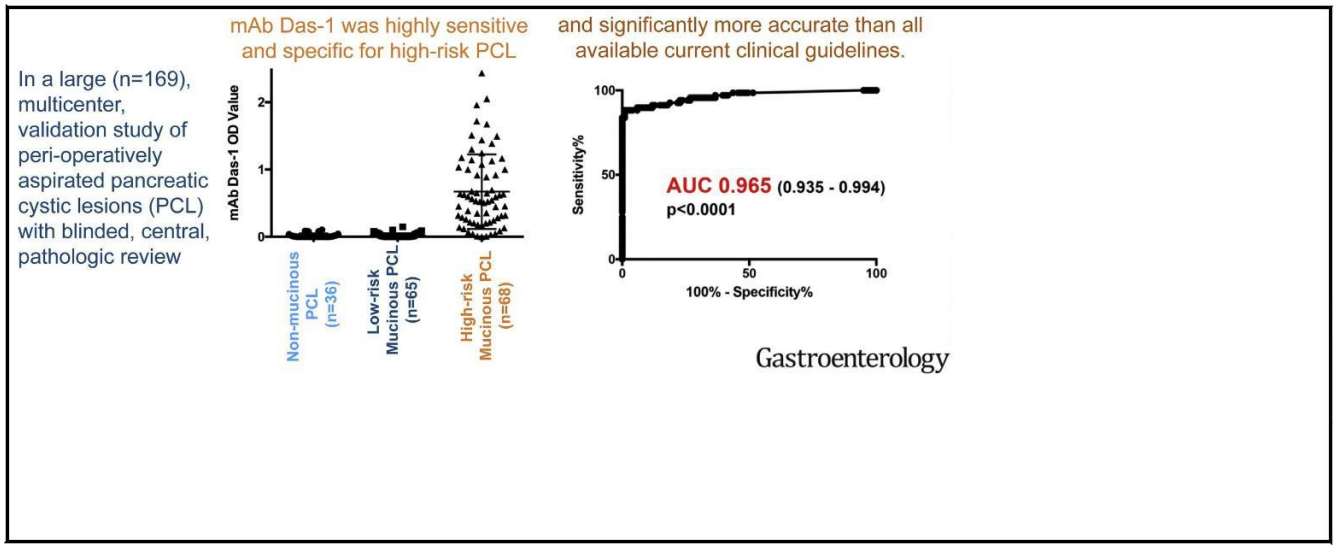
Gastroenterology 2019;157:720–730

CLINICAL—PANCREAS

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

Koushik K. Das,[1] Xin Geng,[2] Jeffrey W. Brown,[1] Vicente Morales-Oyarvide,[3] Tiffany Huynh,[4] Ilaria Pergolini,[3] Martha B. Pitman,[4] Cristina Ferrone,[3] Mohammad Al Efishat,[5] Dana Haviland,[5] Elizabeth Thompson,[6] Christopher Wolfgang,[7] Anne Marie Lennon,[8] Peter Allen,[5] Keith D. Lillemoe,[3] Ryan C. Fields,[9] William G. Hawkins,[9] Jingxia Liu,[9] Carlos Fernandez-del Castillo,[3] Kiron M. Das,[2] and Mari Mino-Kenudson[4]

[1]Division of Gastroenterology, Washington University, St Louis, Missouri; [2]Division of Gastroenterology, Rutgers-Robert Wood Johnson Medical School, New Brunswick, New Jersey; [3]Department of Surgery, Massachusetts General Hospital, Boston, Massachusetts; [4]Deparment of Pathology, Massachusetts General Hospital, Boston, Massachusetts; [5]Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, New York; [6]Department of Pathology, Johns Hopkins School of Medicine, Baltimore, Maryland; [7]Department of Surgery Johns Hopkins School of Medicine, Baltimore, Maryland; [8]Division of Gastroenterology, Johns Hopkins School of Medicine, Baltimore, Maryland; and [9]Department of Surgery, Washington University, St Louis, Missouri



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 ¼ .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.

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.

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 (n¼169), 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.

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

0016-5085/$36.00
https://doi.org/10.1053/j.gastro.2019.05.014

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 ¼ 94], Johns Hopkins University [n ¼ 46], Memorial Sloan Kettering Cancer Center [n ¼ 37], and Washington University [n ¼ 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.

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.

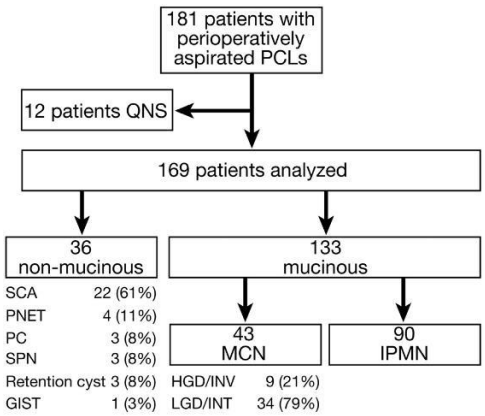


Figure 1. Flow diagram of patients evaluated. GIST, gastrointestinal stromal tumor; HGD/INV, high-grade dysplasia/invasive carcinoma; LGD/INT, low-grade/intermediate-grade dysplasia; MCN, mucinous cystic neoplasm; PC, pseudocyst; PNET, pancreatic neuroendocrine tumor; QNS, quantity not sufficient; SCA,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

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).[2,6,27] Because of the high sensitivity and specificity of mAb Das-1, which led to a small number of false positives (n ¼ 1) and negatives (n ¼ 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 ¼ 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 mg/mL (interquartile range, 1.61–9.56 mg/mL) 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 mg protein) with <150 mL cyst fluid.

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

Cyst fluid from non-mucinous PCLs (n ¼ 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 ¼ 65) had minimal reactivity (OD, 0.019 ± 0.034). Conversely, high-risk IPMN and MCN lesions (n ¼ 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 2A). 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 2B).

Table 1. Patient and Cyst Characteristics

Characteristic	All samples (n ¼ 169)	IPMNs (n ¼ 90)	MCNs (n ¼ 43)	Non-mucinous cystic lesions (n ¼ 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)

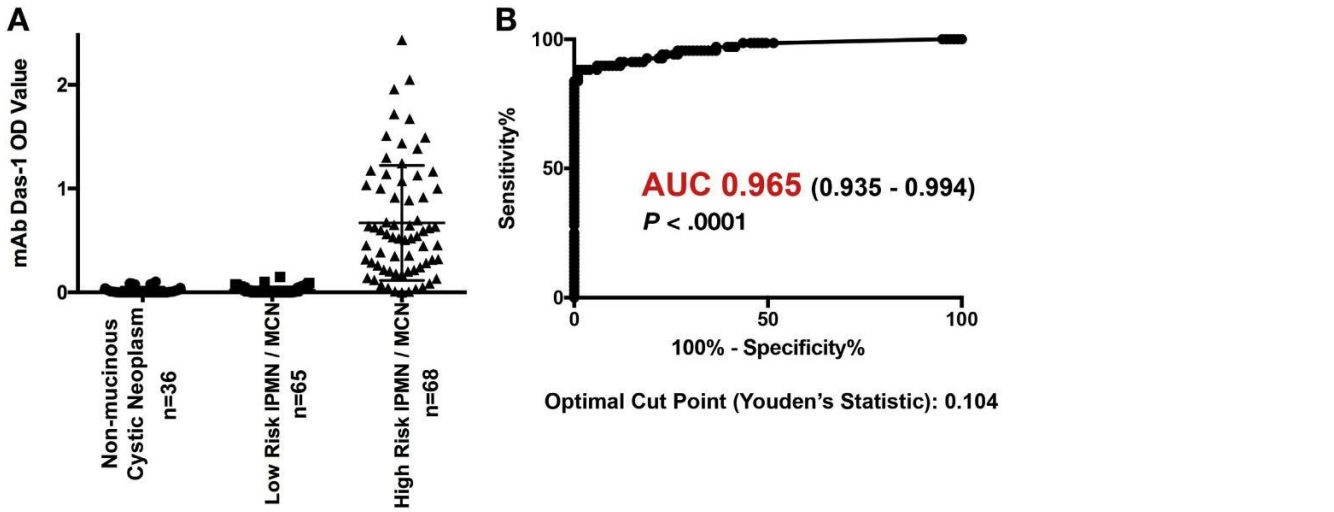


Figure 2. mAb Das-1 is highly sensitive and specific for high-risk pancreatic cystic lesions. (A) Cyst fluid immunoreactivity against mAb Das-1 by ELISA. OD values as determined by ELISA in high-risk IPMNs (invasive IPMN, HGD of any epithelial subtype, and IGD of intestinal subtype) and MCNs with HGD (n ¼ 68), low-risk IPMNs and MCNs (n ¼ 65), non-mucinous cystic neoplasms (serous cyst adenoma, pancreatic neuroendocrine tumors, pseudocysts, solid pseudopapillary neoplasm, retention cysts, and gastrointestinal stromal tumor) (n ¼ 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 ¼ 31) had an OD of 0.025 ± 0.042, non-invasive high-risk IPMN (n ¼ 41) had an OD of 0.610 ± 0.493 ($P < .0001$), and IPMN with an invasive component (n ¼ 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 ¼ 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 ¼ 14; OD, 0.636 ± 0.486) ($P < .0001$) or IPMNs of any type with HGD or with associated invasive carcinoma (n ¼ 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 ¼ 34) were non-reactive (OD, 0.014 ± 0.023) in comparison to MCNs with HGD (n ¼ 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 ¼ 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 ¼ 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 AGAguidelines 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 AGAguidelines, 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 ¼ .0009), any symptoms (OR, 2.01; 95% CI, 1.08–3.76; P ¼ .0280), jaundice (OR, 2.41; 95% CI, 1.11–5.23; P ¼ .0260), and the composite risk indicator (OR, 3.81; 95% CI, 1.63–8.86; P ¼.0013) (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 ¼ 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 ¼ 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 mL cyst fluid, and 94% could be completed with 500 mL. 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.

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Author names in bold designate shared co-first authorship.

Received September 3, 2018. Accepted May 13, 2019.

Reprint requests
Address requests for reprints to: Koushik K. Das, MD, Division of Gastroenterology, Washington University School of Medicine, 660 South Euclid Avenue, Campus Box 8124, St Louis, Missouri 63110. e-mail: k.das@wustl.edu; fax: 314-454-5005; or Mari Mino-Kenudson, MD, Department of Pathology, Harvard Medical School, Massachusetts General Hospital, 55 Fruit Street, Warren 219, Boston, Massachusetts 02114. e-mail: mminokenudson@partners.org; fax: 617-726-7474.

Acknowledgments
The authors would like to thank Timothy Heeren, Professor of Biostatistics at the Boston University School of Public Health, for his invaluable assistance in the statistical analysis and preparation of this manuscript. Data from this study were presented in part at the American Gastroenterological Association, Digestive Disease Week 2017 on May 8, 2017 in Chicago, IL. Author contributions: KKD, XG, JWB, CFC, KMD, MM-K were all involved in the study concept and design, acquisition of data, analysis and interpretation of data, and drafting of the manuscript. VM-O, TH, IP, MBP, CF, MAE, DH, ET, CW, AML, PA, KDL, RCF, WGH were involved in acquisition of data. JL was involved in statistical analysis of the data. Kiron M. Das and Mari Mino-Kenudson contributed equally to this work.

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.

Funding
This work was supported by the American Society for Gastrointestinal Endoscopy (KKD), National Pancreas Foundation (KMD), National Institutes of Health (NIH)/National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)T32DK007130-41 and Digestive Diseases Research Core Centers Pilot & Feasibility Grant as part of P30 DK052574 (JWB), NIH/National Cancer Institute P50CA196510-01A1 Washington University Specialized Program of Research Excellence in Pancreatic Cancer, and NIH/NIDDK P30 DK052574 Digestive Diseases Research Core Centers. Development of mAb Das-1 was supported in part by research grants NIDDK, R01 DK47673 and R01 DK63618 to KMD.

Predictors of Resectability and Survival in Patients with Borderline and Locally Advanced Pancreatic Cancer who Underwent Neoadjuvant Treatment with FOLFIRINOX

Theodoros Michelakos, MD,[Ã] Ilaria Pergolini, MD,[Ã] Carlos Fernández-del Castillo, MD,[Ã] Kim C. Honselmann, MD,[Ã] Lei Cai, MD, PhD,[Ã] Vikram Deshpande, MD,[y] Jennifer Y. Wo, MD,[z] David P. Ryan, MD,[§] Jill N. Allen, MD,[§] Lawrence S. Blaszkowsky, MD,[§] Jeffrey W. Clark, MD,[§] Janet E. Murphy, MD, MPH,[§] Ryan D. Nipp, MD,[§] Aparna Parikh, MD,[§] Motaz Qadan, MD, PhD,[Ã] Andrew L. Warshaw, MD,[Ã] Theodore S. Hong, MD,[z] Keith D. Lillemoe, MD,[Ã] and Cristina R. Ferrone, MD[Ã]

Objective: The aim of this study was to determine (1) whether preoperative factors can predict resectability of borderline resectable (BR) and locally advanced (LA) pancreatic ductal adenocarcinoma (PDAC) after neoadjuvant FOLFIRINOX, (2) which patients might benefit from adjuvant therapy, and (3) survival differences between resected BR/LA patients who received neoadjuvant FOLFIRINOX and upfront resected patients.

Background: Patients with BR/LA PDAC are often treated with FOLFIRINOX to obtain a margin-negative resection, yet selection of patients for resection remains challenging.

Methods: Clinicopathologic data of PDAC patients surgically explored between 04/2011-11/2016 in a single institution were retrospectively collected.

Results: Following neoadjuvant FOLFIRINOX, 141 patients were surgically explored (BR: 49%, LA: 51%) and 110 (78%) were resected. Resected patients had lower preoperative CA 19-9 levels (21 vs 40U/mL, P ¼ 0.03) and smaller tumors on preoperative computed tomography (CT) scan (2.3 vs 3.0 cm, P ¼ 0.03), but no predictors of resectability were identified. Median overall survival (OS) was 34.2 months from diagnosis for all FOLFIRINOX patients and 37.7 months for resected patients. Among resected patients, preoperative CA 19-9 >100 U/mL and >8 months between diagnosis and surgery predicted a shorter postoperative disease-free survival (DFS); Charlson comorbidity index >1, preoperative CA 19-9 >100 U/mL and tumor size (>3.0 cm on CT or >2.5 cm on pathology) predicted decreased OS. DFS and OS were significantly better for BR/LA PDAC patients treated with neoadjuvant FOLFIRINOX compared with upfront resected patients (DFS: 29.1 vs 13.7, P < 0.001; OS: 37.7 vs 25.1 months from diagnosis, P ¼ 0.01).

Conclusion: BR/LA PDAC patients with no progression on neoadjuvant FOLFIRINOX should be offered surgical exploration. Except size, traditional pathological parameters fail to predict survival among resected FOLFIRINOX patients. Resected FOLFIRINOX patients have survival that appears to be superior than that of resectable patients who go directly to surgery.

Keywords: FOLFIRINOX, neoadjuvant therapy, pancreatic cancer

(Ann Surg 2017;xx:xxx–xxx)

Over 30% of the 53,670 patients who will present with pancreatic adenocarcinoma (PDAC) in 2017 will present with borderline resectable (BR) or locally advanced (LA) disease.[1] Historically, this cohort of patients survived 11 months from the time of diagnosis[2] and only a small subset (19% to 30%) was downstaged with chemoradiation, although many of them did not have a margin-negative resection.[2–5] Patients who undergo a resection with a macroscopically positive margin have survival comparable to that of patients who do not undergo an operation.[6,7] On the contrary, patients who are resected with a >1 mm negative resection margin have a significantly longer survival than patients with an R1 resection margin or those who are not resected.[8] This has led to an increased use of neoadjuvant therapy in BR and LA disease with the goal of obtaining a margin-negative resection.

With the encouraging results in the metastatic setting, FOLFIRINOX (5-FU, oxaliplatin, irinotecan) and gemcitabine/nab-paclitaxel have been utilized in patients with BR/LA disease,[9] although no clinical trials have been completed comparing modern neoadjuvant regimens in the setting of BR/LA PDAC. In this group of patients, particularly in LA disease, chemotherapy is often followed by chemoradiation to optimize downstaging before surgical resection is attempted.[10,11] Yet, despite this intensive neoadjuvant regimen, patient selection for resection continues to be difficult, as radiologic imaging is no longer able to predict resectability.[12]

This study reports one of the largest series to date of BR/LA PDAC patients who received FOLFIRINOX before an attempted resection. Our first aim was to determine whether preoperative clinical factors are able to predict which BR/LA PDAC patients who have received FOLFIRINOX will become surgically resectable. Our second aim was to assess whether any preoperative, intraoperative, and postoperative clinicopathologic factors could predict early recurrence, as these patients might benefit from adjuvant therapy. The third aim was to compare clinicopathologic factors, disease-free survival (DFS), and overall survival (OS) between resected BR/LA PDAC patients who received neoadjuvant FOLFIRINOX and contemporary resectable patients who underwent upfront resection.

From the [Ã]Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA; [y]Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, MA; [z]Department of Radiation Oncology, Massachusetts General Hospital, Harvard Medical School, Boston, MA; and [§]Department of Medical Oncology, Massachusetts General Hospital, Harvard Medical School, Boston, MA.

We have no conflicts of interest. We have no financial disclosures.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.annalsofsurgery.com).

Reprints: Cristina R. Ferrone, MD, Associate Professor of Surgery, Harvard Medical School, Department of Surgery, Massachusetts General Hospital, 15 Parkman Street, Boston, MA 02114-3117.
E-mail: cferrone@mgh.harvard.edu.

ISSN: 0003-4932/16/XXXX-0001
DOI: 10.1097/SLA.0000000000002600

METHODS

Inclusion Criteria

Patients who were surgically explored for pancreatic ductal adenocarcinoma with or without neoadjuvant treatment with FOLFIRINOX between April 1, 2011, and November 30, 2016, were included in this study. Of note, data on the initial 40 (28%) patients of this series have been previously reported.[12]

All patients were reviewed at our gastrointestinal oncology multidisciplinary conference (MDC) to assess for resectability and the suitability for neoadjuvant treatment in the case of BR or LA disease. This tumor board includes 2 or more pancreatic surgeons, 1 or more gastrointestinal radiologists, 2 or more medical oncologists, and 1 or more gastrointestinal radiation oncologists. For patients who had received part of their neoadjuvant therapy before being referred to MGH, the stage of the disease was determined by reviewing the imaging at MDC performed at the time of diagnosis. Patients with distant metastasis were excluded. Patients who received neoadjuvant FOLFIRINOX but did not undergo an operation because they progressed, died, or continued their care elsewhere were not included. As a result, this study does not represent an intention-to-treat analysis. LA and borderline lesions were defined according to the Americas Hepato-Pancreato-Biliary Association (AHPBA)/Society of Surgical Oncology (SSO)/Society for Surgery of the Alimentary Tract (SSAT) consensus criteria.[13] Patients were considered LA if there was long segment occlusion of the mesenteric vein/portal vein, more than 1808 involvement of the superior mesenteric artery (SMA) or involvement of the hepatic artery or celiac trunk. BR lesions included lesions with tumor abutment and short segment occlusion of the mesenteric vein/portal vein, gastroduodenal artery encasement up to the hepatic artery without extension to the celiac axis, or <1808 of tumor abutment of the SMA. All patients who had no evidence of metastatic disease after neoadjuvant FOLFIRINOX were surgically explored. Preoperatively, patients did not routinely undergo magnetic resonance imaging with diffusion-weighted sequences or 18-FDG PET scans to detect metastatic disease; a subgroup of patients were laparoscopically explored before laparotomy based on the surgeon's preference. The study was approved by the institutional review board.

Neoadjuvant Regimen

FOLFIRINOX was dosed as previously described,[9] with 5-FU administered as a bolus of 400 mg/m^2, bolus leucovorin 400 mg/m^2, followed by continuous infusion at 1200 mg/m^2 per day for 46 hours, oxaliplatin 85 mg/m^2, and irinotecan 180 mg/m^2. Chemoradiation following FOLFIRINOX, when administered, consisted of 5-FU or capecitabine with 50.4 Gy photon or 25 Gy proton radiotherapy.

Data Collection

Clinicopathologic data were retrospectively collected from a prospectively maintained database as well as the electronic medical record. Variables included date of birth, sex, race (grouped as Caucasian, African-American, Hispanic, and Asian), the Charlson comorbidity index, body mass index (BMI) at diagnosis, surgery and 3 months postoperatively, baseline, post-neoadjuvant and recurrence CA 19–9 levels (U/mL), and size of the tumor on preoperative CT and post-neoadjuvant CT (cm). Regarding the surgical procedure, type of surgery (pancreatoduodenectomy, distal, or total pancreatectomy), estimated intraoperative blood loss (EBL) (mL), intraoperative radiation therapy (IORT), and duration of surgery (minutes) were collected. Pathologic data acquired included tumor grade, tumor size (cm), TNM classification, number of resected and positive nodes, resection margins, perineural invasion, lymphovascular invasion, and American Joint Committee on Cancer stage. A margin wider than 1 mm was considered negative. We also analyzed postoperative outcomes and treatment measures, including hospital length of stay (days), postoperative complications according to Clavien-Dindo classification, postoperative chemo- and radiation therapy, and date and site of progression. Follow-up was obtained from all patients and was updated until March 31, 2017. DFS and OS were calculated from both the date of diagnosis and the date of operation to the date of progression or death, respectively (event), or to the date of the last follow-up visit (censored). Date of death was obtained either from the medical records or from the Social Security Death Index.

Statistics

Continuous data are expressed as median and interquartile range (IQR), whereas categorical data are expressed as frequencies and percentages. Comparison of categorical variables among groups was performed using Fisher exact test. Comparison of continuous variables between or within groups was performed using the Mann-Whitney U test or Wilcoxon signed rank test, as appropriate. Optimal cutoff values were determined using the receiver operating characteristics curve (ROC) analysis.

Follow-up duration was calculated from the time of diagnosis or operation to the time of death or last follow-up taking into account both dead and censored cases. Survival was calculated from the time of diagnosis or the time of operation to the time of disease progression (DFS), death (OS), or last follow-up (censored). Survival curves were plotted using the Kaplan-Meier method. Differences in DFS and OS between groups were analyzed by the log-rank test. Multivariate survival analyses were performed using the backward conditional Cox regression method. Survival analyses were performed using data available at 3 distinct points of care: at diagnosis, preoperatively (after completion of neoadjuvant therapy), and postoperatively. $P < 0.05$ was considered statistically significant. All tests used were 2-tailed. Statistical analysis was performed with the IBM SPSS Statistics software for Windows, Version 20.0 (IBM Corp., Armonk, NY).

RESULTS

FOLFIRINOX Cohort

A total of 141 patients were surgically explored after receiving FOLFIRINOX chemotherapy for LA or BR PDAC (Table 1). Their median age at the time of operation was 63 years (IQR 58 to 69), half of them were female (49%), and the majority were Caucasian (91%). The median Charlson comorbidity index was 1 (IQR 0 to 2). At diagnosis, half of the patients were deemed LA (51%), and the other half BR (49%) by our multi-disciplinary tumor board. Most of the patients received radiation with either photons (50.4 Gy) (79%), or short course protons (25 Gy) (11%) (Fig. 1). The median number of completed FOLFIRINOX cycles was 8 (IQR 6 to 8). Twenty-two (16%) patients demonstrated grade ≥3 toxicity during FOLFIRINOX therapy. The median time from diagnosis to operation was 7.8 months (IQR 6.6 to 9.2).

Effect of Neoadjuvant FOLFIRINOX Therapy on CA 19–9, Tumor Size, and BMI

A decrease in CA 19–9 from 145 U/mL (IQR 28 to 500) to 26 U/mL (IQR 11 to 71) post-FOLFIRINOX was observed ($P < 0.001$). Tumor size decreased from 3.1 cm (IQR 2.6 to 4.0) to 2.4 cm (IQR 1.6 to 3.5) after neoadjuvant therapy ($P < 0.001$). BMI was also lower post-FOLFIRINOX than at the time of diagnosis [25.6 kg/m^2 (IQR 22.3 to 30.1) vs 24.3 (IQR 21.6 to 28.6), $P < 0.001$].

TABLE 1. Demographic, Clinicopathologic, and Operative Characteristics of 141 Patients who Underwent Surgical Exploration After Receiving FOLFIRINOXChemotherapy for Locally Advanced or Borderline Resectable PDAC

	Not Resected N ¼ 31				Resected N ¼ 110				Total N ¼ 141				
	N	%	Median	IQR	N	%	Median	IQR	N	%	Median	IQR	P
Demographic and clinical characteristics													
Age at operation, yrs			66	58–71			63	57–68			63	58–69	0.29
Gender													
Male	21	67.7			51	46.4			72	51.1			0.04
Female	10	32.3			59	53.6			69	48.9			
Race													
White	28	90.3			100	90.9			128	90.8			0.79
Asian	1	3.2			3	2.7			4	2.8			
Hispanic	1	3.2			3	2.7			4	2.8			
Other	1	3.2			4	3.7			5	3.6			
Charlson comorbidity index			1	0–2			1	0–2			1	0–2	0.37
BMI, kg/m^2													
At diagnosis			24.4	22.3–27.1			25.7	22.3–30.3			25.6	22.3–30.1	0.33
At surgery			23.6	20.4–26.5			24.4	21.7–28.9			24.3	21.6–28.6	0.13
Serum CA 19–9, U/mL													
At diagnosis			121	58–436			146	28–500			145	28–500	0.84
Post-neoadjuvant therapy			40	20–228			21	10–56			26	11–71	0.03
Tumor and pathologic characteristics													
Preoperative classification													
Locally advanced	12	38.7			57	51.8			69	48.9			0.23
Borderline	19	61.3			53	48.2			72	51.1			
Tumor size, cm													
Imaging pre-neoadjuvant			3.0	2.5–4.8			3.2	2.7–4.0			3.1	2.6–4.0	0.94
Imaging post-neoadjuvant			3.0	2.0–4.1			2.3	1.5–3.3			2.4	1.6–3.5	0.03
Pathology postoperatively			N/A				2.5	1.9–3.5			2.5	1.9–3.5	N/A
Positive lymph nodes	N/A				40	36.4			40	36.4			N/A
Lymphatic invasion	N/A				30	27.3			30	27.3			N/A
Perineural invasion	N/A				73	67.0			73	67.0			N/A
Negative margins (>1 mm)	N/A				87	80.6			87	80.6			N/A
Operative characteristics													
Operation type													
None	6	19.4			0	0.0			6	4.3			<0.001
Pancreaticoduodenectomy	0	0.0			80	72.7			80	56.7			
Distal pancreatectomy	0	0.0			29	26.4			29	20.6			
Total pancreatectomy	0	0.0			1	0.9			1	0.7			
Gastrojejunostomy	25	80.6			0	0.0			25	17.7			
IORT	14	45.1			36	32.7			50	35.5			0.28
EBL, mL			50	5–150			600	400–800			500	250–800	<0.001
OR duration, min			132	106–218			376	310–444			358	252–421	<0.001
Postoperative characteristics													
LOS, d			4	4–6			6	5–7			6	5–7	<0.001
Clavien-Dindo at 90 d ○83	8	25.8			15	13.7			23	16.3			
Readmission at 90 d	2	6.5			19	17.3			21	14.9			0.37
30-day postoperative mortality	2	6.5			0	0.0			2	1.4			0.05

P values derived from Fisher exact test or Mann-Whitney U test as appropriate.
BMI indicates body mass index; EBL, estimated blood loss; IORT, intraoperative radiation therapy; IQR, interquartile range; LOS, length of stay.

Preoperative Predictors of Resectability

Among the FOLFIRINOX patients who were surgically explored, 110 (78%) were resected, while 31 (22%) were deemed unresectable. The patients who were not resected had either distant (hepatic/peritoneal) metastatic disease identified intraoperatively (20, 14%) or were truly LA due to vascular involvement, most commonly extensive involvement of the common hepatic artery or complete occlusion of the portal vein with significant collaterals (11, 8%). A gastrojejunostomy was performed in the majority (81%) of patients who were not resected, while the remaining underwent only laparoscopy (19%). Of the 11 patients with truly LA disease, 9 (82%) received IORT.

Our first aim was to identify preoperative clinical factors that could predict resectability in patients treated with neoadjuvant FOLFIRINOX. A comparison of resected and nonresected patients is summarized in Table 1. Preoperatively (post-neoadjuvant therapy), resected patients had lower levels of CA 19–9 [preoperative CA 19–9: 21 U/mL (IQR 10 to 56) vs 40 U/mL (IQR 20 to 228), P ¼ 0.03], yet both groups had "normalized" their CA 19–9 levels. Patients who were resected had smaller tumors on preoperative CT scans [2.3 cm (IQR 1.5 to 3.3) vs 3.0 cm (IQR 2.0 to 4.1), P ¼ 0.03]; however, no size, or size change was predictive of resectability. The frequency of grade ○83 toxicity was not different between the 2 groups (12.9 vs 18.0%, P ¼ 0.99). Accordingly, we found no

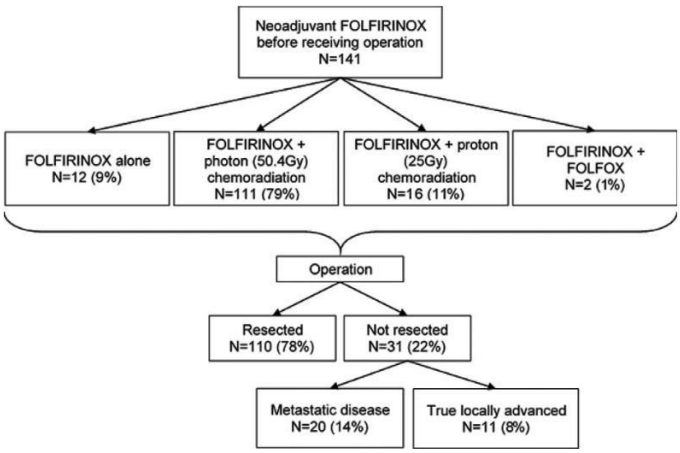

FIGURE 1. Flowchart of included cohort.

preoperative clinical factors that could accurately determine resectability after neoadjuvant therapy.

Disease-Free Survival and Overall Survival

Our second aim was to determine predictors of DFS and OS for patients receiving neoadjuvant FOLFIRINOX. The median follow-up was 21.7 months (IQR 15.9.3 to 33.9) from diagnosis and 13.7 months (IQR 7.6 to 26.8) from the operation, with 5 patients followed up for less than a year since diagnosis.

DFS for resected patients from time of diagnosis was 29.1 months [IQR 15.6 to NR (not reached)] and 20.7 months (IQR 6.5 to NR) from the time of operation (Table 2, Suppl. Table 1, http://links.lww.com/SLA/B351).

Median OS from diagnosis for the entire cohort was 34.2 months (IQR 19.9 to 55.5). For patients who had their tumor resected, median OS from the time of diagnosis was 37.7 months (IQR 23.0 to 55.5) and 31.5 months (IQR 15.1 to NR) from the time of operation. In contrast, patients who were not resected had a median OS of 18.6 months (IQR 14.0 to 38.1) from the time of diagnosis ($P < 0.001$ compared with resected, Fig. 2) and 8.0 months (IQR 5.2 to 31.9) from the time of operation.

Predictors of Survival (Resected FOLFIRINOX Only)

The factors associated with DFS and OS in patients who received neoadjuvant FOLFIRINOX and underwent resection are presented in Table 3. Preoperative CA 19–9 >100 U/mL and time from diagnosis to operation longer than 8 months emerged as independent predictors of worse DFS. Similarly, Charlson comorbidity index >1, preoperative CA 19–9 >100 U/mL, and tumor size >3.0 cm on CT scan and >2.5 cm on pathology were found to be independent predictors of decreased OS. In fact, the ROC curve analysis demonstrated that tumor size on preoperative CT larger than 3.0 cm predicted death at 12 months postoperatively with a sensitivity of 65% and specificity of 77%; pathological size >2.5 cm predicted death at 18 and 24 months postoperatively with a sensitivity of 78% and 77%, and specificity of 60% and 66%, respectively.

Surprisingly, no additional pathological factors, including node positivity or perineural invasion, were significantly associated with DFS on multivariate analyses.

Upfront Resectable PDAC versus BR/LA PDAC Resected Following Neoadjuvant FOLFIRINOX

During the study period, 155 additional patients had upfront resectable tumors and received no neoadjuvant therapy (Table 4). At the time of operation, FOLFIRINOX patients were younger ($P < 0.001$), had a lower BMI ($P = 0.005$), and a lower CA 19–9 level ($P < 0.001$). FOLFIRINOX patients underwent longer operations ($P < 0.001$) with higher blood loss ($P = 0.001$). On pathologic examination, tumors of FOLFIRINOX patients were smaller ($P < 0.001$), although there was no difference between

TABLE 2. Disease-Free (DFS) and Overall Survival (OS) of Patients with Borderline or Locally Advanced PDAC who were taken to the Operating Room After Receiving Neoadjuvant FOLFIRINOX Therapy

	Not Resected (N = 31)	Resected (N = 110)	Total (N = 141)	P
DFS, mo [median (IQR)]				
From diagnosis		29.1 (15.6–NR)		
From operation		20.7 (6.5–NR)		
OS, mo [median (IQR)]				
From diagnosis	18.6 (14.0–38.1)	37.7 (23.0–55.5)	34.2 (19.9–55.5)	<0.001
From operation	8.0 (5.2–31.9)	31.5 (15.1–NR)	27.1 (12.7–46.2)	<0.001

P values derived from log-rank tests.
IQR indicates interquartile range; NR, not reached.

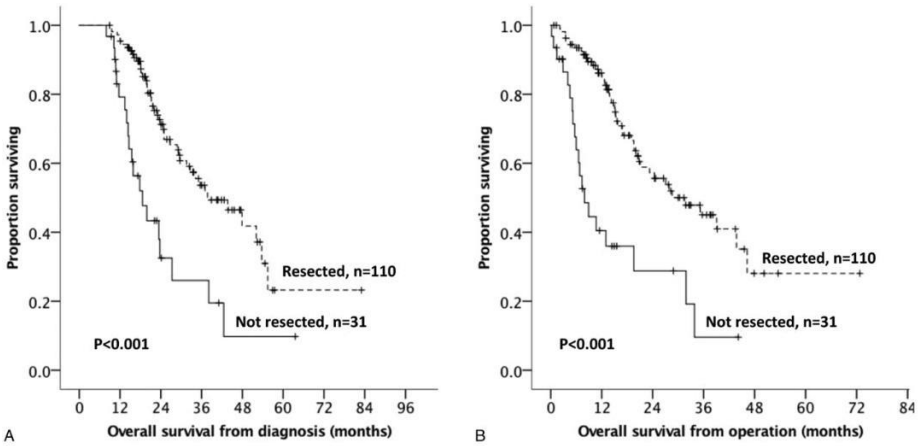

FIGURE 2. Kaplan-Meier overall survival curves: Resected (n ¼ 110, dashed lines) versus not resected (n ¼ 31, solid lines) patients with locally advanced or borderline resectable PDAC who underwent surgical exploration after receiving neoadjuvant FOLFIRINOX therapy. Overall survival was calculated from the time of diagnosis (A) or the time of operation (B). P values were derived from log-rank tests.

groups in tumor size evaluated preoperatively by CT scan (P ¼ 0.88). An R0 resection (margin >1 mm) was achieved more frequently in FOLFIRINOX patients (81% vs 54%, P < 0.001). This was also true when negative margin was defined as absence of tumor cells at the transection margin (FOLFIRINOX 92% vs no neoadjuvant 70%, P < 0.001). Lymph node positivity (P < 0.001), as well as lymphatic (p < 0.001) and perineural invasion (P < 0.001) were all less frequent in FOLFIRINOX patients compared with those who had received no neoadjuvant therapy. Postoperatively, the 2 groups had a similar frequency of Clavien-Dindo ○ß3 complications and 90-day mortality. However, FOLFIRINOX patients had a shorter LOS (P ¼ 0.01) and a lower 90-day readmission rate. Among patients who did not receive neoadjuvant therapy, 40 (26%) and 62 (40%) received adjuvant chemotherapy alone, or adjuvant chemotherapy and radiotherapy, respectively. The most common adjuvant chemotherapy was gemcitabine.

Survival

Median follow-up for the resected cohort was 18.1 (IQR: 11.2 to 27.2) and 14.3 months (IQR 7.5 to 23.8) from diagnosis and

TABLE 3. Factors Associated with Disease-Free and Overall Survival in 110 Patients who Received Neoadjuvant FOLFIRINOX Followed by PDAC Resection

	Univariate		Multivariate	
	Survival from Operation, Median (IQR), mo	P	HR	P
Disease-free survival				
Preoperatively				
Tumor size >3.0 cm (CT scan)	6.8 (4.6–19.0) vs 29.1 (7.3–44.6)	0.01		
CA 19–9 >100 U/mL	8.2 (3.6–19.0) vs 37.7 (6.9–NR)	0.03	2.7	0.05
Diagnosis to operation >8 mo			2.1	0.04
Postoperatively				
Lymph node positivity	11.6 (5.3–29.1) vs 37.5 (7.3–NR)	0.03		
Overall survival				
Preoperatively				
Charlson comorbidity index >1	20.1 (13.7–46.2) vs 43.7 (16.9–NR)	0.03	1.9	0.01
CA 19–9 >100 U/mL	15.7 (4.6–NR) vs 31.5 (16.9–46.2)	0.04	2.1	0.04
Tumor size >3.0 cm (CT)	19.0 (11.1–39.1) vs 31.5 (16.9-NR)	0.02	2.0	0.04
Postoperatively				
Tumor size >2.5 cm (pathology)	19.6 (13.2–39.1) vs 43.7 (16.9–NR)	0.02	1.7	0.03
T stage T2 or higher	23.3 (14.3–46.2) vs median NR	0.03		

P values derived from log-rank test (univariate) of Cox regression models (multivariate).
HR indicates hazard ratio; IQR, interquartile range; NR, not reached.

TABLE 4. Clinicopathologic Characteristics of 265 Patients with BR/LA PDAC who Underwent Resection After Receiving Neoadjuvant FOLFIRINOX Therapy (N ¼ 110) or with Upfront Resectable Tumors who Received no Neoadjuvant Therapy (N ¼ 155)

	FOLFIRINOX N ¼ 110				No Neoadjuvant N ¼ 155				Total N ¼ 265				
	N	%	Median	IQR	N	%	Median	IQR	N	%	Median	IQR	P
Age at operation, yrs			63	57–68			71	65–79			67	61–74	<0.001
Sex													
Male	51	46.4			76	49.0			127	47.9			0.71
Female	59	53.6			79	51.0			138	52.1			
Race/ethnicity													
White	100	90.9			145	93.6			245	92.4			0.30
Asian	3	2.7			3	1.9			6	2.3			
Hispanic	3	2.7			5	3.2			8	3.0			
Other	4	3.7			2	1.3			6	2.3			
Charlson comorbidity index			1	0–2			3	1–4			2	1–3	<0.001
BMI, kg/m^2			24.4	21.7–28.9			26.6	23.7–30.1			25.3	23.0–19.5	0.005
Serum CA 19–9 U/mL			21	10–56			123	26–397			51	14–228	<0.001
Tumor size													
Imaging preoperatively, cm			2.3	1.5–3.3			2.2	1.9–2.8			2.2	1.7–3.0	0.88
Pathology postoperatively, cm			2.5	1.9–3.5			3.2	2.5–4.3			3.0	2.1–3.9	<0.001
Positive lymph nodes	40	36.4			107	69.0			147	55.5			<0.001
Lymphatic invasion	30	27.3			96	61.9			126	47.5			<0.001
Perineural invasion	73	67.0			143	92.3			216	81.5			<0.001
Negative margins (>1 mm)	87	80.6			84	54.2			171	64.5			<0.001
EBL, mL			600	400–800			425	275–800			500	300–800	0.001
OR duration, min			376	310–444			300	240–362			330	264–410	<0.001
LOS, d			6	5–7			7	5–9			6	5–8	0.01

P values derived from Fisher exact test or Mann-Whitney U test as appropriate.
BMI indicates body mass index; EBL, estimated blood loss; IQR, interquartile range; LOS, length of stay.

operation, respectively, with 48 patients being followed for less than a year after diagnosis. DFS and OS from the time of diagnosis were significantly better for the BR/LA PDAC patients treated with neoadjuvant FOLFIRINOX when compared with those with resectable tumors who went directly to the operating room [DFS: 29.1 months (IQR 15.6 to NR) vs 13.7 months (7.2 to 23.0), P < 0.001; OS: 37.7 months (IQR 23.0 to 55.5) vs 25.1 months (15.4 to N/A), P ¼ 0.01]. From the time of operation, DFS was also longer among patients treated with FOLFIRINOX than those going directly to the operating room [20.7 months (IQR 6.5 to NR) vs 13.4 months (IQR 7.0 to 22.0), P ¼ 0.02]. Median postoperative OS was longer for patients treated with FOLFIRINOX, but not statistically significant [31.5 months (IQR 15.1 to NR) vs 24.6 months (15.2 to N/A), P ¼ 0.67] (Fig. 3).

DISCUSSION

FOLFIRINOX chemotherapy has dramatically changed the therapeutic landscape for patients with PDAC. Significant improvement in survival for patients with metastatic disease was demonstrated by the ACCORD trial,[14] and as this study shows, patients with BR/LA disease also appear to benefit from neoadjuvant FOLFIRINOX.

This study presents one of the largest series to date of BR/LA PDAC patients surgically explored following neoadjuvant FOLFIRINOX therapy. Many of our patients also received chemoradiation, in an attempt to increase the R0 resection rate and improve long-term local control. We demonstrate that resectability cannot be predicted by clinical factors preoperatively. Although preoperative CA 19–9 levels and tumor size on CT scan were lower among resected patients, clinically useful cutoffs were not identified. Specifically, CA 19–9 had normalized after neoadjuvant FOLFIRINOX in both groups. Other studies have suggested CA 19–9 as a marker of resectability: Boone et al[15] demonstrated that in BR patients treated with neoadjuvant therapy (gemcitabine-based or FOLFIRINOX), a CA 19–9 response of >50% predicted an R0 resection. Similarly, Aldakkak et al[16] correlated post-neoadjuvant CA-19–9 levels with completion of intended therapy including resection. Additional clinicopathological features such as male sex[17] as well as head/neck location and SMA involvement[18] have been suggested as predictors of unresectability in BR/LA PDAC patients.

Furthermore, we[12] and others[19,20] have previously demonstrated that tumor evaluation on imaging after the completion of neoadjuvant therapy is not a reliable predictor of resectability. On the basis of the absence of reliable clinical markers of resectability and imaging, we advocate for surgical exploration of all BR/LA PDAC patients neoadjuvantly treated with FOLFIRINOX that have no evidence of metastatic disease.

Although this study includes a highly selected group of BR/LA PDAC patients who managed to undergo an operation after having received neoadjuvant FOLFIRINOX, it demonstrates that BR/LA PDAC patients treated with neoadjuvant FOLFIRINOX have a substantially increased OS compared with the median historical survival of 11 months. In this series of patients, the median OS from diagnosis in resected patients was 37.7 months. The operative outcomes and survival of this group are better than that of resectable patients receiving upfront resection.

This study also reports one of the longest survivals observed in patients with BR/LA PDAC treated with FOLFIRINOX. A recent patient-level meta-analysis[21] of LA PDAC patients treated with neoadjuvant FOLFIRINOX demonstrated that across studies, the median OS from the start of FOLFIRINOX ranged from 10.0 to 32.7 months, with a pooled median OS of 24.2 months. Our finding of an OS of 34.2 months from diagnosis might reflect the relatively long

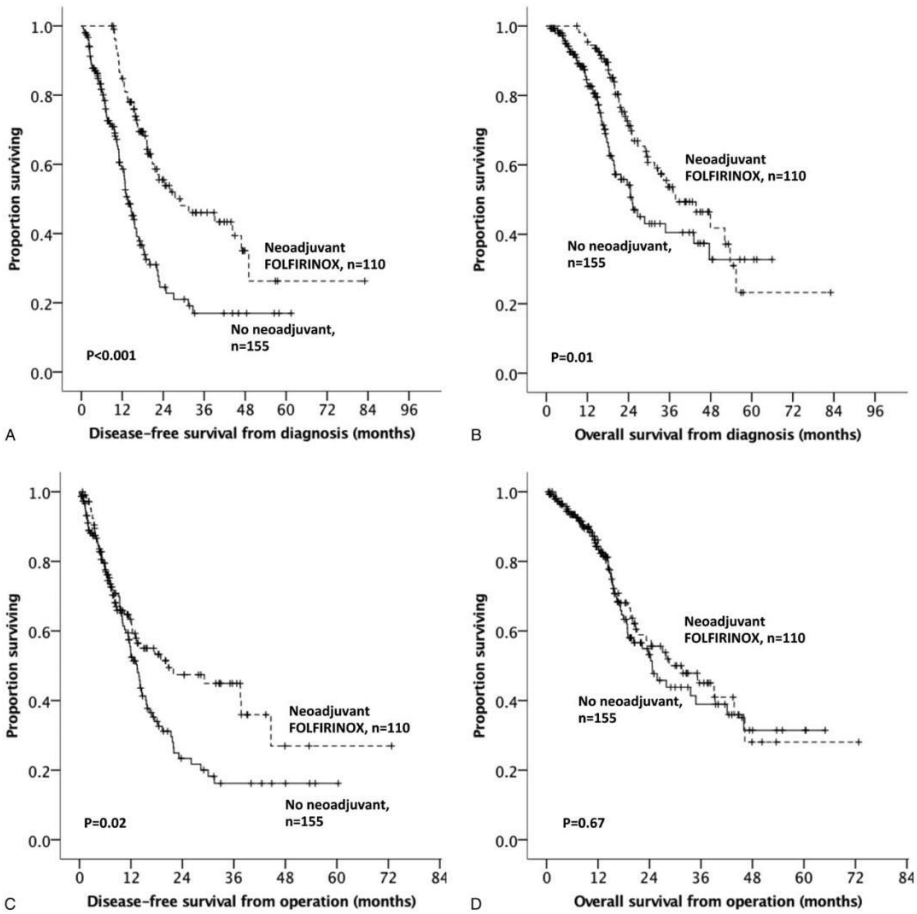

FIGURE3. Kaplan-Meier overall and disease-free survival curves: PDAC patients resected after neoadjuvant FOLFIRINOX(n ¼ 110, dashed lines) or no neoadjuvant therapy (n ¼ 155, solid lines). Disease-free and overall survival were calculated from the time of diagnosis (A and B, respectively) or the time of operation (C and D, respectively). P values were derived from log-rank tests.

experience our institution has in the implementation of neoadjuvant FOLFIRINOX in these patients.

Despite these encouraging results, some patients receiving FOLFIRINOX recur within 12 months of their operation. Preoperative CA 19–9 level >100 U/mL and a period from diagnosis to operation >8 months predicted a shorter DFS. Similarly, preoperative CA 19–9 concentration >100 U/mL, tumor size >3.0 cm on preoperative CT and >2.5 cm on pathology, as well as a Charlson comorbidity index >1 predicted decreased OS. Specifically, although CA 19–9, tumor size, and Charlson comorbidity index are proxies of the disease burden and overall health status, the impact of the diagnosis-to-operation period length is not clear. There were multiple reasons a delay in surgical intervention occurred, including a delay in referral for a surgical opinion, toxicity from FOLFIRINOX, or chemoradiation therapy and obstruction of the biliary stent.

Noteworthy, among resected FOLFIRINOX patients in this study, traditional pathological factors such as lymph node positivity, vascular, and perineural invasion did not emerge as independent predictors of DFS. Tumor size was the only pathologic factor independently predictive of a poor OS. Conversely, time elapsed from diagnosis to resection longer than 8 months was associated with a poor DFS. This delay may be due to toxicity from the chemotherapy. Our data would suggest that prompt surgical exploration may

allow for better disease control; however, data from additional studies are warranted.

Our study also demonstrates an advantage when comparing BR/LA PDAC patients who received neoadjuvant therapy before an operation to contemporary patients who had resectable rumors and underwent upfront resection. Despite longer operations with increased blood loss, length of stay, 90-day morbidity, and mortality were equivalent to upfront resectable patients. Classic pathologic prognostic markers such as LN positivity, resection margins, and tumor size were all improved with neoadjuvant FOLFIRINOX. Our findings strengthen the argument for the extension of neoadjuvant FOLFIRINOX to resectable patients, which several clinical trials are currently exploring (NCT02782182, NCT02178709, NCT02172976, NCT01560949, NCT02959879, NCT02047474, NCT01660711, NCT02345460, NCT02243007, NCT02562716). This finding is in concordance with a previous analysis of National Cancer Database showing improved survival for patients who received neoadjuvant therapy followed by resection compared with propensity-matched patients who underwent upfront resection—with or without adjuvant therapy—among early-stage resectable PDAC patients.[22]

This study cohort comprises a large population of FOLFIRINOX-treated patients none of whom were lost to follow-up. A weakness of this study is the lack of information concerning BR/LA PDAC patients who progressed on neoadjuvant FOLFIRINOX and were not referred for surgical evaluation, which leads to the reporting of a highly selective sample. In addition, a number of patients were not followed for more than a year after diagnosis. Unfortunately, a large proportion of patients receive their neoadjuvant or adjuvant chemotherapy at other institutions. Another limitation is that this is a single-center experience.

In summary, the present study demonstrates a new era of BR/LA PDAC in which PDAC patients receiving neoadjuvant FOLFIRINOX have a favorable prognosis compared with historical series, as well as to patients with resectable tumors receiving upfront resection. In the absence of reliable predictors of resectability, we would advocate that all BR/LA PDAC patients with no progression on neoadjuvant FOLFIRINOX should be offered surgical exploration. Patients with elevated CA 19–9 levels, large tumors, long time from diagnosis to operation, and a high Charlson comorbidity index have a higher likelihood of recurrence, and therefore may benefit from additional adjuvant therapy. While anticipating the results of clinical trials, this study strengthens the rationale for the use of neoadjuvant FOLFIRINOX in patients presenting with resectable disease.

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Multi-Institutional Validation Study of Pancreatic Cyst Fluid Protein Analysis for Prediction of High-Risk Intraductal Papillary Mucinous Neoplasms of the Pancreas

Mohammad A. Al Efishat, MD,ã Marc F. Attiyeh, MD,ã Anne A. Eaton, MS, Mithat Go¨nen, PhD, y Denise Prosser, BS,z Anna E. Lokshin, PhD,z Carlos Ferna´ndez-del Castillo, MD,§ Keith D. Lillemoe, MD,§ Cristina R. Ferrone, MD,§ Ilaria Pergolini, MD,§ Mari Mino-Kenudson, MD,ô Neda Rezaee, MD,jj Marco Dal Molin, MD,jj Matthew J. Weiss, MD,jj John L. Cameron, MD,jj Ralph H. Hruban, MD,ãã Michael I. D'Angelica, MD,ã T. Peter Kingham, MD,ã Ronald P. DeMatteo, MD,ã William R. Jarnagin, MD,ã Christopher L. Wolfgang, MD, PhD,jj and Peter J. Allen, MDã

Objective: Preliminary work by our group suggested that proteins within the pancreatic cyst fluid (CF) may discriminate degree of IPMN dysplasia. We sought to externally validate these markers and determine whether their inclusion in a preoperative clinical nomogram could increase diagnostic accuracy.

Summary Background Data: IPMN is the most common radiographically identifiable precursor to pancreatic cancer; however, the timing and frequency of its malignant progression are unknown, and there are currently no reliable preoperative tests that can determine the grade of dysplasia in IPMN.

Methods: Clinical and radiographic data, as well as CF samples, were obtained from 149 patients who underwent resection for IPMN at 1 of 3 institutions. High-risk disease was defined as the presence of high-grade dysplasia or invasive carcinoma. Multianalyte bead array analysis (Luminex) of CF was performed for 4 protein markers that were previously associated with high-risk disease. Logistic regression models were fit on training data, with and without adjustment for a previously developed clinical nomogram and validated with an external testing set. The models incorporating clinical risk score were presented graphically as nomograms.

Results: Within the group of 149 resected patients, 89 (60%) had low-risk disease, and 60 (40%) had high-risk disease. All 4 CF markers (MMP9, CA72–4, sFASL, and IL-4) were overexpressed in patients with high-risk IPMN ($P < 0.05$). Two predictive models based on preselected combinations of CF markers had concordance indices of 0.76 (Model-1) and 0.80 (Model-2). Integration of each CF marker model into a previously described clinical nomogram leads to increased discrimination compared with either the CF models or nomogram alone (c-indices of 0.84 and 0.83, respectively).

Conclusions: This multi-institutional study validated 2 CF protein marker models for preoperative identification of high-risk IPMN. When combined with a clinical nomogram, the ability to predict high-grade dysplasia was even stronger.

Keywords: biomarkers, cyst fluid, dysplasia, intraductal papillary mucinous neoplasms, pancreas

(Ann Surg 2017;xx:xxx–xxx)

Intraductal papillary mucinous neoplasms (IPMN) of the pancreas are a heterogeneous group of lesions that grow within the ductal system of the pancreas and may involve the main pancreatic duct (MD-IPMN), branch ducts (BD-IPMN), or both (Mixed-IPMN).[1] Since the recognition of this entity over 3 decades ago,[2,3] IPMN have gained increased attention because of their frequent identification on routine cross-sectional imaging, and because they are the most common radiographically identifiable precursors of pancreatic cancer.[4,5]

The pathway of IPMN progression from noninvasive to invasive disease is believed to be responsible for approximately 20% to 30% of pancreatic cancer cases.[5] These cystic adenomatous lesions are believed to progress from low-grade dysplasia, to high-grade dysplasia, to invasive cancer.[6] Once invasive disease develops, survival outcomes are similar to conventional pancreatic cancer when controlled for stage.[7,8] Therefore, most clinicians believe that resection of high-grade dysplasia presents an opportunity to cure a lesion before the development of an incurable invasive disease. However, previous recommendations for the routine resection of IPMN have led to some overtreatment, with many reports identifying high-risk pathology in less than half of all patients who have undergone pancreatectomy.[9,10]

From the ãDepartment of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY; yDepartment of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY; zDepartment of Pathology, University of Pittsburgh Cancer Institute, Pittsburgh, PA; §Department of Surgery, Massachusetts General Hospital, Boston, MA; ôDepartment of Pathology, Massachusetts General Hospital, Boston, MA; jjDepartment of Surgery, Johns Hopkins Hospital, Baltimore, MD; and ããSol Goldman Pancreatic Cancer Research Center, Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD.

This study was supported in part by NIH/NCI research project (R01) grant, project 5R01CA182076-02, and the NCI SPORE grant CA62924.

This work has been presented as an oral presentation at the 2016 American College of Surgery (ACS) Clinical Congress, Washington, DC, October 16–20, 2016.

Authors' contributions are as follows:

Conception and Design: Al Efishat, Attiyeh, Ferna´ndez-del Castillo, Go¨nen, Lokshin, Lillemoe, Weiss, Cameron, Hruban, D'Angelica, Kingham, DeMatteo, Jarnagin, Wolfgang, Allen.

Development of Methodology: Al Efishat, Gonen, Ferna´ndez-del Castillo, Lokshin, Wolfgang, Allen.

Acquisition of Data: Al Efishat, Attiyeh, Pergolini, Rezaee, Dal Molin, Mino-Kenudson, Prosser.

Analysis and Interpretation of Data: Al Efishat, Attiyeh, Gonen, Eaton, Allen.

Drafting of Manuscript: Al Efishat, Allen.

Review and Critical Revision of the Manuscript: Al Efishat, Attiyeh, Eaton, Gonen, Prosser, Lokshin, Lomakin, Ferna´ndez-del Castillo, Lillemoe, Ferrone, Mino-Kenudson, Pergolini, Rezaee, Dal Molin, Weiss, Cameron, Hruban, D'Angelica, Kingham, DeMatteo, Jarnagin, Wolfgang, Allen.

Administrative, Technical, and Material Support: Al Efishat, Attiyeh, Pergolini, Rezaee, Ferrone, Dal Molin, Mino-Kenudson, Prosser, Lomakin, Allen.

Study Supervision: Allen.

The authors declare no potential conflicts of interest.

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Reprints: Peter J. Allen, MD, Professor of Surgery, Memorial Sloan-Kettering Cancer Center, 1275 York Ave- Office C896, New York, NY 10021. E-mail: allenp@mskcc.org.

ISSN: 0003-4932/16/XXXX-0001
DOI: 10.1097/SLA.0000000000002421

The ability of current laboratory, radiographic, and endoscopic tests to distinguish between low-risk (low and moderate dysplasia) and high-risk (high-grade dysplasia and invasive) IPMN is limited.[11,12] The presence of a dilated main pancreatic duct (MPD) on preoperative imaging remains the most commonly implemented criterion for prediction of high-grade dysplasia or invasive disease, as it has been shown that approximately 60% of patients with resected MD-IPMN harbor high-grade dysplasia or invasive disease.[13] When the main duct is not dilated (BD-IPMN), only 10% to 15% of resected patients will be found to have high-grade disease.[14] The most recent consensus guidelines for management of IPMN have recommended resection for all IPMN patients with a dilated MPD >1 cm or an enhancing solid component.[15] Reports have shown, however, that in this setting, 60% of resected patients will have high-risk disease. If only 60% of these patients have high-risk disease, then 40% of patients will undergo a major operation for low-risk disease.[16] Hence, improved markers of high-risk disease are needed.

Over the past several years, our group has investigated several serum and cyst fluid (CF) protein markers in patients who have undergone resection for IPMN. These studies have demonstrated consistent differences in the patterns of CF protein expression between low-risk and high-risk patients.[17,18] In the most recent study,[19] we analyzed the CF of 78 patients with resected IPMN utilizing antibody bead array (Luminex) with 87 different protein markers. High-risk disease was found to have a positive correlation with 29 of the measured proteins, of which 23 (79%) were inflammatory markers. In this previous study, none of the proinflammatory or neoplastic markers were found to be significantly overexpressed in the low-risk group. Multivariate modeling was performed on these data, and resulted in 2 different mathematical prediction models with 2 different sets of markers (MMP9 b, CA72–4, and sFASL b, IL-4) that each had a discrimination index (c-index) of 86% for predicting high-risk disease. In these 78 patients, if MPD dilation was used as the criterion for high-risk disease (as recommended by the current guidelines), then the c-index would have been only 73%.

In this current study, we sought to validate, on an independent multi-institutional patient set, these 2 predictive models using antibody bead array (Luminex). We employ a larger set of CF samples from 3 high-volume academic hospitals (Johns Hopkins Hospital, Massachusetts General Hospital, Memorial Sloan Kettering). We also aimed to incorporate these protein-level models into a recently developed clinical and radiographic prediction model (nomogram) to investigate whether a combined model could result in a highly accurate prediction tool for high-risk IPMN.

METHODS

Patients

Following institutional review board (IRB) approval, and using prospectively maintained pancreatic databases from the Pancreatic Surgery Consortium, each 1 of the 3 participating institutions [Memorial Sloan Kettering (MSK), Massachusetts General Hospital (MGH), and Johns Hopkins Hospital (JHH)] randomly selected 50 patients who had undergone resection for a pathologically proven IPMN, between January 2004 and September 2015. Eligible patients were required to have had adequate banked CF (at least 250 mL) available for the study. Patients with concurrent malignancies (eg, cholangiocarcinoma, neuroendocrine tumor) were excluded.

All patients had previously signed one of the corresponding institutional review board-approved tissue banking protocols (MSK IRB# 00-032, JHH IRB# AM00028936, MGH IRB# 2003p001289), and a waiver of authorization was obtained from each institutional IRB prior to accessing the electronic medical records. Demographic data, clinical data, laboratory, radiographic, and pathologic features were extracted from the clinical databases. Main duct IPMN (MD-IPMN) was recorded if the main pancreatic duct (MPD) was dilated (>0.5 cm) on radiographic evaluation or main-duct involvement was reported on pathologic assessment, and branch duct (BD-IPMN) was defined if there was cystic disease in the absence of main duct dilation (○ß0.5 cm) or involvement. A diagnosis of mixed-type (a combination of both main duct dilation and cystic disease) was classified as main duct disease for the purposed of this study.

Histopathologic assessment of resected specimens was performed as per institutional protocol by a dedicated gastrointestinal pathologist. Grade of dysplasia was defined as the highest degree of dysplasia identified within the examined specimen.[6] Based on the outcome of interest, patients were classified as "high-risk" if their pathology report showed high-grade dysplasia, or invasive carcinoma. Lesions with low-grade or intermediate-grade dysplasia were defined as "low-risk."

Cyst Fluid Samples

Cyst fluid samples were aspirated with an 18- to 21-gauge needle either intraoperatively by the surgeon or upon arrival to the surgical pathology suite by a pathologist. Samples were then aliquoted, and stored at À80°C. Based on the recorded time between resection and freezing, only samples that had been refrigerated within 60 minutes of resection and underwent no previous freeze-thaw cycles were eligible. All the samples, deidentified to study number only, were shipped overnight on dry ice to University of Pittsburgh Cancer Institute (UPCI) Luminex core facility, where all multiplex assays were performed.

Multiplex Biomarker Analysis

Multianalyte analysis was performed using commercially available plates that included the 4 prespecified proteins.[19] Luminex Multiplex Bead Immunoassays were performed in 96-well microplate format as per standard protocol (See supplementary files SF1 and SF2, http://links.lww.com/SLA/B291 for the detailed steps and protocols of all assays).[19–21] Briefly, dilutions were followed according to each Vendor's protocol recommendations, unless otherwise noted. Millipore research human cytokine/chemokine Panel 1 (5-Plex) (Catalog # HCYTOMAG-60K-05) was diluted 2-fold to increase bead recovery. Additional Millipore assay conducted was Milli Research Human Cancer Biomarker Panel 1 (1-Plex) (Catalog # HCCBP1MAG-58K-01) at 6-fold. R&D systems assays conducted were LMPM000 and LMPM911 at 50-fold. And finally, the UPCI Core-developed assay conducted (5plex) at 5-fold. (See Supplementary table ST1, http://links.lww.com/SLA/B293 for a summary of these multiplex assays and dilutions). Quality control data for each core-developed assay, including correlation with commercial ELISA, can be found on the UPCI Luminex Core Facility website (http://upci.upmc.edu/cpf/luminex.cfm).

Upon completion, all Luminex assay plates were then read on a BioRad Bioplex 100 or 200 instrument utilizing BioPlex Manager4.1.1. Quantification of markers was either exported directly from this software or via XML files analyzed by the scaler program. All the resulting concentrations were normalized according to a scaling procedure, previously developed by UPCI group, to account for variations across different experiments (batch effect).[20]

Statistical Analysis

Our sample size was selected based on the precision with which wecouldestimate the sensitivity for the grade of dysplasia, and thus we did not use traditional sample size paradigms. With a sample size of 150, we could estimate sensitivity using a 95% confidence interval to within Æ9.2% if true sensitivity was 80%. The precision would increase to Æ6.2% if true sensitivity was 90%. Of note, we chose

TABLE 1. Performance Metrics for the Training and Validation Sets in Single and Combined Models

Predictive Model	Variable	Odds Ratio	P	Training Set c-Index (n ¼ 104)	Validation Set c-Index (n ¼ 45 Patients)
Clinical nomogram alone	Nomogram LP	2.43 (1.50–3.93)	0.0003	0.80	0.77
Model-1[A]	Log CA72–4	1.46 (1.12–1.91)	0.0056	0.76	0.80
	Log MMP9	1.28 (1.05–1.57)	0.0134		
Clinical nomogram plus Model-1	Nomogram LP	1.96 (1.23–3.13)	0.0050	0.88	0.84
	Log CA72–4	1.55 (1.11–2.17)	0.0097		
	Log MMP9	1.34 (1.05–1.73)	0.0204		
Model-2[y]	Int-risk[z]	16.07 (5.10–50.68)	<0.0001	0.80	0.79
	High-risk[z]	13.27 (3.94–44.59)	<0.0001		
Clinical Nomogram Plus Model-2	Nomogram LP	1.64 (0.98–2.76)	0.0604	0.82	0.83
	Int-risk[z]	4.33 (1.03–18.28)	0.0462		
	High-risk[z]	4.80 (1.16–19.82)	0.0300		

nLP indicates linear predictor; Int-risk ¼ intermediate risk (main duct IPMN AND non-elevated sFASL and IL4). High-risk (sFASL >90th percentile or IL4 >84th percentile).
ALog (MMP9) and log (CA-72–4) as continuous predictors.
yThree-level categorical predictor from a decision tree based on IL-4, sFASL, and main/branch duct.
zVersus low risk (branch duct IPMN, sFASL, and IL4 not elevated).

sensitivity over specificity because the clinical emphasis was felt to be weighted toward avoiding nonoperative management of high-risk lesions that should ideally be resected to prevent progression to cancer. This translates into a need to decrease false-negative results and improve both negative predictive value and sensitivity.

Protein concentrations were log-transformed before analysis to produce more normally distributed data. Continuous variables, including protein concentrations, were summarized using median and range and compared between the low-risk and high-risk groups using the Mann-Whitney U test. Categorical variables were summarized using frequency and percentage and compared using Fisher exact test. Dependence among proteins was evaluated using Pearson correlation coefficient, accepting correlation coefficients of <0.7 to represent independence between the selected markers.

The data were randomly split into a training (n ¼ 104) and testing dataset (n ¼ 45). Multivariable logistic regression was used to assess relationships between preselected combinations of protein markers and high-risk disease in the training dataset. The markers to be included in the models were selected based on multivariate modelingfromour previouswork.[19] Model-1 was based on MMP9 and CA72–4 log concentrations, whereas Model-2 was a 3-leaf decision tree based on sFASL, IL-4, and the IPMN duct type (decision tree classification details are shown in Table 1). For Model-2, sFASL and IL-4 were expressed as percentiles in their respective datasets.

Predictions from our clinical nomogram were added as a predictor in each model to determine if the markers remained independently associated with outcome.[22] Model performance was assessed on the test dataset using concordance indices (c-indices), with values ranging from 0.5 (as good as chance) to 1.0 (perfect discrimination). The final multivariable models were visually represented as nomograms and validated using the testing dataset. All statistical analysis was done in R 3.1.1 using the rms, Hmisc, pROC, and readxl packages, and P values <0.05 were considered significant.

RESULTS

Patient Characteristics

In total, 154 patients who underwent resection of IPMN, and had CF banked per protocol, were initially included from the 3 participating institutions (MSK, MGH, and JHH). However, during the multiplex assays, 5 samples were found to be too viscous for the assay (all 5 samples were high-risk), and thus had to be excluded due to inadequate protein measurement. The remaining 149 patients constituted our study cohort (Fig. 1).

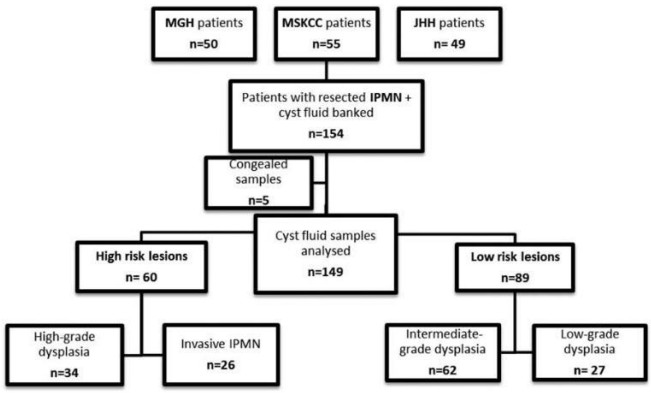

FIGURE 1. Study cohort. Number of patients included from each institution, excluded patients, and the breakdown of numbers between the low-risk and high-risk groups.

TABLE 2. Clinical, Biologic, Radiographic, and Pathologic Characteristics with Univariate Analysis, Stratified by Low-Risk and High-Risk Groups (N ¼ 149)

Characteristics	Total (n ¼ 149)	High-Risk[Ã] (n ¼ 60)	Low-Risk (n ¼ 89)	P
Age at operation	71 (32, 88)	70.5 (44, 88)	71 (32, 88)	0.434
Sex				0.007
Female	63 (42)	17 (28)	46 (52)	
Male	86 (58)	43 (72)	43 (48)	
Institution				0.041
JH	47 (32)	18 (30)	29 (32)	
MGH	48 (32)	26 (43)	22 (25)	
MSK	54 (36)	16 (27)	38 (43)	
BMI	26 (18, 45)	27.76 (18, 40)	26.07 (18, 45)	0.067
Symptoms	50 (34)	26 (43)	24 (27)	0.051
Weight loss	28 (19)	17 (28)	11 (12)	0.019
Diabetes	40 (27)	21 (35)	19 (21)	0.089
Smoking	71 (47.7)	39 (65)	32 (36)	<0.001
Jaundice	6 (4)	5 (8)	1 (1)	0.039
Largest cyst size, cm				0.409
○ 3	56 (38.1)	21 (35.6)	35 (39.8)	
>3	83 (56.5)	33 (55.9)	50 (56.8)	
None seen	8 (5.4)	5 (8.5)	3 (3.4)	
Serum CA 19–9, units/mL	22 (1, 6618)	29 (1, 6618)	16 (1, 113)	0.058
Cyst fluid CEA, ng/mL	150 (7.2, 100800)	155.5 (7.2, 50000)	150 (10, 100800)	0.487
MPD size, cm	0.5 (0, 2.1)	1 (0, 2.1)	0.3 (0, 1.8)	<0.001
MPD size range				<0.001
0.5 cm < MPD ○ 1.0 cm	41 (28)	26 (43)	15 (17)	
MPD ○ 0.5 cm	87 (58)	15 (25)	72 (81)	
MPD>1.0cm	21 (14)	19 (32)	2 (2)	
Radiographic duct type				<0.001
Branch duct	87 (58)	15 (25)	72 (81)	
Main duct	62 (42)	45 (75)	17 (19)	
Abrupt MPD change	12 (9)	10 (18)	2 (3)	0.005
Solid component	22 (15)	18 (30)	4 (5)	<0.001

Continuous variables are summarized using median and range (P values from rank sum test), rounded to the nearest one.
Categorical variables are summarized using frequency and percentage (P values from Fisher exact test).
Median (low, high) or N (%).
[Ã]High-risk disease included high-grade dysplasia alone (n ¼ 34) and high-grade dysplasia with invasion (n ¼ 26).

The clinical, pathologic and radiographic characteristics of the cohort (stratified by disease risk) are summarized in Table 2. High-risk disease was identified in 60 patients (40%), and 89 (60%) had low-risk disease (low and intermediate-grade dysplasia). Median age at resection was similar in both groups (71 years). Males represented 58% (n¼ 86) of the cohort, and constituted 72% of the high-risk group. Female patients weresignificantlymore likely to harbor low-risk disease. BD-IPMN was present in 87 patients (58%) and the majority of BD-IPMN patients (n ¼ 72, 83%) had low-risk disease. Within the group of 62 patients (42%) with MD-IPMN, the majority (n ¼ 45, 73%) had high-risk disease on final pathological analysis. Main-duct IPMN was significantly associated with high-risk disease (P < 0.001).

Weight loss, jaundice, a history of smoking, an abrupt change in MPD diameter and the presence of solid component were more common in the high-risk group (P ○ 0.05). The training dataset (n ¼ 104) had 65 low-risk and 39 high-risk patients (63% high-risk), while the validation dataset (n ¼ 45) had 21 high-risk and 24 low-risk patients (47% high-risk).

Multiplex Assays and Univariate Analysis of Cyst Fluid Biomarker Levels Between the High-Risk and Low-Risk Groups

Correlation between the 4 markers that were included in the predictive models (MMP9, CA72–4, sFASL and IL-4) was low to moderate, with correlation coefficients of 0.25–0.68 (supplementary table ST2, http://links.lww.com/SLA/B294). Univariate analysis showed differential expression (P ○ 0.05) of all 4 protein markers between the high-risk and low-risk groups (Table 3, Fig. 2).

TABLE 3. Differentially Expressed Cyst Fluid Biomarkers Between the Low-Risk and High-Risk Groups

Protein Marker[Ã]	Overall Concentration	Low-Risk	High-Risk	P
Log sFASL, pg/mL	3.408 (2.766, 9.334)	3.258 (2.766, 7.66)	3.77 (2.874, 9.334)	<0.001
Log MMP-9, pg/mL	9.255 (5.72, 15.56)	7.898 (5.72, 13.93)	10.4 (5.743, 15.56)	<0.001
Log IL-4, pg/mL	0.6419 (0.1398, 8.006)	0.5878 (0.1398, 8.006)	0.8064 (0.1398, 6.385)	0.020
Log CA72–4, U/mL	1.135 (À0.837, 11.93)	0.5735 (À0.837, 4.635)	3.144 (À0.3567, 11.93)	<0.001

[Ã]All are cyst fluid inflammatory markers except sFASL.
Analysis of CF proteins was performed using Mann-Whitney test.
Log concentrations presented as median; interquartile range.

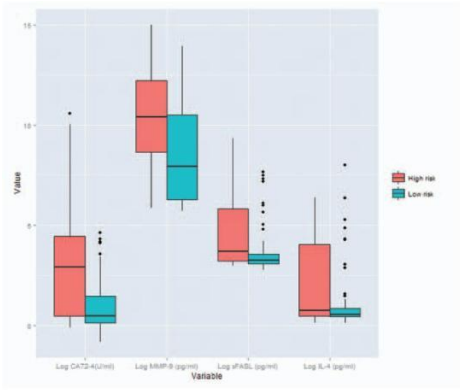

FIGURE 2. Differential expression of protein markers between low-risk and high-risk groups. Log scales of standardized concentrations are presented; representative biomarkers with differential expression between low-risk and high-risk groups.

We then performed further analysis of each IPMN duct subtype (MD-IPMN and BD-IPMN) separately to see whether these biomarkers had different associations with high-risk disease in MD-IPMN and BD-IPMN. However, given the relatively small number in each subgroup, and the known low power of interaction tests, these tests were not precise, and the ultimate decision not to stratify the cohort based on duct type was based on these considerations.

Validation of Predictive Cyst Fluid Biologic Models

The 2 preselected multivariate models maintained high predictive ability in this multi-institutional cohort. Model-1, which included the log concentration of CF MMP9 and CA-72–4 as continuous predictors, had a c-index of 0.76 in the training set and 0.80 in the validation (testing) set. Model-2 had a c-index of 0.80 in the training set and 0.79 in the validation set. Odds ratios and P values for the different components in each model are summarized in Table 1.

Cyst fluid protein markers remained independently associated with the degree of dysplasia when predictions from our previous clinical nomogram (c-index of 0.80 in the training set and 0.77 in the validation set) were added as a predictor in each model (all P value $<$ 0.05).[22] Furthermore, diagnostic accuracy was further improved when the 2 CF models were merged with the clinical nomogram compared with either of the CF models or the nomogram alone (Table 1). Combining Model-1 with the clinical nomogram (tested by receiver-operating characteristic curve analysis) yielded the highest discriminative potential in both the training and validation set with c-indices of 0.88 and 0.84, respectively. Similarly, adding the clinical nomogram to Model-2 resulted in an improved predictive performance in the training and validations groups (c-indices 0.82 and 0.83, respectively). The combined clinical and protein-level nomograms are visually presented in Figure 3.

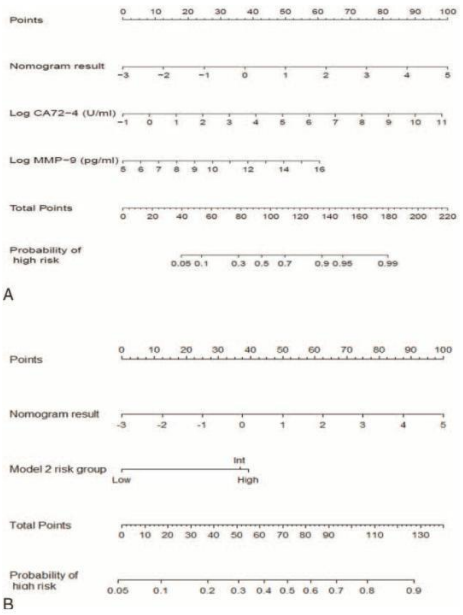

FIGURE 3. Combined clinical and protein-level nomogram for predicting high-risk IPMN. (A) Based on Model-1. (B) Based on Model-2. Nomogram score is calculated per the method described in our previous work,[22] log concentrations of the CF protein markers (CA72–4 and MMP9 in Mode1, sFASLand IL-4 in Model 2) are recorded and given points according to the model's point scale bar, and the total points are added and translated into a probability of high-risk disease.

DISCUSSION

The distinction between high-risk and low-risk IPMN is of utmost importance; as resection of IPMN in the setting of high-risk disease is considered indicated and appropriate. Similarly, if low-risk disease could be reliably identified, these patients would be radiographically monitored, and could avoid the morbidity of operation until high-risk disease developed.[23] In the current study, we developed and independently validated 2 separate CF biomarker models of dysplasia from 149 patients, who underwent resection at 3 different institutions, using antibody bead array (Luminex). Additionally, we demonstrated that incorporation of these 2 predictive CF marker models into a recently developed clinical and radiographic nomogram resulted in an even higher diagnostic discrimination for high-risk IPMN.[22] We believe that this is the first study to demonstrate the benefit of combining clinical, radiographic, and CF protein expression in defining high-risk IPMN.

Because of the limitations of imaging and cytology in defining high-risk IPMN, the diagnostic utility of CF analysis has been extensively evaluated.[11] A variety of protein markers including carcinoembryonic antigen (CEA), CA19–9, CA15–3, M1 mucin and amylase; as well as DNA and miRNA markers have been studied as potential diagnostic markers of pancreatic cyst subtype. Although some of these markers have shown the ability to identify mucinous lesions and differentiation of histopathologic subtype, their ability to discriminate grade of dysplasia in IPMN has not been encouraging.[24–29] For example, CF CEA level has

been consistently shown to be a useful predictor for identification of mucinous lesions with up to 79% accuracy and 85% accuracy when combined with the presence of extracellular mucin.[28,30] However, the degree of elevation of CF CEA has not been found to be predictive of the grade of dysplasia in these patients.[31–33] In a recent retrospective study from Japan, multivariate analysis showed a weak association between CEA level in the pancreatic fluid and invasive carcinoma in mixed and MD-IPMN (OR, 1.002; 95% CI, 1.000–1.003; P ¼ 0.048). The area under the curve (AUC) value for pancreatic fluid CEA level in mixed IPMN was 0.796 (P < 0.001), whereas the AUC for pancreatic fluid CEA in MD- IPMN was 0.877 (P < 0.001).[34] Multiple other studies developed clinical and radiographic nomograms to predict invasive disease in MD-IPMN and BD-IPMN with variable diagnostic accuracy.[35–37]

Our group has focused on CF protein expression as a tool for discrimination of grade of dysplasia in IPMN. Previous experiments have demonstrated consistent patterns of protein expression between low-risk and high-risk lesions.[17–19] For example, we previously evaluated the CF for inflammatory markers as a surrogate for tumor-associated neutrophils (TAN), as tissue studies from our group have identified an increased number of TAN in high-risk lesions.[38] These initial evaluations identified overexpression of inflammatory markers such as IL-1b, IL-5, and IL-8 in high-risk lesions; CF IL-1b remained a significant predictor of high-risk disease on multivariate analysis that included IPMN subtype; and CF inflammatory markers were associated with the degree of TAN.[17] High-mobility group A2 protein was also found to be significantly higher in CF of high-grade compared with low-grade IPMN.[39] Other groups found an association between neutrophil-to-lymphocyte ratio and risk of invasive disease in IPMN.[40–42]

More recently,[19] we evaluated the association of TAN with malignant progression in 78 patients with resected IPMN, and performed a multiplexed assay for 87 different CF proteins, including CF inflammatory markers (CFIM), as possible surrogate markers for parenchymal inflammation. The majority (96%) of the low-risk lesions demonstrated no TAN, whereas 89% of invasive lesions expressed high levels of TAN. The grade of dysplasia was also found to have positive correlation with 29 of the measured proteins, of which 23 (79%) were CFIM. These findings suggested that CFIM may be an excellent surrogate marker for the identification of the TAN-dysplasia association. Because many of the individual markers were overexpressed in high-risk lesions, we used multivariate modeling to develop predictive models and chose the 2 most promising models to validate in the current study.

This current study expands on and validates this previous work and, to our knowledge, is the first to describe a highly predictive biologic model for identification of high-risk IPMN based on CF protein expression analysis and clinical and radiographic factors. Our multi-institutional cohort of resected IPMN patients was typical of what is reported in the literature (31). The minority of patients had high-risk disease (n ¼ 60, 40%), and degree of dysplasia was associated with main duct involvement. The majority of patients with BD-IPMN (n ¼ 72, 83%) had low-risk disease, whereas most of MD-IPMN (n ¼ 45, 75%) were found to have high-risk disease on final pathological analysis. Other factors associated with high-risk disease included weight loss, jaundice, history of smoking, and the presence of solid component (P ○8 0.05). These are all well-known risk factors for high-risk disease and coincide with our findings in a clinical nomogram study as well as what others have previously published literature.[22,43,44]

The performance of the 2 multivariate models in the training and validation sets was encouraging as both Model-1 and Model-2 demonstrated a high predictive ability for identifying high-risk disease (c-indices 0.76, 0.80 and 0.80, 0.79, respectively for the training and validations sets). Additionally, all 4 markers were independently associated with the degree of dysplasia when variables from our clinical nomogram (which accounts for duct subtype) were added as a predictor in each model. Importantly, combining these protein-level models with our clinical-radiographic nomogram resulted in a higher predictive ability than any of the models or nomogram alone (Table 1).

The most recent consensus guidelines (2012 International Consensus Guidelines) for the management of IPMN rely on clinical and radiographic features, rather than biologic markers, as the primary predictive tools for identifying high-risk disease, and are typically referenced as a factor in the treatment decision making for patients with IPMN.[15] These guidelines consider the presence of pancreatitis, cyst diameter >3 cm in BD-IPMN, thickened or enhancing cyst walls, main duct diameter 5 to 9 mm, nonenhancing mural nodule, or abrupt change in caliber of pancreatic duct with distal pancreatic atrophy as "worrisome features." Factors considered to be "high-risk stigmata" include obstructive jaundice, enhancing solid component within cyst, and main duct dilation ≥10 mm. Resection is recommended for all patients with dilated MPD ≥10 mm, that is, MD-IPMN, and for any BD-IPMN >3 cm with "high-risk stigmata." Multiple reports however have shown that patients who undergo resection for MD-IPMN by these guidelines will have only a 50% to 60% chance of having high-grade dysplasia at the time of resection.[45] Conversely, high-grade dysplasia is present in approximately 10% to 20% of patients who undergo resection in the absence of a dilated pancreatic duct (branch duct IPMN).[12,14] Additionally, Aso et al investigated the associations between the malignant grade of IPMN and the number of high-risk stigmata and worrisome features (as outlined by the consensus guidelines), as well as the statistical significance of each factor for predicting malignancy, and found a high prevalence of malignancy in patients with MD-IPMN (64%) regardless of the presence or absence of high-risk stigmata.[44] The overall sensitivity and specificity of high-risk stigmata were 57% and 90% for predicting malignant IPMN (high-grade dysplasia or invasion) and 69% and 83% for predicting invasive carcinoma, respectively.[44] Similarly, a recent study from Japan found that the accuracy of the 2012 consensus guidelines for predicting malignancy in IPMN is 45%.[46] These findings highlight the shortcomings of the current consensus guidelines and suggest a persistent need for a better tool to identify those with high-risk IPMN. Given the findings of the current study, we believe that the combination of CF protein assessment and clinical and radiographic features may allow for a robust prediction model that can decrease the risk of "undertreatment" for high-risk IPMN lesions, which need to be resected to prevent progression into malignancy.

The most critical flaw in this study is that the samples were not obtained preoperatively. Most CF samples are obtained clinically using endoscopic ultrasound guidance. These fluid samples often contain contaminants from the gastrointestinal tract that may alter protein levels.[47] In addition, this study includes only patients with pathologically proven IPMN. The ability to preoperatively identify the histopathologic subtype of the given lesion (serous cystadenoma vs IPMN vs mucinous cystic neoplasm) is an additional dilemma that is only partially solved with CF CEA measurement. Previous work by our group has not found these markers to be elevated in other histopathologic subtypes; however, prospective assessment on all patients being considered for resection is needed, and a prospective study is ongoing.[26] Although prospective evaluation with EUS obtained samples will allow us to validate this approach in those undergoing resection, its applicability to patients who are selected for radiographic surveillance will remain unknown. The defined problem is that we cannot determine grade of dysplasia without resection,

and therefore those who are selected for radiographic surveillance cannot have their grade (or even histopathologic subtype) determined. Upcoming prospective studies will define a 3- to 5-year period of surveillance without progression as "success"; however, even this prospective evaluation will fall short and require large numbers of patients for validation.

CONCLUSIONS

This multi-institutional study validated 2 models, with high objective predictive ability, for the identification of high-risk IPMN based on CF protein expression. When combined with our clinical nomogram, the selected markers provided a stronger ability to predict high-risk disease than either the nomogram or CF markers alone. This study was relatively large in size, multi-institutional in nature, and included an independent dataset for validation. The latter served to decrease the risk of overfitting the models to our data and provided a fair and unbiased test of the models. Measurement of CF proteins can provide an accurate assessment of the degree of dysplasia, and with further development may be helpful for surgical decision-making in these patients. Given the consistent findings of elevated inflammatory markers in the CF of high-risk lesions in multiple studies, an anti-inflammatory strategy may be a reasonable approach to prevent IPMN progression.

ACKNOWLEDGMENTS

This work would not have been possible without the generous and unconditional collaboration between the 4 contributing institutions (Memorial Sloan Kettering, Johns Hopkins Hospital, Massachusetts General Hospital, and University of Pittsburgh Cancer Institute).

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Petrone et al. Clinicaland Translational Gastroenterology (2018)9:158
DOI 10.1038/s41424-018-0026-3

Clinicaland Translational Gastroenterology

ARTICLE Open Access

Corrected: Correction

Long-term follow-up of low-risk branch-duct IPMNsof the pancreas: is main pancreatic duct dilatation the most worrisome feature?

Maria Chiara Petrone[1], Pietro Magnoni[1], Ilaria Pergolini[2], Gabriele Capurso[3], Mariaemilia Traini[1], Claudio Doglioni[4], Alberto Mariani[1], Stefano Crippa[5] and Paolo Giorgio Arcidiacono[1]

Abstract

Objectives: The management of branch-duct IPMNremains controversial due to the relatively low rate of malignant degeneration and the uncertain predictive role of high-riskstigmata (HRS)and worrisome features (WFs) identified by the 2012 International Consensus Guidelines.Our aim was to evaluate the evolution of originallylow-risk(Fukuoka-negative)BD-IPMNsduring a long follow-upperiod in order to determine whether the appearance of any clinicalor morphological variables may be independently associated with the development of malignancy over time.

Methods: A prospectively collected database of all patients with BD-IPMNreferring to our Institute between 2002 and 2016 was retrospectively analyzed. Univariateand multivariate analysisof association between changes during follow-up, including appearance of HRS/WFs,and development of malignancy (high-grade dysplasia/invasive carcinoma) was performed.

Results: A total of 167 patients were selected for analysis,and seven developed malignant disease (4.2%).During a median follow-up time of 55 months, HRSappeared in only three cases but predicted malignancy with 100% specificity. Worrisome features, on the other hand, appeared in 44 patients (26.3%).Appearance of mural nodules and MPDdilatation >5 mm showed a significant association with malignancy in multivariate analysis ($p = 0.004$ and $p = 0.001$, respectively).MPDdilatation in particular proved to be the strongest independent risk factor for development of malignancy (OR= 24.5).

Conclusions: The riskof pancreatic malignancy in this population is low but definite. The presence of major WFs, and especially MPDdilatation, should prompt a tighter follow-up with EUSand a valid cytological analysis whenever feasible.

Introduction

Pancreatic cystic neoplasms (PCNs) have become very frequent incidental findings especially in the elderly population, thanks to technical improvements and extensive use of cross-sectional imaging[1,2]. Of all PCNs, intraductal papillary mucinous neoplasms (IPMNs) are

Correspondence: Maria Chiara Petrone (petrone.mariachiara@hsr.it)
[1]Pancreato-BiliaryEndoscopy and Endosonography Division,Pancreas Translational& ClinicalResearch Center, Vita-SaluteSan RaffaeleUniversity, IRCCSSan RaffaeleScientific Institute, Milan,Italy
[2]Department of Surgery, Università Politecnica delle Marche, Ancona, Italy
Full list of author information is available at the end of the article.

now the most frequent as their prevalence has been steadily increasing during the last decade[3,4].

IPMNs are well-acknowledged precursor lesions for pancreatic ductal adenocarcinoma (PDAC), with markedly different risk of malignant degeneration based on the pattern of ductal involvement. IPMNs arising in the main pancreatic duct (MD-IPMNs) have a significant malignant potential and an indication for surgical resection[5,6]. However, the vast majority of IPMNs arising in side ductal branches (BD-IPMNs) degenerate far less frequently, with a rate of ~3.7% and an estimated annual risk of 0.7% for patients undergoing non-operative management[7]. Although BD-IPMNs do represent a precancerous condition offering the opportunity to cure a pancreatic neoplasm before an incurable invasive cancer develops, their rate of degeneration is so low that the risks associated with pancreatic surgery might outweigh the benefits of resection. Therefore, the current management of BD-IPMNs consists of surveillance with therapeutic options based on the presence of specific morphologic features that are associated with the risk of malignancy.

International Consensus Guidelines (ICG) for the evaluation and management of IPMN were published in 2006 and later revised in 2012 after the 14th meeting of the International Association of Pancreatology in Fukuoka, Japan[5]. On that occasion, predictors of malignancy were stratified into two sets of variables bearing a different risk of degeneration, namely high-risk stigmata (HRS) and worrisome features (WFs). Similar definitions of predictive factors were proposed by the European guidelines for the management of PCNs in 2013[6]. These predictors mostly consist of basic morphological and clinical criteria. HRS include the presence of a mural nodule with demonstrated enhancement on either cross-sectional imaging or endoscopic ultrasound (EUS), jaundice, and a MPD dilatation ≥10 mm, and their presence warrants surgical resection in fit patients. The presence of less pronounced changes, i.e., BD-IPMN size ≥3 cm, mural nodules without demonstrated enhancement, cyst wall thickening, MPD dilatation between 5 and 9 mm and episodes of acute pancreatitis, define the diagnosis of WFS. In such cases the current guidelines suggest an evaluation with EUS, the imaging technique that has the highest resolution of the pancreas, offers the chance of fine-needle aspiration (EUS-FNA) and could help the selection of patients with an indication for surgical resection.

The problem clinicians are facing today is that diagnosis with FNA is reported to be successful in half of cases at most[8] and decision making mainly relies on imaging alone. Management of BD-IPMN is also controversial because the natural history of the disease and the effective role of each of the HRS/WFs in predicting the risk of malignant degeneration have yet to be fully understood. Considering that most IPMNs are incidental findings, it is not infrequent to diagnose cysts that already harbor at least one of the WFs (especially a size ≥3 cm), thus limiting the possibility to evaluate their role as predictive factors during the natural history of the disease.

The present study aims at evaluating the role of possible predictive variables of malignancy development in a carefully selected population of patients with BD-IPMNs which are "naïve" (i.e., Fukuoka-negative for the presence of HRS or WFs) at the time of diagnosis. The primary endpoint is to evaluate the association between development of malignancy and any clinical and radiological features, with particular regard to morphological changes during follow-up including the appearance of HRS and WFs defined as per the 2012 ICG.

Methods

Study design

This is a single-center retrospective cohort study performed on a prospectively collected database. We initially interrogated the database for patients enrolled in the Gastroenterology and Gastrointestinal Endoscopy Unit of San Raffaele Scientific Institute, Milan, Italy, who were at least once classified as having a certain or highly probable diagnosis of branch-duct IPMN between September 2002 and December 2016. Certain diagnosis was defined by a conclusive result of EUS-FNA cytology or histological examination of surgical specimens. A highly probable diagnosis was considered in presence of cystic lesions ≥5 mm communicating with the MPD, as clearly demonstrated by MRI/MRCP and/or EUS[9].

Only patients with BD-IPMNs without HRS or WFs (i.e., Fukuoka-negative) were selected for the analysis, and a follow-up endpoint of at least 24 months was established for patients who were managed non-operatively. Criteria for exclusion were therefore: (a) the presence of HRS or WFs at the time of diagnosis; (b) an inadequate follow-up, defined as the lack of any follow-up examinations, a follow-up time <24 months, or a lack of continuity in the follow-up (i.e., gaps greater than 24 months between consecutive examinations); (c) misdiagnosis, established either clinically by consecutive examinations or histologically on surgical specimens.

Data collected at the time of diagnosis included sex, age, family history of PDAC, history or presence of symptoms (abdominal pain, nausea and/or vomiting, unintentional weight loss), and baseline morphological features: unifocal/multifocal disease, main cyst location, main cyst size, and MPD caliber. Follow-up was performed by means of EUS, cross-sectional imaging and/or transabdominal ultrasound (US) depending on physician's preference, and the results were recorded in a dedicated database. The use of US was reserved for consistently low-risk BD-IPMNs showing no changes over a considerably

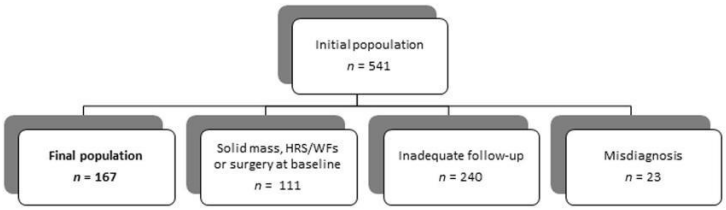

Fig. 1 Flow diagram illustrating the exclusion criteria applied to select cases for analysis. HRS high-risk stigmata, WFs worrisome features

Table 1 Demographic and clinical variables, diagnostic work-up, and baseline features of our low-risk branch-duct IPMN population

Baseline variables	Value
Age at diagnosis, median with range (years)	66 (33–84)
Sex	
Female, No. (%)	105 (62.9%)
Male, No. (%)	62 (37.1%)
Family history of PDAC, No. (%)	6 (3.6%)
History or presence of symptoms, No. (%)	31 (18.6%)
Pain, No. (%)	25 (15.0%)
Nausea/Vomiting, No. (%)	2 (1.2%)
Weight loss, No. (%)	4 (2.4%)
Incidental finding, No. (%)	136 (81.4%)
Unifocal disease, No. (%)	61 (36.5%)
Multifocal disease, No. (%)	106 (63.5%)
Two cysts, No. (%)	36 (21.6%)
≥three cysts, No. (%)	70 (41.9%)
Main BD cyst location	
Head and uncinate process, No. (%)	72 (43.1%)
Neck and body, No. (%)	78 (46.7%)
Tail, No. (%)	17 (10.2%)
Main BD cyst size, mean ± SD (mm)	14.5± 5.7
MPD caliber, mean ± SD (mm)	2.6± 0.8

BD branch-duct, EUS endoscopic ultrasound, FNAC fine-needle aspiration cytology, PDAC pancreatic ductal adenocarcinoma, SD standard deviation.

long span of time, so that the relatively low accuracy of the technique would not influence relevant outcomes.

The appearance of a defined pancreatic solid neoplasm, HRS or WFs during the follow-up were the main outcomes of interest. The following changes occurring during the follow-up were also recorded: (a) cyst size growth ≥5 mm between consecutive examinations; (b) appearance of new cysts; (c) size growth of any mural nodules ≥2 mm; and (d) appearance of new symptoms. The finding of high-grade atypia on EUS-FNA, when performed, was not equated to other clinical and radiological ICG criteria but rather considered as a definitive diagnosis of malignancy, and its role was assessed separately. In operated patients, lesions were classified as benign (low-/moderate-grade dysplasia) or malignant (high-grade dysplasia or invasive carcinoma) by histological examination.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 23.0 (Armonk, NY: IBM Corp). Kaplan–Meier estimates were used to describe progression-free survival in our population, considering appearance of WFs, and development of malignancy as the event of interest, respectively. Subgroup comparisons on categorical variables were performed using Pearson's χ^2 test or Fisher's exact test as appropriate. Features showing a significantly different frequency between subgroups were selected for analysis of association with malignancy. Binary logistic regression was used for univariate and multivariate analysis with a forward selection model. Results were considered to be statistically significant when p values were <0.05. Collinearity between significant predictors was tested by calculating the Spearman correlation coefficient and results <0.8 were considered acceptable.

Results

Descriptive statistics

From a total of 541 patients resulting from our initial research, 167 patients with BD-IPMNs without HRS or WFs and with available follow-up data were included in the study (see Fig. 1). Patients' characteristics and baseline features are summarized in Table 1.

Only 31 patients presented with symptoms, which makes most of the diagnosed BD-IPMNs "incidentalomas" (81.4%). Single lesions were found in 61 patients, whereas 106 patients (63.5%) had multifocal disease. The mean lesion size was 14.5 ± 5.7 mm, ranging from 5 to 29 mm (i.e., the cut-offs for diagnosis of BD-IPMN and the consideration of cyst size as "worrisome", respectively).

Table 2 Results of follow-up including frequency of changes and appearance of high-risk stigmata/worrisome features

Follow-up Variables	Value
Follow-up time, median with range (months)	55 (13–134)
Changes during follow-up, No. (%)	97 (58.1%)
Appearance of high-risk stigmata, No. (%)	3 (1.8%)
Enhanced mural nodule, No. (%)	1 (0.6%)
MPD caliber ≥10 mm, No. (%)	1 (0.6%)
Jaundice, No. (%)	1 (0.6%)
Time to develop HRS, median with range (months)	56 (45–60)
Appearance of worrisome features, No. (%)	44 (26.3%)
Cyst size ≥3 cm, No. (%)	21 (12.6%)
Cyst wall thickening, No. (%)	12 (7.2%)
Mural nodule, No. (%)	13 (7.8%)
MPD caliber 5–9 mm, No. (%)	10 (6.0%)
Acute pancreatitis, No. (%)	4 (2.4%)
Time to develop WFs, median with range (months)	26 (4–132)
Additional features	
Cyst growth ≥5 mm, No. (%)	59 (35.3%)
Appearance of new cysts, No. (%)	56 (33.5%)
Mural nodule growth ≥2 mm, No. (%)	2 (1.2%)
Appearance of new symptoms, No. (%)	7 (4.2%)
Diagnosis of high-grade atypia on EUS-FNA, No. (%)	4 (2.4%)

EUS-FNA endoscopic ultrasound-guided fine-needle aspiration, HRS high-risk stigmata, MPD main pancreatic duct, WF worrisome feature

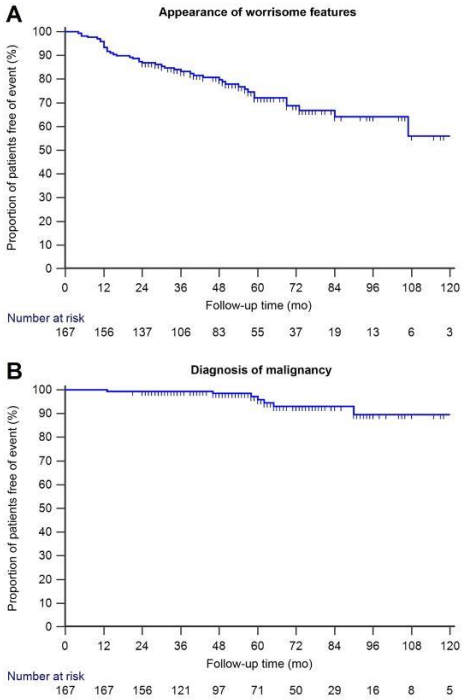

Fig. 2 Kaplan–Meier curves showing progression-free survival in our low-risk branch-duct IPMN population over a 10-year period. The events of interest were appearance of worrisome features (a) and development of malignancy (b), respectively

The mean MPD diameter was 2.6 ± 0.8 mm. These values comply with our selection criteria for low-risk BD-IPMNs.

The median follow-up time was 55 months ranging up to a maximum of 134 months (Table 2). Overall changes were reported in 97 patients (58.1%) after a median time from diagnosis of 25 months. Such changes mostly consisted of dimensional growth (35.3%) or appearance of new lesions (33.5%). HRS appeared in only three patients (1.8%) after a median follow-up time of 45 months. WFs, on the other hand, appeared in 44 patients (26.3%) after a median time of 26 months, with an extremely wide range (4–132 months). EUS-FNA was performed in 63 out of 167 patients (37.7%), and a positive cytology result (high-grade atypia) was obtained in four cases (2.4%).

Surgery was performed in eight patients (4.8%) after a median follow-up time of 52 months (range 13–90 months). The histological examination demonstrated benign disease in two of them, whereas malignancy was diagnosed in six cases (3 high-grade dysplasia, 3 carcinoma), which corresponds to 75% of operated patients. A single patient developed an inoperable solid mass, bringing the count to seven malignancies (4.2% of the overall study population). All other patients without a histological demonstration of malignant disease served as the control group for the analysis of association with malignancy. The progression-free survival curves for the appearance of WFs and the diagnosis of malignancy during follow-up are shown in Fig. 2.

In seven out of eight cases, EUS had a primary role in establishing the main indications for surgery: presence of mural nodules (5) with or without finding of high-grade atypia on EUS-FNA (3 out of 5), presence of a solid mass associated with jaundice and MPD dilatation (1), and rapid MPD caliber enlargement to ≥10 mm documented over two consecutive EUS examinations (1). The remaining case consisted of a large (40 mm) and rapidly growing (>5 mm/year) cyst studied by MRI/MRCP. Although most operated patients had BD-IPMNs with mural nodules detected by EUS, two cases of false positivity for malignancy and overtreatment were also

Table 3 Preoperative and operative details regarding nine patients who underwent surgery and/or received a final diagnosis of malignancy

Pt. #	Age (y)	Year of diagnosis	Time from diagnosis (mo)	Indication	Primary role of EUS	FNAC	Procedure	Histology
1	63	2005	13	WFs: nodule, cyst size ≥3 cm, cyst wall thickening	Yes	HGA	TP	IPMN-PDAC
2	65	2005	90	WFs: nodule, cyst wall thickening	Yes	HGA	DP	HGD (gastric type)
3	66	2007	58	HRS: jaundice WF: MPD 5–9 mm	Yes (detected solid mass)	LGA	PD	IPMN-PDAC
4[a]	72	2009	21	WFs: nodule, acute pancreatitis	Yes	—	DP	LGD (gastric type)
5[a]	46	2010	21	WFs: nodule, cyst size ≥3 cm	Yes	—	PD	IGD (intestina type)
6[b]	81	2010	50	WF: MPD 5–9 mm	Yes (detected solid mass)	HGA	——	
7	52	2011	60	WF: cyst size ≥3 cm (10 mm) Cyst size growth >5 mm/y	No	—	PD	HGD (intestinal type)
8	60	2011	46	WF: MPD 5–9 mm rapidly escalated to HRS: MPD ≥10 mm	Yes	—	PD	IPMN-PDAC
9	57	2011	62	HRS: enhancing nodule	Yes	HGA	PD	HGD (gastric type)

The indications for surgery were established by endoscopic ultrasound in seven out of eight cases. Presence of any other worrisome features was reported, although they would not drive surgical decision per se
DP distal pancreatectomy, *EUS* endoscopic ultrasound, *FNAC* fine-needle aspiration cytology, *LGA* low-grade atypia, *LGD* low-grade dysplasia, *HGA* high-grade atypia, *HGD* high-grade dysplasia, *HRS* high-risk stigmata, *IGD* intermediate-grade dysplasia, *MPD* main pancreatic duct, *PD* pancreaticoduodenectomy, *PDAC* pancreatic ductal adenocarcinoma, *TP* total pancreatectomy, *WF* worrisome feature
[a]Surgical overtreatment of patients with low- to intermediate-grade dysplasia on histological examination
[b]Inoperable patient with pancreatic ductal adenocarcinoma distinct from IPMN

registered in this subgroup (1 low-grade dysplasia, 1 intermediate-grade dysplasia). In those two cases, EUS-FNA had not been performed. EUS was also able to detect solid masses that had been described by contrast-enhanced CT as undefined parenchymal changes in two cases, consisting of one case of operated IPMN-carcinoma and one case of inoperable PDAC distinct from IPMN which developed over a 6-month time span. Further details about preoperative and operative data are listed in Table 3.

Analysis of association with malignancy

Among all features described during the follow-up, a statistically significant difference in frequency in patients with malignant disease was found for presence of MPD dilatation ($p = 0.005$ for MPD 5–9 mm, $p = 0.042$ for MPD ≥10 mm), mural nodules ($p = 0.011$ for mural nodules without demonstrated enhancement, $p = 0.042$ for enhanced mural nodules, $p = 0.002$ for mural nodule growth) and jaundice ($p = 0.042$). A cyst size ≥3 cm in particular did not impact the frequency of malignancy ($p = 0.215$) (Table 4).

Significant variables were included in the univariate analysis adjusted for sex and age. The role of HRS was clear as they predicted malignancy with 100% specificity, but the fact that each of them was present in only one patient spoiled their statistical power in logistic regression. Therefore, only mural nodules and MPD dilatation 5–9 mm were selected by the forward logistic model in multivariate analysis. As discussed above, the role of EUS-FNAC was assessed separately. All four cases of cytological high-grade atypia were proven to harbor malignant disease, which made cytology (when available) the strongest predictor of malignancy with 100% specificity. Nonetheless, inclusion of a positive cytology result in the multivariate analysis did not alter the model and both mural nodules and MPD dilatation remained as independent risk factors associated with the development of malignancy (OR 17.0, $p = 0.004$ and OR 24.5, $p = 0.001$, respectively) (Table 5). A Spearman correlation coefficient <0.8 excluded collinearity between the two predictors. MPD dilatation 5–9 mm resulted to be the strongest predictor for diagnosis of malignancy.

Discussion

The aim of the present study was to retrospectively evaluate the long-term follow-up of patients with originally low-risk (Fukuoka-negative) BD-IPMNs, in order to determine the significance of the appearance of several clinical and morphological variables as predictors of the development of pancreatic malignancy.

Our series confirms the importance of BD-IPMN as a relatively common incidental finding (81.4% of patients were asymptomatic). More than half of our population

showed changes over a median follow-up period of ~4.5 years. Most of these changes were proven harmless, consisting of dimensional growth or appearance of new lesions, which were not found to be significant risk factors for malignancy per se and were likely influenced by inter-observer variability and by the resolution of different imaging modalities. Nonetheless, 4.2% of patients developed a malignant disease during follow-up, in some cases even after a long time span. Therefore, as recently reported elsewhere[10,11], there is currently insufficient data to safely cease surveillance of BD-IPMN, no matter how stable or innocuous the lesion may appear. At the same time our study shows that many BD-IPMNs do not progress over time, so intensive follow-up protocols may prove not to be cost-effective.

Though not specifically aimed at comparison of guidelines, the present study paves the way for validation of the 2012 International Consensus Guidelines. Our analysis of HRS as predictors of malignancy provided significant results but was hampered by the little proportion of patients showing such features (3 out of 167). This may be informative by itself, as it shows that very few patients with apparently innocuous BD-IPMNs are diagnosed with HRS over time.

Cyst size ≥3 cm is generally considered the most controversial worrisome feature[12]. There is no shortage of studies denying a role for this variable as a risk factor for malignant degeneration in BD-IPMNs without other WFs[13,14]. However, recent meta-analyses present cyst size ≥3 cm as a significant risk factor, with values of OR widely ranging from 2.3[15] to 62.4[16]. In our cohort, trespassing the 3-cm threshold was found to have no significant association with malignancy. This would support the consideration of cyst size as the weakest morphological predictor of malignancy when using the present 3-cm cut-off set by the ICG. Nonetheless, there was a single case of BD-IPMN displaying only a large cyst size as WF that proved to harbor malignancy. The cyst had reached a maximum diameter of 40 mm and had shown a >5 mm/year growth rate prior to surgery. This fact remains noteworthy and prompts the choice of a different cut-off and/or inclusion of growth rate as a collateral parameter as advocated by the European guidelines[6], as well as by the latest revision of the ICG in 2017[17].

The appearance of mural nodules and the increase in MPD diameter during the follow-up were the only WFs, which were selected by our forward logistic model and were found to be significantly associated with development of malignancy both in univariate and multivariate analysis ($p = 0.001$ and $p = 0.004$, respectively). Results reported by Jang et al. in a large surgical series (n = 350) were very similar to ours and identified a significant association for MPD dilatation >5 mm, presence of a mural nodule and cyst wall thickening in univariate analysis (all p values <0.001). MPD dilatation and mural nodules were confirmed by multivariate analysis (HR 4.54, $p < 0.001$ and HR 6.26, $p < 0.001$, respectively)[18]. A recent meta-analysis by Ricci et al. found the highest values of pooled diagnostic odds ratio (DOR) for jaundice (6.3), presence of mural nodules (4.8), cyst wall thickening (4.2), and MPD dilatation (4.0)[19]. Similarly, another meta-analysis by Kim et al. found the highest pooled DOR for mural nodules (6.0) followed by MPD dilatation (3.4), and

Table 4 Different frequency of changes and appearance of high-risk stigmata/worrisome features in patients with malignant disease compared to controls

Variable	Control Group (n = 160)	Malignancy (n = 7)	p value
High-riskstigmata	0	3	
Enhanced mural nodule	0	1	0.042
MPD caliber ≥10 mm	0	1	0.042
Jaundice	0	1	0.042
Worrisome features	37	7	
Cyst size ≥3 cm	19	2	0.215
Cyst wall thickening	10	2	0.081
Mural nodule	10	3	0.011
MPD caliber 5–9 mm	7	3	0.005
Acute pancreatitis	4	0	1.000
Additional features			
Family history of PDAC	6	0	1.000
History of symptoms	31	0	0.351
Multifocaldisease	101	5	1.000
Cyst growth	57	2	1.000
Appearance of new cysts	56	0	0.097
Mural nodule growth	0	2	0.002
New symptoms	6	1	0.263
High-grade atypia on EUS-FNA	0	4	<0.001

p values refer to subgroup comparisons with Pearson's χ^2 test or Fisher's exact test

EUS-FNAendoscopic ultrasound-guided fine-needle aspiration, PDACpancreatic ductal adenocarcinoma

p values which were <0.05 implying statistical signi$_{fi}$cance were highlighted in bold.

Table 5 Logistic regression analysis of possible predictors of malignancy

Variable	Multivariate analysis		
	OR	95% CI	p value
Mural nodule	17.02	2.44–118.59	0.004
MPD caliber 5–9 mm	24.48	3.40–176.31	0.001

Only mural nodules and MPD dilatation 5–9 mm were retained by the forward selection model for multivariate analysis
CI confidence interval, MPD main pancreatic duct, OR odds ratio
p values which were <0.05 implying statistical significance were highlighted in bold

thickened/enhancing cyst walls (2.3). Therefore, the authors advocated a more aggressive approach to mural nodules and a watchful waiting for the other features[15]. When compared to these meta-analyses, our results indicate that MPD dilatation is the strongest predictor of malignancy with an odds ratio which is markedly higher than those of other examined factors (OR 24.5).

The finding of mural nodules on EUS represented the most common indication for surgery in our cohort. The unparalleled sensitivity of EUS in detecting nodules is now widely acknowledged and was recently underlined by Ridtitid et al. in a large single-center study of 364 patients with BD-IPMN, where mural nodules identified by EUS were missed by CT/MRI in 28% of cases in the malignant group[20]. However, a pitfall lies in the classification of nodules as enhanced (HRS) or non-enhanced (WF) with EUS, as the use of intravenous ultrasound contrast agents was introduced relatively recently and is far less routinary than it is for cross-sectional imaging. Considering that our cohort encompasses cases studied more than a decade ago, it is likely that at least some of the nodules (presumably, the ones in the subgroup of operated patients) would have been found positive had an enhancement study been performed. The importance of demonstrating nodule enhancement in an attempt to increase the positive predictive value (PPV) of this predictor is emphasized by the slight change made in the 2017 revision of the ICG. Only enhanced mural nodules on cross-sectional imaging are now mentioned, and their classification as HRS or WF is now based on nodule size with a 5-mm cut-off[17].

The role of MPD dilatation as a predictor of malignancy directly relates to the controversy that lies in the definition of this variable both as a worrisome feature for BD-IPMN and a criterion to define mixed-type IPMN according to the 2012 ICG. If we consider that mixed IPMN shares the high rates of malignant degeneration of MD-IPMN and should be similarly committed to surgical resection, the appearance of MPD dilatation in a lesion formerly diagnosed as BD-IPMN leads to a therapeutic dilemma. A dilatation greater than 10 mm bears a markedly higher risk of malignancy, but it is a late sign which is actually found in very few cases. On the other hand, a dilatation above 5 mm is a far more frequent finding, but its meaning is not univocal and its PPV for malignancy is limited[21]. This inverse relationship between feature prevalence and PPV was recently confirmed in a study by Ma et al. considering 239 patients with PCNs (including 163 IPMNs) who underwent surgical resection. In their series MPD dilatation ≥10 mm had a high PPV (72.7%) but was present in only 11 patients, whereas MPD dilatation 5–9 mm had a markedly lower PPV (44.1%) but was a much more frequent finding (59 out of 239 operated patients, 24.7%)[22]. Remarkably, MPD dilatation is currently valued as a risk feature regardless of the underlying cause, which may be direct tumor involvement or ductal hypertension caused by mucin, protein plugs, or focal pancreatitis. The occurrence of pure BD-IPMNs with MPD dilatation but without MPD disease can be demonstrated on pathology only, but this information cannot be obtained by imaging for all patients undergoing surveillance, which represents the most common clinical setting. Crippa et al. recently showed that classifying a BD-IPMN with MPD >5 mm as a mixed-type may lead to significant overdiagnosis of mixed-IPMN (8/93, 8.6%) and overtreatment of otherwise harmless BD-IPMNs (2/93, 2.1%)[23]. On the other hand, minimal MPD involvement may be demonstrated as an incidental finding on histology in many cases of radiologically diagnosed BD-IPMNs, although it may not imply a more aggressive biology than pure BD-IPMNs[24]. If we consider the findings in our cohort, in one case of malignancy an initial MPD dilatation was rapidly followed by progressive enlargement to more than 10 mm. This would strongly suggest active MPD involvement, which was found on histological examination. In the other two cases, a thorough EUS evaluation proved MPD dilatation to result from passive retrograde dilatation caused by a solid mass that had not been clearly defined by previous imaging. These findings would lead to question the consideration of MPD dilatation as an "independent" risk factor rather than a sentinel for the presence of other factors which are eluding present understanding of the clinical condition. The topic remains controversial and warrants further clarification of the role of MPD dilatation in the natural history of the disease.

Differently than clinical and radiological features, EUS-FNA allows to directly assess the presence of malignancy within the specimen, the main limitation of this procedure being its low sensitivity. However, once a representative sample is obtained, its results may be considered as a definitive diagnosis with almost absolute specificity. Indeed, high-grade atypia on cytology was found to be a strong predictor of malignancy with 100% concordance. In the above-mentioned meta-analysis by Ricci et al. a positive cytology result had a DOR of 5.5 and the sensitivity, specificity, PPV, and negative predictive value of

EUS-FNAC were estimated to be 34.7%,87.8%,56.1%,and 75%, respectively[19]. This would lead to the consideration that the finding of WFs in BD-IPMN patients should prompt all attempts to obtain a valid cytological analysis in order to individualize management.

The present study was not designed for comparison or validation of guidelines. Nonetheless, it may be interesting to consider our results in light of the recently published American Gastroenterological Association Institute guidelines on the diagnosis and management of asymptomatic pancreatic cystic neoplasms[25]. After extensive technical review of the literature[26], these guidelines proposed a conservative approach with indication for surgery only in the presence of two at least out of three high-risk features (cyst size ≥3 cm, MPD dilatation, solid component) confirmed by EUS and/or in presence of a positive cytology result. Mural nodules and MPD dilatation, which had high pooled specificity values reported in the review (80% and 91%, respectively), are also the two significant risk factors outlined by our study. The authors of the AGA guidelines estimated a specificity of 95% for malignancy using their combination criterion. However, Singhi et al. retrospectively applied them to a study cohort of 41 patients with pancreatic cystic lesions referred for EUS-FNA and available pathology results (including 23 IPMNs), and found only a sensitivity of 62% and a specificity of 79% for malignant disease. In their series, application of the AGA guidelines would have deferred surgery for five out of 11 (45.4%) cases of malignant IPMNs (4 carcinoma, 1 high-grade dysplasia) and would not have prevented unnecessary surgery for two out of 12 cases of IPMN with low-grade dysplasia (16.7%)[27]. Similar results were provided by Ge et al. in a multicenter study of 300 patients with PCNs (including 198 IPMNs), where retrospective application of the AGA guidelines had 83.3% sensitivity and 69.1% specificity for malignancy. Although the guidelines accurately recommended surveillance in 95% of patients, nine invasive cancers (5%) would have been missed[28]. If retrospectively applied to our subgroup of operated patients, the AGA guidelines would not have spared the two patients with low-/intermediate-grade dysplasia from overtreatment because of the copresence of mural nodule and cyst size ≥3 cm in one case and symptoms (acute pancreatitis) in the other. Conversely, two cases of malignancy would have been missed (one case with rapidly growing cyst size and one case with rapidly enlarging MPD caliber). These results would indicate that even just one of the morphological high-risk features may be worrisome per se and the limitation of having at least two to consider surgery may be too strict.

The most important caveat with the AGA guidelines emerges from the advocated surveillance protocol, which is very loose for cysts not classified as being "high-risk". The proposed follow-up consists of MRI in one year and every two years thereafter for a total of only five years, provided that the cyst remains unchanged. However, recent evidence suggests that HRS and WFs may appear well after this time span. A study on long-term follow-up of BD-IPMN by Pergolini et al. found that 20 out of 363 patients (5.5%) developed malignancies after five years of surveillance. More importantly, malignancies developed in 12 out of 282 patients (4.3%) who had absence of HRS/WFs at the five-year threshold and would develop them later, with a median time of 93 months [29]. Similar results were reported in a multicenter study of IPMNs followed-up for more than five years by Crippa and colleagues[11]. In our cohort, most changes developed within the first five years (the median time was 25 months), but there were also cases of WFs appearing after this time span in previously unchanged BD-IPMNs. Discontinuation of follow-up after five years would have missed eight patients developing WFs, including one case with MPD dilatation and one with appearance of a mural nodule. Although all eight patients were still in follow-up and in the control group for analysis at the time of the study, the authors would recommend against generalizing results and setting a threshold for discontinuation of follow-up.

The present study has major flaws due to its retrospective nature. Our study period encompassed 14 years during which clinical decision making and patient management changed progressively according to publication of guidelines and further understanding of the natural history of the disease. Also, some considerations should be made for a careful interpretation of our results. As expected, the rate of malignancy arising in this "naïve", low-risk BD-IPMN population was low, providing us with few cases developing the outcomes of interest, and ultimately limiting the power of our statistical analysis. Follow-up was performed by means of EUS in our Unit, but any credible reports of cross-sectional imaging and trans-abdominal ultrasound were accepted as well. It may be argued that US has lower accuracy and cannot be considered equally reliable. However, follow-up with US was only employed for consistently low-risk and unchanged cysts. This approach is somewhat justified by the 2014 AIGO-AISP Italian Consensus Guidelines, which suggest the use of US for single lesions that are clearly visible in alternation with MRI/MRCP [30]. It was not our goal to compare the sensitivity and specificity of different techniques, and we valued actual clinical practice over inter-observer and inter-technique variability.

In conclusion, this study shows that more than half of patients with low-risk BD-IPMN develop changes over a long period of time. Although many of these changes are actually harmless, consisting of cyst size growth and finding of new lesions, over one quarter of patients develop HRS/WFs, and the risk of malignancy in this population remains low but not negligible (4.2%). This

would discourage discontinuation of surveillance after five years as proposed by the 2015 AGA guidelines. The appearance during follow-up of WFs as indicated by the 2012 ICG resulted to be worrisome indeed, and would justify a tighter follow-up with EUS. MPD dilatation in particular proved to be the strongest independent factor associated with malignancy. In presence of such features, considering the safety and feasibility of EUS-guided FNA in tertiary referral centers, all efforts should be made to obtain an adequate cytological analysis whenever feasible.

Study Highlights

What is current knowledge

- Management of branch-duct IPMN has become more and more conservative.
- Surgical decision mainly relies on the presence of clinical and morphological predictors of malignancy described by different guidelines.
- The 2012 International Consensus Guidelines first stratified such predictors into high-risk stigmata and worrisome features, but their role in defining the risk of pancreatic malignancy remains unclear.

What is new here

- More than one quarter of patients with initially low-risk BD-IPMN developed high-risk stigmata or worrisome features over time and even after a long follow-up period.
- High-risk stigmata and positive cytology results were rare but associated with malignancy with 100% concordance.
- Among worrisome features, mural nodules and especially MPD dilatation >5 mm proved to be the strongest predictors of pancreatic malignancy.

Author details
[1]Pancreato-BiliaryEndoscopy and Endosonography Division,Pancreas Translational &ClinicalResearch Center, Vita-SaluteSan RaffaeleUniversity,IRCCSSan Raffaele Scientific Institute, Milan, Italy. [2]Department of Surgery, Università Politecnica delle Marche, Ancona, Italy. [3]Digestive and Liver Disease Unit, S. Andrea UniversityHospital,Rome, Italy. [4]Department of Pathology, IRCCSSan Raffaele Scientific Institute, Milan, Italy. [5]Pancreas Translational & Clinical Research Center, Divisionof Pancreatic Surgery, Università Vita-Salute,IRCCS San RaffaeleScientific Institute, Milan,Italy

Competing interests

Guarantor of the article: Maria Chiara Petrone, MD.

Specific author contributions: Planning and conducting the study, collecting and interpreting data, and drafting the manuscript: Maria Chiara Petrone, Pietro Magnoni; performing the statistical analysis and interpreting data, Ilaria Pergolini; performing the statistical analysis, interpreting data, and critical review of the manuscript: Gabriele Capurso; conducting the study, collecting and interpreting data: Mariaemilia Traini, Claudio Doglioni, Alberto Mariani, Stefano Crippa; planning and conducting the study, interpreting data, and critical review of the manuscript: Paolo Giorgio Arcidiacono. All listed authors approved the final draft submitted.

Financial support: Gabriele Capurso received granting from AIRC(IG Grant 2015, 17177).

Potential competing interests: None.

Received: 5 December 2017 Revised: 14 March 2018 Accepted: 23 April 2018
Published online: 13 June 2018

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J Gastrointest Surg
DOI 10.1007/s11605-017-3602-z

2017 SSAT PLENARY PRESENTATION

Intraductal Papillary Mucinous Neoplasm of the Pancreas in Young Patients: Tumor Biology, Clinical Features, and Survival Outcomes

Vicente Morales-Oyarvide[1] &Mari Mino-Kenudson[2] &Cristina R. Ferrone[1] & Andrew L. Warshaw[1] &Keith D. Lillemoe[1] &Dushyant V. Sahani[3] &Ilaria Pergolini[1] & Marc A. Attiyeh[4] &Mohammad Al Efishat[4] &Neda Rezaee[5] &Ralph H. Hruban[7,6] & Jin He[5] &Matthew J. Weiss[5] &Peter J. Allen[4] &Christopher L. Wolfgang[5] & Carlos Fernández-del Castillo[1]

Published online: 18 October 2017

Received: 11 May 2017 /Accepted: 25 September 2017

Abstract

Aim The aim of this paper is to describe the characteristics of intraductal papillary mucinous neoplasms (IPMNs) of the pancreas in young patients.

Methods We evaluated 1693 patients from the Pancreatic Surgery Consortium who underwent resection for IPMN and classified them as younger or older than 50 years of age at the time of surgery. We assessed the relationship of age with clinical, radiological, pathological, and prognostic features.

Results We identified 90 (5%) young patients. Age was not associated with differences in main pancreatic duct size (P = 0.323), presence of solid components (P = 0.805), or cyst size (P = 0.135). IPMNs from young patients were less likely to be of gastric type (37 vs. 57%, P = 0.005), and more likely to be of oncocytic (15 vs. 4%, P = 0.003) and intestinal types (44 vs. 26%, P = 0.004). Invasive carcinomas arising from IPMN were less common in young patients (17 vs. 27%, P = 0.044), and when present they were commonly of colloid type (47 vs. 31% in older patients, P = 0.261) and had better overall survival than older patients (5-year, 71 vs. 37%, log-rank P = 0.031).

Conclusion Resection for IPMN is infrequent in young patients, but when they are resected, IPMNs from young patients demonstrate different epithelial subtypes from those in older patients and more favorable prognosis.

Keywords IPMN · Pancreatic cystic neoplasms · Young

Presented at the Society for Surgery of the Alimentary Tract (SSAT) Plenary Session at the Digestive Disease Week 2018 on May 7th, 2017 in Chicago, IL.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11605-017-3602-z) contains supplementary material, which is available to authorized users.

* Carlos Fernández-del Castillo
cfernandez@partners.org

[1] Department of Surgery, Massachusetts General Hospital and Harvard Medical School, 55 Fruit Street, Wang Ambulatory Care Center 460, Boston, MA 02114, USA

[2] Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

[3] Department of Radiology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

[4] Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[5] Department of Surgery, The Sol Goldman Pancreatic Cancer Research Center, Johns Hopkins University School of Medicine, Baltimore, MD, USA

[6] Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

[7] Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Introduction

Intraductal papillary mucinous neoplasms (IPMNs) were first described by Ohashi et al. in the 1980s and were commonly reported in older men showing main pancreatic duct (MPD) dilatation with mucin extrusion through the ampulla of Vater.[1–4] In recent years, there has been mounting evidence of biological heterogeneity within IPMN, and four epithelial subtypes have been described: gastric, pancreatobiliary, intestinal, and oncocytic. Each appears to be associated with unique genetic and clinicopathological features.[5–14] Invasive carcinomas arising from these different subtypes also exhibit unique features with prognostic relevance.[6,7,13,14] Thus, a better understanding of the patient characteristics associated with each of the IPMN subtypes may help to tailor treatment and surveillance strategies.

Genetic and epidemiological studies of other cancer types including colorectal, lung, and breast cancers have shown that tumors are genetically different in young and older patients.[15–17] IPMNs are usually diagnosed and resected in patients in the seventh decade of life, but growing awareness of this entity and widespread use of cross-sectional imaging modalities have made it increasingly clear that IPMNs are not only present in older men with a florid clinical presentation. In fact, most IPMNs currently diagnosed are incidentally discovered.[18] While diagnosis of an IPMN remains uncommon in young individuals, it is unclear whether they have different clinical and pathological features compared to IPMNs in older patients. In the present study, we sought to evaluate the clinical, radiological, pathological, and prognostic characteristics of young patients undergoing resection for IPMN at three academic institutions in the USA.

Materials and Methods

Study Population

The study population was derived from prospective databases of patients undergoing surgical resection for IPMN at the institutions conforming the Pancreatic Surgery Consortium, namely: Massachusetts General Hospital (MGH; Boston, MA), the Johns Hopkins Hospital (JHH; Baltimore, MA), and Memorial Sloan Kettering Cancer Center (MSKCC; New York, NY). Surgery was performed at MGH for 472 patients between March 9, 1990 and July 30, 2015; at JHH for 766 patients between January 31, 1995 and January 15, 2016; and at MSKCC for 455 patients between February 21, 1989 and August 24, 2015. Institutional Review Board approval was granted or waived at each institution.

Definition of Age Groups

We classified patients based on their age at the time of surgery. We first examined the age distribution in our study cohort using a frequency histogram (Fig. 1). The median age at the time of surgery in our patient population was 69 years, which is comparable to other contemporary series of resected IPMNs.[19–21] Seeking to capture the youngest patients in our population while maintaining statistical power, we divided our cohort as younger or older than 50 years of age, which corresponds approximately to the 5th percentile. In post hoc sensitivity analyses, we replicated the main analyses using age cutoffs of 55 and 60 years of age.

Clinical and Radiological Assessments

From medical records, we collected data about patient sex, history of abdominal pain and jaundice as presenting symptoms, weight loss, type of pancreatic resection, and year of surgery. Patients without preoperative abdominal pain, jaundice, or weight loss were considered Basymptomatic.^ We obtained preoperative radiological assessments from the most recent abdominal computed tomography (CT), magnetic resonance (MR), or endoscopic ultrasound (EUS) prior to surgery. We evaluated the main pancreatic duct (MPD) diameter, size of the largest cyst, presence of enhancing solid components/mural nodules within the largest cyst, and evidence of cyst wall thickening or enhancement. Since cross-sectional imaging techniques (CT and MR) and EUS may exhibit discrepancies in measurements of dimensions, we only considered MPD diameter and largest cyst size measurements obtained from the most recent CT or MR.

Pathological Evaluation

All resected specimens underwent comprehensive gross and microscopic pathological examination following the same methodology across centers. The grade of dysplasia was classified as low-/moderate-grade dysplasia, high-grade dysplasia (formerly carcinoma in situ), or invasive carcinoma arising in association with an IPMN. High-grade dysplasia was defined as severe architectural complexity with micropapillary structures and/or cribriforming, significant nuclear atypia with loss of polarity and/or nuclear pleomorphism, and mitotic activity without evidence of invasion. Invasive carcinoma arising in association with an IPMN was defined as lesions with micro- or macroscopic invasion into the stroma beyond the epithelial basement membrane in direct continuity with areas of high-grade dysplasia. The predominant histological subtype of the precursor component was classified as gastric, intestinal, oncocytic, or pancreatobiliary; tumors with no clear predominant subtype were classified as Bmixed^.[6,7] Invasive

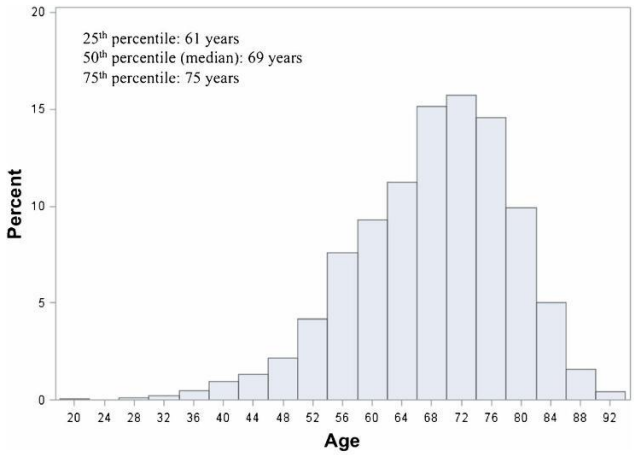


Fig. 1 Frequency distribution of age at the time of surgery in 1693 patients with resected intraductal papillary mucinous neoplasms

carcinomas arising from IPMN were classified as tubular, colloid, or oncocytic.[6,7]

Outcome Measures

The main outcome measures in this study were (1) highest grade of dysplasia, (2) predominant histological subtype of the precursor lesions and of the invasive carcinoma arising from IPMN, and (3) disease-specific (DSS) and overall survival (OS). For analyses of the highest grade of dysplasia, we evaluated high-grade dysplasia and invasive carcinomas as two distinct outcomes (i.e., not combined into a single category). For analyses of predominant histological subtypes, the comparisons were made between each individual subtype vs. all others combined (e.g., gastric-type vs. other precursor subtypes combined, colloid carcinoma vs. other invasive subtypes combined). DSS analyses were performed in patients with non-invasive IPMN (i.e., tumors with low-/moderate- or high-grade dysplasia), and it was defined as time between surgery and pancreatic cancer-related death; OS was defined as time between surgery and death from any cause; given that death from non-IPMN related causes is expected to be higher among older patients—particularly among older patients with non-invasive tumors—we restricted OS analyses to patients with invasive carcinomas arising in association with an IPMN.

Statistical Analyses

We evaluated the associations of age at surgery with grade of dysplasia and predominant histological subtypes using chi-square and Fisher's exact tests. Survival analyses were conducted using the log-rank test and Cox proportional hazards regression obtaining hazard ratios (HR) and 95% confidence intervals (CI). Survival statistics are also presented using Kaplan-Meier curves and 2- and 5-year survival rates. All hypothesis tests were two-sided and statistical significance was set at $P \leq 0.05$. Statistical analyses were performed using SAS software (version 9.4; SAS Institute, Cary, NC).

Results

The baseline characteristics of our study population overall and by age at surgery are presented in Table 1. There were no sex differences between the two age groups (women, 50 vs. 50%). Young patients were significantly more likely to present with abdominal pain (54 vs. 34%, $P < 0.001$), less likely to present with obstructive jaundice (0 vs. 11%, $P = 0.003$), and had similar rates of weight loss (24 vs. 30%, $P = 0.349$) compared with older patients. Overall, 41% of the patients in our study were asymptomatic and they were distributed similarly between young and older patients (34 vs. 42%, $P = 0.240$).

Location of the IPMN in the pancreatic head and uncinate process was significantly more common among young patients, and accordingly, patients in this age group were significantly more likely to have undergone a pancreaticoduodenectomy (80 vs. 63%, $P = 0.007$) compared to older ones. Radiologically, there were no significant differences between the two age groups in terms of MPD diameter, presence of enhancing solid components/mural nodules, thickened or enhancing cyst walls, and largest cyst size (Table 1).

Our study population spans a 27-year period, over which surgical indications and surveillance strategies for patients with IPMN have experienced important changes.[2,22–24] At our institutions, we have mostly followed the recommendations of the International Consensus Guidelines put forth first in

Table 1 Baseline characteristics of 1693 resected IPMN based on age at resection

	Overall	Age		
		< 50 years	≥ 50 years	P[a]
No. of patients	1693	90	1603	
Women, n (%)	851 (50%)	45 (50%)	806 (50%)	0.959
Abdominal pain, n (%)				
Yes	511 (35%)	39 (54%)	472 (34%)	< 0.001
No	935 (65%)	33 (46%)	902 (66%)	
Unknown	247	18	229	
Jaundice, n (%)				
Yes	155 (11%)	0 (0%)	155 (11%)	0.003
No	1289 (89%)	72 (100%)	1217 (89%)	
Unknown	249	18	231	
Weight loss, n (%)				
Yes	369 (29%)	16 (24%)	353 (30%)	0.349
No	888 (71%)	50 (76%)	838 (70%)	
Unknown	436	24	412	
Type of pancreatic resection, n (%)				
Pancreaticoduodenectomy (Whipple procedure)	1070 (64%)	70 (80%)	1000 (63%)	0.007
Middle or distal pancreatectomy	498 (30%)	15 (17%)	483 (30%)	
Total pancreatectomy	111 (6%)	3 (3%)	108 (7%)	
Unknown	14	2	12	
MPD size, n (%)				
MPD < 5 mm	498 (50%)	28 (61%)	470 (50%)	0.323
5 mm ≤ MPD < 10 mm	289 (29%)	11 (24%)	278 (29%)	
MPD ≥ 10 mm	206 (21%)	7 (15%)	199 (21%)	
Unknown	700	44	656	
Mural nodule/solid component, n (%)				
Yes	245 (22%)	11 (21%)	234 (22%)	0.805
No	862 (78%)	42 (79%)	820 (78%)	
Unknown	586	37	549	
Thickened/enhancing cyst walls, n (%)				
Yes	110 (10%)	4 (8%)	106 (10%)	0.551
No	997 (90%)	49 (92%)	948 (90%)	
Unknown	586	37	549	
Cyst size (cm), median (IQR) (n = 646)	2.90 (1.79)	2.54 (1.55)	2.90 (1.80)	0.135
Predominant precursor epithelial subtype, n (%)				
Gastric	544 (55%)	19 (37%)	525 (57%)	< 0.001
Intestinal	265 (27%)	23 (44%)	242 (26%)	
Oncocytic	49 (5%)	8 (15%)	41 (4%)	
Pancreatobiliary	47 (5%)	1 (2%)	46 (5%)	
Mixed	76 (8%)	1 (2%)	75 (8%)	
Unknown	712	38	674	
Highest grade of dysplasia, n (%)				
Low/moderate grade	837 (50%)	47 (54%)	790 (50%)	0.103
High grade	381 (23%)	25 (29%)	356 (23%)	
Invasive carcinoma	440 (27%)	15 (17%)	425 (27%)	
Unknown	35	3	32	
Histological type of invasive carcinoma, n (%)				
Tubular	275 (66%)	7 (47%)	268 (67%)	0.155
Colloid	134 (32%)	7 (47%)	127 (31%)	

Table 1 (continued)

	Overall	Age		
		< 50 years	≥ 50 years	P[a]
Oncocytic	8 (2%)	1 (6%)	7 (2%)	
Unknown	23	–	23	

IPMN intraductal papillary mucinous neoplasm, IQR interquartile range, MPD main pancreatic duct

[a] Chi-square or Fisher exact test for categorical variables and Wilcoxon rank-sum test for continuous variables, not considering missing values

2006[23] and updated in 2012,[24] but management strategies may have varied prior to that. Notably, the proportion of young patients with resected IPMN was similar from 1989 to 2005 (pre-Sendai), from 2006 to 2011 (Sendai), and from 2012 to 2016 (Fukuoka): 6.0, 5.0, and 4.8%, respectively (P = 0.615). Moreover, when we evaluated the differences in baseline clinical and radiological characteristics stratified by these three treatment eras (Supplemental Table 1), we only found significant differences in the pre-Sendai era, when young patients were significantly more likely to present with abdominal pain and less likely to present with jaundice.

Highest Grade of Dysplasia

The prevalence of high-grade dysplasia was similar in both age groups (29 and 23% in young and older patients, respectively). Invasive carcinoma arising in association with an IPMN was less frequent among young patients (17 vs. 27%) (Table 1). To further explore the risk of invasive carcinoma by age group, we dichotomized cases as invasive and non-invasive (low-, moderate-, or high-grade dysplasia). Young patients had a 44% lower risk of invasive carcinoma arising in association with an IPMN compared to patients age 50 and older (95% CI 0.32–0.99, P = 0.044). We observed a similar trend of lower risk of invasive carcinoma in younger patients using age thresholds of 55 years (P = 0.091) and 60 years (P = 0.031) (Supplemental Table 2).

Histological Subtypes of Precursor Lesions and Invasive Carcinomas Arising from IPMN

Overall, the most common histological subtype in precursor lesions was the gastric type (55%) followed by intestinal type (27%) (Table 1). Young patients were significantly less likely to harbor gastric-type IPMN (37 vs. 57%, OR 0.44, 95% CI 0.25–0.79, P = 0.005), and more likely to harbor oncocytic-type IPMN (15 vs. 4%, OR 3.94, 95% CI 1.74–8.90, P = 0.003) and intestinal-type IPMN (44 vs. 26%, OR 2.25, 95% CI 1.28–3.97, P = 0.004). Associations between younger age and distinct epithelial subtypes of the precursor lesions were still present (statistically significant or as a trend) when we used age thresholds of 55 and 60 years, especially for the oncocytic subtype (Supplemental Table 2). Among patients with invasive carcinomas arising in association with an IPMN, the most common histological subtype overall was tubular carcinoma (66%), followed by colloid carcinoma (32%) (Table 1). Colloid carcinoma was more common in young patients compared to older ones, but the association was not statistically significant (47 vs. 31%, OR 1.89, 95% CI 0.67–5.34, P = 0.261). Lymph node metastases were less common in invasive carcinomas arising in association with an IPMN in young patients than in older ones (20 vs. 39%) but this difference did not reach statistical significance (P = 0.106). Colloid carcinomas in the entire study population (i.e., irrespective of age) rarely had LN metastases (20 vs. 56% in tubular carcinomas, P < 0.001).

Disease-Specific and Overall Survival

Of the 1218 patients with non-invasive IPMN, follow-up data was available in 1122. Of these, the cause of death was known for 1010 patients, who constituted the study population for DSS. Among young patients, there were no disease-specific deaths, compared to 13 in patients age 50 and older. This translated into 5-year DSS rates of 100 and 98.3% in young and older patients, respectively (log-rank P = 0.300) (Table 2, Fig. 2a). Of the 440 patients with invasive carcinoma arising from IPMN in the entire cohort, 410 had follow-up data and these comprised the study population for OS. Patients with invasive carcinoma arising in association with an IPMN had a median OS of 38.9 months (95% CI 31.6–48.8 months). While limited by sample size in the young age group, survival analysis showed that young patients with invasive carcinoma had longer OS than patients age 50 and older (5-year OS 71.4 vs. 36.9%, HR 0.24, 95% CI 0.06–0.98, log-rank P = 0.031) (Table 2, Fig. 2b). Young and older patients with invasive carcinoma arising from IPMN were followed for a similar duration of time after surgery (median follow-up time among patients who were alive at the end of the study, 22 and 25 months, respectively; P = 0.968). We conducted secondary OS analyses using alternative cutoffs of 55 and 60 years of age (Supplemental Fig. 1) and found that younger patients had longer OS using both these cutoffs, but the association was statistically significant only with the 55 years cutoff. After

Table 2 Disease-specific and overall survival by patient age at the time of resection

	Disease-specific survival (DSS)				
	No. at risk[a]	No. of events	2-year	5-year	Log-rank P
Age					
< 50 years	58	0	100.0%	100.0%	0.300
≥ 50 years	952	13	99.0%	98.3%	
	Overall survival (OS)				
	No. at risk[b]	No. of events	2-year	5-year	Log-rank P
Age					
< 50 years	12	2	71.4%	71.4%	0.031
≥ 50 years	398	240	63.1%	36.9%	

IPMN intraductal papillary mucinous neoplasm, HR hazard ratio for unadjusted Cox proportional hazards regression, CI confidence intervals

[a] In patients with non-invasive IPMN and excluding those with missing follow-up data and unknown cause of death

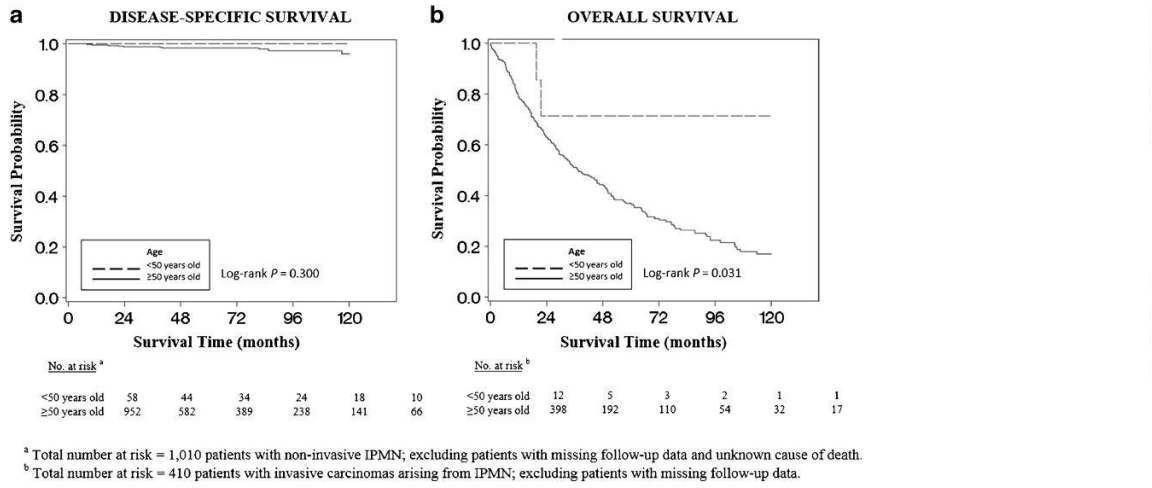
[b] In patients with invasive carcinoma arising from IPMN and excluding those with missing follow-up data

adjusting for the invasive carcinoma histological subtype (tubular vs. colloid vs. oncocytic), age was no longer significantly associated with improved OS (HR 0.30, 95% CI 0.07–1.24) and colloid type carcinoma was found to be associated with longer OS independent of patient age (HR 0.58, 95% CI 0.43–0.77). These results suggest that at least some of the favorable prognosis seen in young patients with invasive carcinomas may be attributed to their high prevalence of colloid-type carcinoma.

Discussion

In a large, multi-institutional series of resected IPMNs, we found that patients who underwent surgery before age 50 were more likely to have IPMN of oncocytic and intestinal epithelial subtypes located in the pancreatic head and uncinate process. Notably, young patients were not more or less likely to be symptomatic or show radiological worrisome features or high-risk stigmata of malignancy. Young patients were less likely to harbor invasive carcinoma arising from IPMN, and when they did, it was frequently of the colloid type and was associated with improved prognosis compared to older patients with invasive carcinomas.

IPMNs are commonly diagnosed in individuals in the seventh decade of life.[25] The actual prevalence of IPMN in the general population is unknown, but under the premise that most incidentally found pancreatic cysts are IPMN, the prevalence would be at the very least 0.8/100,000 and as high as 10% in older patients.[26–28] The incidence of pancreatic cysts appears to increase with age; in a study of 2803 individuals with a median age of 51 years old undergoing magnetic resonance imaging as part of a preventive medical examination, 1.3% of individuals between ages 40 and 49 had a pancreatic cyst, while the prevalence in subjects age 70 and older was around 10%.[29]



[a] Total number at risk = 1,010 patients with non-invasive IPMN; excluding patients with missing follow-up data and unknown cause of death.
[b] Total number at risk = 410 patients with invasive carcinomas arising from IPMN; excluding patients with missing follow-up data.

Fig. 2 Kaplan-Meier curves for survival by age at the time of surgery for intraductal papillary mucinous neoplasm (IPMN). a Disease-specific survival in patients with non-invasive IPMN. b Overall survival among patients with invasive carcinoma arising from IPMN

The reason why we observed a lower risk of invasive carcinoma in association with an IPMN resected at a younger age is not entirely clear. IPMNs exhibit a progressive spectrum of dysplasia from low-/moderate-grade to invasive carcinoma, thus it is possible that the lower prevalence of invasive carcinoma was a function of earlier diagnosis. Due to a longer life expectancy, young patients could have undergone surgery for tumors with fewer high-risk features compared to older patients, leading to lower rates of invasive carcinoma in the young group. This interpretation, however, is debatable given the other differences between the groups that are outlined below.

Our results suggest that indications for surgery in young and older patients in our study may have been similar. There were no significant differences in terms of MPD size, cyst size, presence of mural nodules/solid component, or thick walls, which are known worrisome features and high-risk stigmata of malignancy. If IPMNs from younger patients were truly diagnosed and resected at an earlier stage in their natural history, one could expect to see differences in these parameters. Collectively, the rate of asymptomatic lesions was similar in young and older patients. Individually, we did find that obstructive jaundice—which is associated with invasive carcinoma—was more common among older patients. Conversely, while abdominal pain is not part of the consensus guidelines algorithm, it was more common in younger patients and it could have led to patients with benign lesions and no worrisome radiological signs to have surgery, especially before the guidelines were in place. In fact, the associations of age with abdominal pain and obstructive jaundice were only observed among patients who underwent surgery in the pre-Sendai era.

Indications for surgical resection of IPMNs have evolved over the past two decades. Before clinical guidelines were published, the decision to operate rested heavily on individual and institutional experience, making it prone to wide variations across centers. As our understanding of the disease improved, it became clear that certain clinical and radiological characteristics could help us stratify patients based on their risk of cancer, leading to the publication of the International Consensus Guidelines in 2006[23] and its updated version in 2012.[24] In our study population, 614 (36%) patients had surgery before 2006. Remarkably, the proportion of patients under age 50 who underwent resection was similar between 1989 and 2005, 2006 and 2011, and from 2012 onward. Taken together, these data suggest that while indications for surgery have changed over time, they were likely applied to young and older patients similarly.

The finding that most strongly supports the notion that IPMNs in young patients may have underlying biological differences is the marked contrast in the epithelial subtypes in IPMN of young and older patients. Young patients were significantly more likely to have oncocytic- and intestinal-type precursor epithelia, whereas IPMNs from older patients were predominantly of the gastric type. These differences may have significant importance. Gastric-type IPMN (also referred to as Bnull type^[5]) are frequently confined to the side branches of the pancreatic ductal system and usually have low to moderate dysplasia; however, when they become invasive, they give rise to tubular-type carcinomas, which have a very poor prognosis similar to that of conventional pancreatic ductal adenocarcinoma.[6,7] Intestinal-type IPMNs are commonly found in the pancreatic head and uncinate process involving the MPD and they often show high-grade dysplasia or invasive carcinoma; when intestinal-type IPMNs become invasive, they often give rise to colloid-type carcinomas which have a lower frequency of LN metastases and a more favorable prognosis than tubular-type carcinomas.[6,7] Oncocytic-type IPMNs and their associated oncocytic-type carcinomas are very rare, accounting for approximately 5% of resected IPMN.[7,30] In our study, 15% of IPMNs from young patients were of the oncocytic type, compared to 4% in older patients. This IPMN subtype is predominantly located in the pancreatic head and uncinate process with MPD involvement. By definition, all oncocytic-type IPMNs have high-grade dysplasia with or without invasive carcinoma, yet they have an excellent prognosis, with a 5-year DSS of 93 to 100% in patients with invasive carcinoma.[30,31]

It could be hypothesized that intestinal- and oncocytic-type IPMNs develop earlier but take longer time to undergo malignant transformation than other subtypes, hence their high prevalence in young patients and the lower risk of invasive carcinoma in this age group. This does not appear to be the case, since the age differences of patients with and without invasive carcinoma were not larger for intestinal- and oncocytic-type IPMNs in our series (average difference, 3.6 years in gastric-type, 2.9 years in intestinal-type, − 0.5 years in oncocytic-type, and 1.1 years in pancreatobiliary-type). Additionally, it is highly unlikely that intestinal- and oncocytic-type IPMNs go on to become gastric-type tumors as patients become older. While we did not perform molecular analyses on our study population, recent studies have shed light on the genetic events occurring in these different subtypes and strongly suggest that they represent distinct pathways of carcinogenesis. KRAS mutations are very frequent in gastric-type IPMN and tubular carcinomas,[10,13,14] whereas intestinal-type IPMN and colloid carcinomas are characterized by a high prevalence of GNAS mutations and markers of intestinal differentiation.[5,10,11,13,14] In contrast, most oncocytic-type IPMNs are KRAS and GNAS wild types and have been found to have BRAF mutations.[11,14,31,32] Collectively, our results indicate that resected IPMNs from young patients have predominantly intestinal and oncocytic differentiation and therefore may have distinct molecular features. Given that invasive carcinomas arising from these subtypes have been found to be more indolent and have a lower rate of LN

metastases,[6,7] this might be the reason young patients with invasive carcinomas had a more favorable prognosis. Next-generation sequencing may soon allow us to better characterize IPMN preoperatively using cyst fluid and fine-needle aspiration biopsies,[12] which may help identify gene alterations associated with distinct IPMN subtypes and improve risk stratification of patients.

The present study has multiple strengths. It is to the best of our knowledge the first study to explore the differences in the characteristics of IPMN based on patient age at the time of surgery. Given the small number of patients undergoing resection at a very young age, a large study sample is needed to observe substantial differences. This was made possible in our study by the collaborative effort of three academic pancreatic surgery reference centers. Our study has highly annotated radiological data that allows us to conduct analyses of known imaging features associated with high-risk lesions; in the case of two of the most important parameters (MPD diameter and cyst size), measurements were obtained from the same imaging modality, minimizing variability. Moreover, surgical specimens were re-reviewed by pathologists with a special interest in IPMN to identify the histological subtypes of the precursor and invasive components.

Our study also has limitations. We do not know the exact indication for surgery for every patient in our study or how much weight was given to life expectancy when deciding whether to operate, although the proportion of asymptomatic patients and the radiological characteristics associated with high-risk lesions were similar between age groups. Given that our study is limited to resected IPMNs, we recognize that it fails to capture younger patients with IPMNs who did not meet the criteria for surgery—which may represent a larger proportion of patients under age 50 with suspected IPMN—and older patients who had indications for resection but were not surgically fit, as well as patients of any age diagnosed with an unresectable disease. Despite our best efforts, a fraction of patients lacked radiological and histological data. Nevertheless, missing data was likely randomly distributed between the two age groups and there is no indication that this introduced bias in our results. Lastly, while survival analyses revealed that young patients with invasive carcinomas arising from IPMNs show improved survival compared to older patients, these results must be interpreted with caution given the small number of young patients with invasive carcinomas.

Conclusion

In summary, resection for IPMN is uncommon in individuals aged 50 or younger. These patients are more likely to have oncocytic- and intestinal-type IPMNs and less likely to harbor an associated invasive carcinoma. Our results suggest that when an invasive carcinoma arising from IPMN is present, young patients may have a more favorable prognosis than older ones, and this could be attributable to a higher frequency of colloid-type carcinomas in young patients, but these observations need to be further validated. Understanding the biological and prognostic differences of IPMNs between young and older patients could be a valuable step towards achieving management strategies tailored to different groups of patients.

Author Contributions Conception and design: V Morales-Oyarvide, C Fernández-del Castillo

Acquisition, analysis, or interpretation of the data: All the authors

Drafting of the manuscript or critical revision for important intellectual content: All the authors

Final approval of the version to be published: All the authors

Agreement to be accountable for all aspects of the work: All the authorsGrant Support and FundingGrant support from NIH CA62924 and the Michael Rolfe Foundation for R.H.H.

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Development and Validation of a Multi-Institutional Preoperative Nomogram for Predicting Grade of Dysplasia in Intraductal Papillary Mucinous Neoplasms (IPMNs) of the Pancreas

A Report from The Pancreatic Surgery Consortium

Marc A. Attiyeh, MD,ã Carlos Fernández-del Castillo, MD, Mohammad Al Efishat, MD,ã Anne A. Eaton, MS,z Mithat Go¨nen, PhD,z Ruqayyah Batts, BA,ã Ilaıa Pergolini, MD,y Neda Rezaee, MD,§ Keith D. Lillemoe, MD,y Cristina R. Ferrone, MD,y Mari Mino-Kenudson, MD,ô Matthew J. Weiss, MD,§ John L. Cameron, MD,§ Ralph H. Hruban, MD,jj Michael I. D'Angelica, MD,ã Ronald P. DeMatteo, MD,ã T. Peter Kingham, MD,ã William R. Jarnagin, MD,ã Christopher L. Wolfgang, MD,§ and Peter J. Allen, MD ã

Objective: Previous nomogram models for patients undergoing resection of intraductal papillary mucinous neoplasms (IPMNs) have been relatively small single-institutional series. Our objective was to improve upon these studies by developing and independently validating a new model using a large multi-institutional dataset.

Summary Background Data: IPMNs represent the most common radiographically identifiable precursor lesions of pancreatic cancer. They are a heterogenous group of neoplasms in which more accurate markers of high-grade dysplasia or early invasive carcinoma could help avoid unnecessary surgery in 1 case and support potentially curative intervention (resection) in another.

Methods: Prospectively maintained databases from 3 institutions were queried for patients who had undergone resection of IPMNs between 2005 and 2015. Patients were separated into main duct [main and mixed-type (MD)] and branch duct (BD) types based on preoperative imaging. Logistic regression modeling was used on a training subset to develop 2 independent nomograms (MD and BD) to predict low-risk (low- or intermediate-grade dysplasia) or high-risk (high-grade dysplasia or invasive carcinoma) disease. Model performance was then evaluated using an independent validation set.

Results: We identified 1028 patients who underwent resection for IPMNs [MD: n ¼ 454 (44%), BD: n ¼ 574 (56%)] during the 10-year study period. High-risk disease was present in 487 patients (47%). Patients with high-risk disease comprised 71% and 29% of MD and BD groups, respectively (P <0.0001). MD and BD nomograms were developed on the training set [70% of total (n ¼ 720); MD: n ¼ 318, BD: n ¼ 402] and validated on the test set [30% (n ¼ 308); MD: n ¼ 136, BD: n ¼ 172]. The presence of jaundice was almost exclusively associated with high-risk disease (57 of 58 patients, 98%). Cyst size >3.0cm, solid component/mural nodule, pain symptoms, and weight loss were significantly associated with high-risk disease. C-indices were 0.82 and 0.81 on training and independent validation sets, respectively; Brier scores were 0.173 and 0.175, respectively.

Conclusions: For patients with suspected IPMNs, we present an independently validated model for the prediction of high-risk disease.

Keywords: cancer, dysplasia, intraductal papillary mucinous neoplasm, IPMN, nomogram, pancreas, the pancreatic surgery consortium

(Ann Surg 2016;xx:xxx–xxx)

Intraductal papillary mucinous neoplasms (IPMNs) of the pancreas are radiographically identifiable precursors of invasive pancreatic cancer. The incidence of IPMNs is rising mostly due to the increasing use of high-resolution cross-sectional imaging.[1,2] These cystic neoplasms have been shown to evolve from low-grade dysplasia to high-grade dysplasia to invasive carcinoma, and this pathway of progression is believed to account for 20% to 30% of pancreatic cancers.[3] The timing and frequency of malignant progression are unknown, and therefore the management of patients with IPMNs is controversial.[4,5] This controversy exists because current laboratory, endoscopic, cytologic, and imaging technologies are unable to reliably distinguish between IPMNs that are at low-risk (low- to intermediate-grade dysplasia) from those that are at high-risk (high-grade dysplasia) of progressing to invasive cancer.

Presently, the most accurate factor associated with high-risk IPMNs is dilation of the main pancreatic duct on preoperative imaging [main duct IPMNs (MD-IPMN)]. Patients who undergo resection for MD-IPMN have a 50% to 60% chance of having high-grade dysplasia or invasive carcinoma at the time of resection.[6] Conversely, high-grade dysplasia is present in only 10% to 15% of patients who undergo resection in the absence of a dilated pancreatic duct [branch duct IPMNs (BD-IPMN)].[5] The 2012 International Consensus Guidelines (ICG2012), therefore, recommend resection for patients with MD-IPMN and observation for the majority of patients with BD-IPMN.[4,7,8] The identification of more accurate markers of high-grade dysplasia could allow for more rational treatment decision-making. Low-risk patients could avoid a potentially morbid and life-threatening operation, and high-risk patients

From the ãDepartment of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY; yDepartment of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA; zDepartment of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY; §Department of Surgery, The Sol Goldman Pancreatic Cancer Research Center, Johns Hopkins University School of Medicine, Baltimore, MD; ôDepartment of Pathology, Massachusetts General Hospital, Boston, MA; and jjDepartment of Pathology, The Sol Goldman Pancreatic Cancer Research Center, Johns Hopkins University School of Medicine, Baltimore, MD.

Reprints: Peter J. Allen, MD, Department of Surgery, C896, Memorial Sloan Kettering Cancer Center, New York, NY 10021. E-mail: allenp@mskcc.org.

The work presented in this paper was supported in part by NIH funding (R01 CA182076).

Study conception and design: MAA, CF-dC, MAE, AAE, MG, RB, KDL, CRF, MM-K, MJW, JLC, RHH, MID, RPD, TPK, WRJ, CLW, PJA.

Acquisition of data: MAA, MAE, RB, IP, NR.

Analysis and interpretation of data: MAA, MAE, AAE, MG, PJA.

Drafting of the manuscript: MAA, PJA.

Critical revision: MAA, CF-dC, MAE, AAE, MG, IP, NR, KDL, CRF, MM-K, MJW, JLC, RHH, MID, RPD, TPK, WRJ, CLW, PJA.

The authors report no conflicts of interest.

ISSN: 0003-4932/14/26105-0821

DOI: 10.1097/SLA.0000000000002015

could undergo resection hopefully prior to the development of invasive disease.

Previously published data from Memorial Sloan Kettering described a nomogram-derived objective risk score that could be used to assess the probability of patients with IPMNs having high-risk disease.[9] While only a single-institutional study, the nomograms for MD-IPMN and BD-IPMN each had a relatively strong concordance index of 0.74, demonstrating a significant association between nomogram-predicted and actual risk of having high-risk disease.

The current study sought to build on these and other previous nomograms by expanding our patient population to include a large multi-institutional dataset.[10,11] These data were gathered from 3 high-volume institutions, and previous factors that were found to be associated with the presence of high-risk IPMNs were included in the analysis.

METHODS

Prospectively maintained databases from 3 of the institutions of The Pancreatic Surgery Consortium were included in the study. The Consortium is composed of 5 independent groups from 4 high-volume institutions [Memorial Sloan Kettering (MSK), Johns Hopkins Hospital (JHH), Massachusetts General Hospital (MGH), and University of Verona (UV)]. The current study was conceived and designed by investigators from MSK, JHH, and MGH. Data from MSK, JHH, and MGH were combined into a cumulative database that was queried for patients who had undergone resection of pathologically proven IPMNs between 2005 and 2015. Patients resected for a recurrent IPMN, pancreatic adenocarcinoma in the absence of an IPMN, and patients who had postoperative pathological findings of concurrent malignancies (eg, cholangiocarcinoma, neuroendocrine tumor) were excluded. Preoperative imaging reports were reviewed to ensure that all cases were radiographically described as predominantly cystic in nature.

Demographic, clinical, laboratory, radiological, and pathological factors were extracted from the databases. The presence of symptoms was interpreted as any episode of abdominal pain or gastrointestinal (GI) disturbance in the upper abdomen, and the symptoms of weight loss and jaundice were recorded separately. Factors such as alcohol use or smoking were defined as any past or current use. Laboratory results were recorded from those obtained at preoperative testing. If multiple cysts were seen on imaging, the cyst size and location were recorded as that of the largest cyst. Main duct dilation measurements were stratified across 3 categories: ○≤0.5, >0.5 and ○≤1.0, and >1.0 cm. IPMN subtypes were assigned based on main duct dilation. (○≤0.5 cm: BD-IPMN; >0.5 cm: MD-IPMN).[1] Any findings on imaging described as a solid component, thickened or enhanced cyst, concurrent lesion, and/or mural nodule were initially recorded separately but later combined into a single variable ("solid component/mural nodule") as the composite was thought to be more replicable across observers. A concurrent lesion was defined as a concurrent noncystic finding (eg, a mass in the head of the pancreas and a cyst in the tail). A radiological diagnosis of mixed-type was classified as main duct.

Pathological analysis was performed by dedicated gastrointestinal pathologists at each of the 3 institutions, and all pathology had been previously reviewed. The determination of risk was based on the highest grade of dysplasia noted in the resected lesion: low- and intermediate-grade were classified as "low-risk," while high-grade dysplasia and invasive carcinoma were classified as "high-risk." Any incidence of adenocarcinoma on pathology with a concurrent IPMN was recorded as an invasive IPMN and therefore "high-risk." A breakdown of the study cohort can be found in Figure 1.

Statistical Analysis

The outcome of interest was the level of risk (low- vs. high-risk) determined by the grade of dysplasia on pathologic analysis. The data were split into a training set (70% of patients) and a validation set (30% of patients), stratified by MD- and BD-IPMN. Univariate and multivariate models were built from the training set to predict the probability of high-risk disease in future patients. Based on the significant difference between the levels of high-risk in the 2 duct-type groups, separate nomograms for MD-IPMN and BD-IPMN were created. In addition, a history of jaundice had an extremely high positive predictive value (57 of 58 patients with jaundice in the training dataset had high-risk disease). We therefore designed our model to assign a predicted probability of high-risk disease of 1 to patients with jaundice, and those patients were consequently excluded from further model building.

Patient characteristics were summarized separately for MD-IPMN and BD-IPMN using median and range for continuous covariates, and frequency and percentage for categorical covariates. Differences between patients with low- and high-risk disease were assessed using the Wilcoxon rank sum test and Fisher exact test. Variable selection was based on univariate significance, clinical importance, and results from prior studies. Multivariable modeling was done using logistic regression and assessed using concordance indices (c-indices), calibration plots, and Brier scores (mean squared prediction error). The concordance index is a measure of model discrimination and represents the probability that given a pair of patients, the model assigns a higher risk to the patient who is truly high risk compared with the patient who is truly low risk. Calibration plots show the true (observed) rate of high-risk disease in groups of patients defined by model-predicted risk of high-risk disease; in a well-calibrated model, the observed and expected rates are very similar. The final multivariable model was visually represented using nomograms and validated using the test datasets. All statistical analysis was done in R 3.1.1 using the rms, Hmisc, pROC, and readxl packages, and P values less than 0.05 were considered significant.

RESULTS

A total of 1073 patients underwent pancreatic resection for IPMNs at 1 of the 3 institutions between 2005 and 2015. Resection was performed for recurrence in 20 patients, and these patients were excluded. In addition, 25 additional patients were excluded because the IPMNs were identified at the time of resection for a separate pathologically distinct malignancy (eg, distal cholangiocarcinoma). The remaining 1028 patients constituted our study group. Sex was equally distributed (49% male; 51% female). Median age at resection was 68 years (IQR 60–75 yrs). High-risk disease was identified on final pathological analysis in 487 patients (47%). Patients with MD-IPMN had a significantly higher likelihood of having high-risk disease (high-risk disease: 71% MD vs. 29% BD; P <0.0001). The training and validation sets contained 720 (70%) and 308 (30%) patients, respectively. The distributions of MD-IPMN and BD-IPMN were comparable between the 2 groups (44% MD-IPMN and 56% BD-IPMN in each of the training and validation sets).

Univariate analysis identified 7 variables that were significantly different between low- and high-risk groups in the MD-IPMN and BD-IPMN subsets (Table 1). Patients with isolated main duct dilation were more likely to have high-risk disease (compared with mixed-type), and mixed-type lesions were more likely to have high-risk disease when the cyst size was greater than 3.0 cm (ie, mixed-type with large branch duct component). High-risk disease was also associated with a solid component/mural nodule, a history of weight loss, pain and/or GI symptoms, and main pancreatic duct dilatation greater than 1.0 cm. For BD-IPMN, high-risk disease was associated

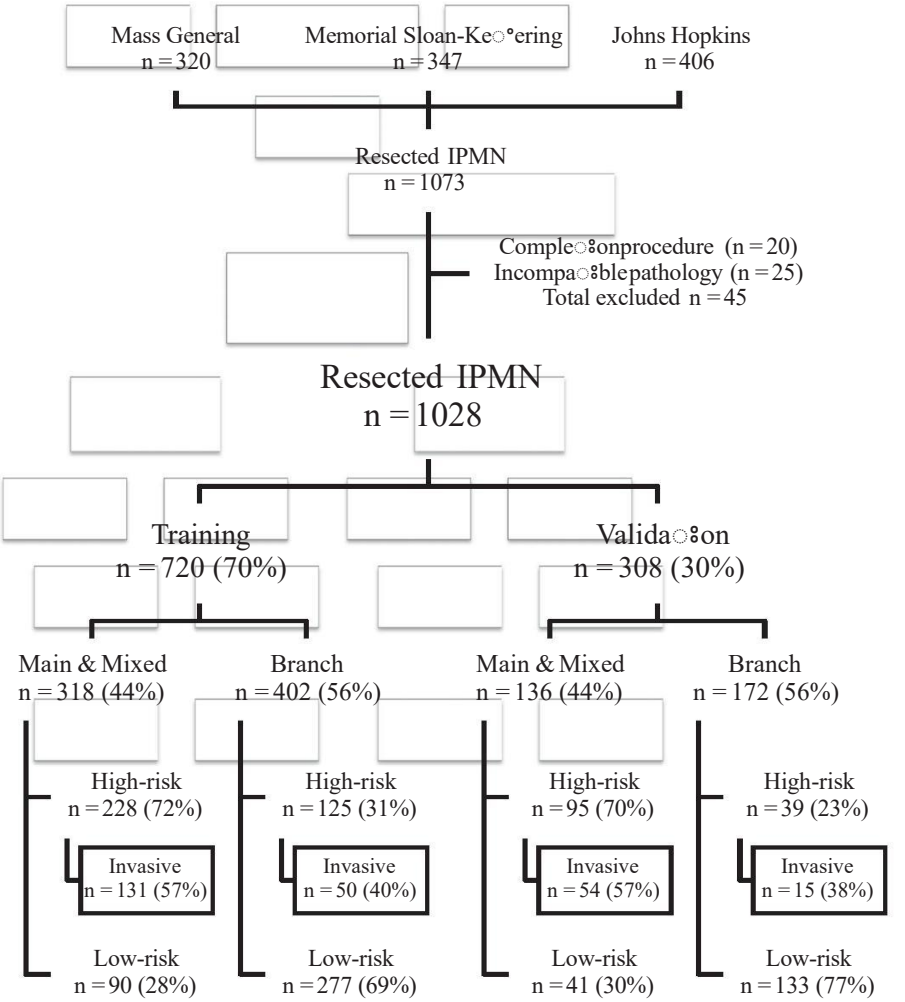

FIGURE 1. Study cohort.

with a cyst size greater than 3.0 cm, solid component/mural nodule, pain and/or GI symptoms, older age, and male sex. Preoperative CA 19-9 levels were only available for approximately 60% of the patients in each of the main and branch duct groups, and despite their significance in univariate analysis, they were excluded from further modeling.

Based on univariate results, a multivariate logistic regression model was built for MD- and BD-IPMN (Table 2), and nomograms were created to predict high-risk disease (high-grade dysplasia or invasive carcinoma) (Fig. 2). Patients with jaundice were assigned a high-risk probability of 1, and the rest were assigned a high-risk probability based on the nomogram that matched their radiological diagnosis (MD-IPMN or BD-IPMN). For example, a 70-year-old nonjaundiced asymptomatic male with a 3.5 cm BD-IPMN without high-risk imaging features would have a score of 136, resulting in a probability of high-risk disease of 32%. We initially tested the model using our training set, and the c-index was 0.82 (Brier score 0.173). The validation set was then applied to the model, and the c-index was 0.81 (Brier score 0.175). Calibration plots for training and validation sets can be seen in Figure 3. The data points on the training calibration plot are expected to be close to the equivalence line since they were used to build the model. The strength of the model is displayed in the validation calibration plot as the equivalence line generally falls within the 95% confidence interval of the observed rate of high-risk disease for each group indicating accurate prediction of high-risk disease on new unseen data.

DISCUSSION

Currently, our ability to accurately identify high-risk disease in patients with IPMNs is limited. Resection is generally recommended for patients with MD-IPMN, yet up to 40% of these patients will have low-risk disease at the time of resection.[12] The consequences of these limitations should not be understated as pancreaticoduodenectomy continues to be associated with a 2% to 4% risk of mortality and a 20% to 25% risk of major morbidity at institutions with the largest operative volumes.[13] A recent report from MSK highlighted the difficulty in identifying those at high-risk for progression to invasive cancer.[12] In this study of 186 patients who underwent resection for IPMNs, there were 75 patients (40%) who proved to have only low- or intermediate-grade dysplasia. The median age of patients in this study was 69 years, the risk of dying from operative complications was 2%, and the major operative complication rate was 37%. Improving our ability to predict high-risk

TABLE1. Patient Characteristics From Training Population and Univariate Analysis (n ¼ 662)

	Training Set (n ¼ 662)							
	Main Duct and Mixed-type (n ¼ 281)				Branch Duct (n ¼ 381)			
	Total (n)	High-risk (n ¼ 191)	Low-risk (n ¼ 90)	P Value	Total (n)	High-risk (n ¼ 105)	Low-risk (n ¼ 276)	P Value
Institution								
MSK	99 (35%)	71 (37%)	28 (31%)	0.331	129 (34%)	40 (38%)	89 (32%)	0.569
JHH	93 (33%)	65 (34%)	28 (31%)		152 (40%)	39 (37%)	113 (41%)	
MGH	89 (32%)	55 (29%)	34 (38%)		100 (26%)	26 (25%)	74 (27%)	
Age	68 (18–92)	67 (18–92)	69 (30–89)	0.244	67 (34–92)	70 (41–92)	66 (34–88)	0.011Ã
Body mass index	25.8 (15.5–46.1)	25.9 (15.5–46.1)	25.4 (17.5–38.1)	0.922	26.0 (15.0–47.0)	25.7 (17.6–47.0)	26.3 (15.0–43.2)	0.383
Sex								
Male	154 (55%)	112 (59%)	42 (47%)	0.072	160 (42%)	50 (48%)	110 (40%)	0.201Ã
Female	127 (45%)	79 (41%)	48 (53%)		221 (58%)	55 (52%)	166 (60%)	
Diabetes								
Yes	78 (28%)	56 (29%)	22 (24%)	0.476	58 (15%)	15 (14%)	43 (16%)	0.873
No	203 (72%)	135 (71%)	68 (76%)		323 (85%)	90 (86%)	233 (84%)	
Pancreatitis								
Yes	91 (32%)	62 (32%)	29 (32%)	1.000	81 (21%)	25 (24%)	56 (20%)	0.484
No	190 (68%)	129 (68%)	61 (68%)		300 (79%)	80 (76%)	220 (80%)	
Personal history of cancer								
Yes	64 (23%)	39 (20%)	25 (28%)	0.174	68 (18%)	20 (19%)	48 (17%)	0.765
No	217 (77%)	152 (80%)	65 (72%)		313 (82%)	85 (81%)	228 (83%)	
Family history of pancreatic cancer								
Yes	31 (11%)	20 (10%)	11 (12%)	0.686	64 (17%)	7 (7%)	57 (21%)	<0.001
No	250 (89%)	171 (90%)	79 (88%)		317 (83%)	98 (93%)	219 (79%)	
Symptomatic								
Yes	159 (57%)	117 (61%)	42 (47%)	0.028Ã	167 (44%)	53 (50%)	114 (41%)	0.133Ã
No	122 (43%)	74 (39%)	48 (53%)		214 (56%)	52 (50%)	162 (59%)	
Weight loss								
Yes	93 (33%)	73 (38%)	20 (22%)	0.010Ã	51 (13%)	16 (15%)	35 (13%)	0.505
No	188 (67%)	118 (62%)	70 (78%)		330 (87%)	89 (85%)	241 (87%)	
CA 19-9(serum) >40y								
Yes	42 (23%)	36 (29%)	6 (10%)	0.003	35 (16%)	16 (28%)	19 (12%)	0.007
No	141 (77%)	86 (71%)	55 (90%)		185 (84%)	42 (72%)	143 (88%)	
Solid component/mural nodulez								
Yes	122 (43%)	94 (49%)	28 (31%)	0.005Ã	102 (27%)	41 (39%)	61 (22%)	0.001Ã
No	159 (57%)	97 (51%)	62 (69%)		279 (73%)	64 (61%)	215 (78%)	
Number of cysts								
0	52 (19%)	42 (22%)	10 (11%)	0.001Ã	N/A	N/A	N/A	0.381
1	158 (56%)	110 (58%)	48 (53%)		223 (60%)	69 (66%)	154 (58%)	
2	29 (10%)	21 (11%)	8 (9%)		53 (14%)	14 (13%)	39 (15%)	
3	42 (15%)	18 (9%)	24 (27%)		94 (26%)	22 (21%)	72 (27%)	
Largest cyst size§								
○3.0 cm	113 (42%)	63 (34%)	50 (58%)	<0.001Ã	253 (67%)	54 (52%)	199 (72%)	<0.001Ã
>3.0 cm	106 (39%)	80 (43%)	26 (30%)		127 (33%)	50 (48%)	77 (28%)	
None seen	52 (19%)	42 (23%)	10 (12%)		N/A	N/A	N/A	
MPD size								
0.5 cm ≤ and ○1.0 cm	220 (78%)	144 (75%)	76 (84%)	0.091Ã	N/A	N/A	N/A	N/A
>1.0 cm	61 (22%)	47 (25%)	14 (16%)		N/A	N/A	N/A	

Patients with a history of jaundice (n ¼ 58) were excluded from training set. Median (low–high) or n (%).
Avariables used in subsequent multivariate analysis.
yPreoperative CA 19-9 was only available for 183/281 (65%) MD-IPMN and 220/381 (58%) BD-IPMN patients.
zSolid component, thickened, or enhanced cyst, mural nodule, or concurrent lesion.
§Ten MD-IPMN patients and 1 BD-IPMN patient did not have cyst size information available.
IQR indicates interquartile range; JHH, Johns Hopkins; MGH, Massachusetts General Hospital; MPD, main pancreatic duct; MSK, Memorial Sloan Kettering.

IPMNs would improve clinical care. Patients with low-risk lesions could be monitored and avoid a life-threatening operation until high-risk disease developed, and patients with high-risk lesions could undergo resection hopefully prior to the development of pancreatic cancer.

In the current study, we developed and independently validated a preoperative clinical model for IPMN that strongly predicts the risk of having high-grade dysplasia or invasive cancer. Analysis of our predictive model suggests that it may be better than the ICG2012 at identifying the presence of high-risk disease. The c-index of the model on validation data was 0.81 which highlights the model's ability to discriminate between low- and high-risk disease in a large group of patients 81% of the time. Currently, the reported rate of high-risk disease in patients with main duct dilation undergoing resection for presumed MD-IPMN is approximately 60%.[6,12] In addition, a separate model that determined

TABLE 2. Main and Branch Duct Nomogram Models (Multivariable Logistic Regression Models Fit on the Training Data, Excluding Patients With Jaundice)

	Main Duct and Mixed-type (n ¼ 271)		Branch Duct (n ¼ 380)	
	Odds Ratio (95% CI)	P Value	Odds Ratio (95% CI)	P Value
Largest cyst size[Ã]				
>3.0 cm	2.19 (1.20–4.05)	0.002	2.24 (1.37–3.65)	0.001
None seen	3.61 (1.65–8.53)		N/A	N/A
Solid component/mural nodule	2.44 (1.39–4.39)	0.002	2.08 (1.25–3.45)	0.005
Weight loss	1.92 (0.92–4.12)	0.086	N/A	N/A
Symptomatic	1.39 (0.73–2.64)	0.316	1.51 (0.94–2.44)	0.087
Main duct >1.0 cm	1.13 (0.55–2.40)	0.742	N/A	N/A
Agey	N/A	N/A	1.02 (1.00–1.05)	0.119
Sex (male)	N/A	N/A	1.14 (0.70–1.85)	0.593

Odds ratios refer to the odds of having high-risk disease (vs. low-risk).

[Ã]Odds ratio compared to reference category, ○ß3.0 cm.

yOdds ratio per 1 year increase in age.

CI indicates confidence interval.

high-risk probability based solely on the presence of main versus branch duct disease was run on our validation dataset and the c-index was 0.74. Therefore, our model is able to predict higher-risk disease better than the presence of main duct dilation alone.

The strengths of this study include the large sample size, the multi-institutional nature of the data, and the use of an independent validation dataset. Prior studies have typically been single-institutional and without independent validation. Validation on an independent dataset decreases the risk of over-fitting the model to an individual dataset, and the similarity between the c-indices of the training (0.82) and validation (0.81) sets suggests that this model is widely applicable. An additional advantage of this model, and nomograms in general, is that they assign risk probabilities on a continuous scale as an individualized risk score rather than splitting patients into 2 broad risk groups. This allows for additional stratification of risk and for patients and doctors to tailor treatment decisions based on patients' individual risks.

The prevalence of high-risk disease in the present study is in accord with the existing literature: 71% of resected MD-IPMN were found to have high-risk disease (defined as having high-grade dysplasia or invasive carcinoma) compared with only 29% of resected BD-IPMN. In patients with MD-IPMN, cyst size greater than 3.0 cm carried an odds ratio of 2.19 and an even higher odds ratio of 3.61 when there were no cysts seen (P ¼ 0.002). These results are similar to previously published reports that have demonstrated a slightly lower risk of high-grade or invasive IPMNs in patients undergoing resection for mixed-type IPMNs when compared with pure main duct disease.[14,15] Interestingly, to our knowledge, the association in mixed-type IPMNs between a larger cyst size and higher-risk disease has not been reported.

The presence of a solid component, mural nodule, concurrent lesion, or thickened or enhancing cyst on imaging was associated with the presence of high-risk disease in both MD- and BD-IPMN. We combined these findings into a single variable ("solid component/mural nodule") as previous studies have documented the difficulty in distinguishing between these features.[16] As part of a study by Do et al investigating interobserver agreement, 4 independent radiologists reviewed pancreatic protocol CT studies for 84 patients who had undergone resection for IPMNs. They classified the lesion as main, branch, or mixed and provided their estimation of the presence of malignant features such as a solid component or a mural nodule. The study results showed that while the radiologists' estimations of cyst size and MPD diameter were comparable, their assessment of malignant features was more variable. The authors suggested that these markers could be better determined as part of a tumor board conference where a consensus can be reached especially for factors that consensus guidelines have established as indications for resection.

Jaundice is considered a marker of high-risk disease in patients with a cystic lesion of the head of the pancreas. The ICG2012 recommend resection in such cases.[4] Similarly, in our study, jaundice was found to be a very strong predictor of high-risk disease. For MD and BD patients who presented with a history

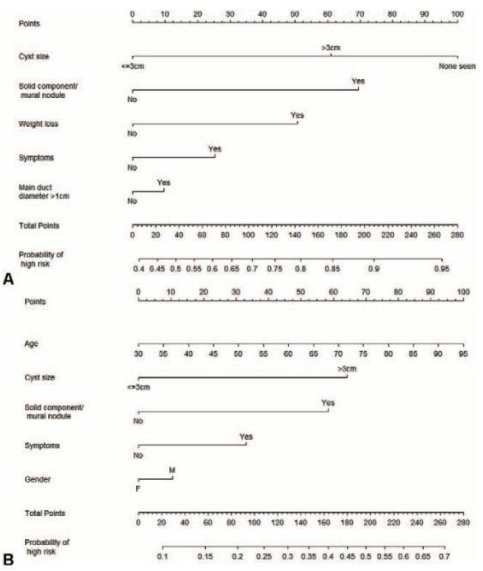

FIGURE2. Clinical nomograms for predicting high-risk disease in nonjaundiced patients with MD-IPMN (A) and BD-IPMN (B). If a patient has symptoms of jaundice, assign probability ¼ 1. A, Main duct nomogram. B, Branch duct nomogram.

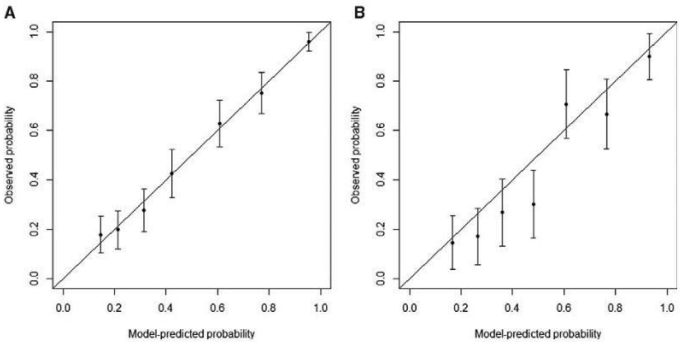

FIGURE3. Observed probability of high-risk disease by model-predicted probability of high-risk disease in training data (A) and validation data (B). Error bars represent 95% confidence intervals for observed probabilities. A, Training calibration plot. C-index 0.82. Brier score 0.173. B, Validation calibration plot. C-index 0.81. Brier score 0.175.

of jaundice, 37 of 37 (100%) and 20 of 21 (95%) of patients were found to have high-risk disease following resection, respectively. (The single low-risk patient with a history of jaundice also had prior episodes of biliary stricture and cholangitis that we believe to be the cause of his symptom.) Our model automatically assigns a predicted probability of high-risk disease of 1 to these patients and excludes them from the logistic regression analysis, allowing us to more accurately measure associations between the remaining factors and high-risk disease. Therefore, our model agrees with previously published guidelines stating that patients with jaundice should undergo resection.

Currently, standard recommendations for the management of IPMN are based on metaanalyses first published in 2006 and later updated in the ICG2012.[4,7] These guidelines attempt to stratify patients into higher-risk groups and aid surgeons with treatment recommendations. Recommendations include resection for all patients with MD-IPMN and resection for BD-IPMN with "high-risk stigmata" on imaging (eg, mural nodules). Observation is generally recommended for BD-IPMN without radiological findings of a solid component or mural nodularity. A review of the 2006 guidelines by Nagai et al[17] found that while the guidelines had near perfect sensitivity (97%), their low specificity (30%) resulted in many patients with low-risk IPMNs being resected. In 2015, a validity study examined the conclusions drawn from the ICG2012 and found that many mixed-type IPMNs were actually low-risk which further reduced the likelihood of high-risk disease in the MD-IPMN group.[18] Their results for sensitivity and specificity for the ICG2012 guidelines were 88% and 65%, respectively. These findings suggest a continued need for an improved ability to discriminate between patients with low- versus high-risk IPMNs.

A nomogram is a graphical representation of a complex statistical formula that accepts multiple input variables and provides an easy-to-understand answer to a focused question. As a prognostic tool, nomograms provide an individualized risk score for a given patient. Both preoperative (eg, estimating risk of severity of disease) and postoperative (eg, predicting recurrence-free or overall survival) nomograms have been described in the literature on topics such as breast, GI, and prostate cancer. A recent review highlighted the strengths and pitfalls of using clinical nomograms.[19] The authors highlighted 4 key performance metrics: validation, discrimination, calibration, and clinical usefulness. With respect to validation, our study used an independent dataset to validate the model to ensure a more fair and unbiased assessment of the model. With respect to discrimination, nomograms are typically scored using a c-index ranging from 0.5 (as good as chance) to 1.0 (perfect discrimination). The c-index of our model was 0.81 on a validation dataset meaning that 81% of the time, the model assigned a lower probability to a patient with truly low-risk disease than a patient with high-risk disease. With respect to calibration, the accuracy of a nomogram is best depicted by a calibration plot showing the relationship between predicted risk and actual risk. An ideal plot would show a diagonal line (y ¼ x). The calibration plot of our model on the training set (Fig. 3A) demonstrated a strong association between the nomograms and the data. The validation plot (Fig. 3B) was expectedly weaker (ie, larger confidence intervals, data points further away from the "ideal line") but was still able to show high accuracy for patients in the lower (< 10%–30%) and higher (> 90%) risk groups. Finally, with respect to clinical usefulness, our results suggest that our model may be a better predictor of high-risk disease and therefore could be a useful adjunct to clinical decision-making. It provides a risk assessment on a continuous scale, as opposed to the ICG2012's categorical criteria, that is easier to apply to an individual patient in the context of associated comorbidities and life expectancy. Further work will include a prospective analysis to determine whether it significantly improves patient outcomes when compared with clinician-directed management.

In 2013, researchers from MSK published an IPMN nomogram that sought to predict high-grade dysplasia and invasive carcinoma based on more limited data.[9] The nomograms developed in this previous study contained the same factors presented here, namely, solid component/mural nodule, lesion size, and weight loss. The endpoint in that study was a 3-level ordinal outcome: benign, high-grade dysplasia, and invasive carcinoma. In the present study, we elected to use the simpler and more clinically useful endpoint of high-risk disease, a composite of high-grade dysplasia and invasive carcinoma. By expanding our sample size to include 2 other large pancreatic centers and by including independent validation, our study serves not only to support prior results but also to expand and strengthen the model by identifying other possible markers of high-risk disease. In 2010, researchers in Japan created and later validated a nomogram that could predict the probability of carcinoma in IPMNs.[11,20] While this study had the benefit of being externally validated, the sample size used (n ¼ 81 for the training set; n ¼ 180 for validation) was relatively small. In addition, 1 factor they found to be significant was cytology grade. As part of our data collection, we attempted to collect data on cytology in a similar manner, but the

variability as well as lack of specificity in reports led to the exclusion of this variable from the final model. Practically speaking, the use of a nonstandardized cytology grade in the model renders a nomogram difficult to apply to other centers.

The present study has several weaknesses. First, our cohort only included patients who underwent resection resulting in a selection bias in our data; it is unknown whether these factors would remain significant in unresected patients. This makes it difficult to apply the nomogramsto patients whocarry a diagnosisof anincidentalcystic lesion. Additional studies are currently being developed tovalidate our model on unresected patients undergoing surveillance. Second, as mentioned previously, the clinical utility our model offers has yet to be demonstrated to be significant due to the lack of any prospective analysis.We anticipatefuturestudieswillfurthervalidateourmodelby applying it to patients with IPMNs and determining its accuracy and usefulness. However, for this particular type of study, patients determined to likely be low-risk and therefore managed conservatively will not have a pathological diagnosis to support or refute the model's prediction. Assuming the patient does not undergo resection during their follow-up period, 1 possible solution is to define a length of time (eg, at least 5 years) after which it can be safely stated that in the absence of clinical or radiographicevidenceof disease progression, the patient likely had a low-risk lesion. Third, our study found preoperativeCA19-9tobepredictiveofhigh-riskdiseaseonunivariate analysis. However, due to its specificity for malignancy and that approximately 40% of our cohort did not have CA 19-9 levels available,itwasexcludedfromsubsequentanalysis.Finally,our model is not meant to replace a clinician's decision-making with regard to resecting an IPMN. Although nomograms can predict the likelihood of identifying a high-risk lesion, only the surgeon and the patient can best balance risks and benefits and decide the threshold for which resection is indicated.

In conclusion, for patients with suspected IPMNs, we present an independentlyvalidated model containing 2 nomograms forpredicting high-risk disease. Our study is the largest to date to identify significant factors contributing to high-risk disease in IPMNs, and our model displays strong objective predictive power when validated with independent data. As noted previously, future studies will need to expand these nomograms to include unresected patients so their applicability can go beyond preoperative patients. Finally, studies investigating the useofcystfluidcharacteristicsasdiagnosticand/orprognosticmarkers may enhance our model even further.

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Pancreatology xxx (2017) 1–7

Contents lists available at ScienceDirect

Pancreatology

journal homepage: www.elsevier.com/locate/pan

Diabetes mellitus in intraductal papillary mucinous neoplasm of the pancreas is associated with high-grade dysplasia and invasive carcinoma

Vicente Morales-Oyarvide [a], Mari Mino-Kenudson [b], Cristina R. Ferrone [a], Dushyant V. Sahani [c], Ilaria Pergolini [a], Adria´n A. Negreros-Osuna [c], Andrew L. Warshaw [a], Keith D. Lillemoe [a], Carlos Ferna´ndez-del Castillo [a, *]

[a] Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, USA
[b] Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, USA
[c] Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Boston, USA

ARTICLE INFO

Article history:
Received 26 May 2017
Received in revised form
21 July 2017
Accepted 30 August 2017
Available online xxx

Keywords:
Pancreatic cystic neoplasms
Intraductal papillary mucinous neoplasms
Pancreatic cancer
Diabetes mellitus

ABSTRACT

Background: While the association between Diabetes Mellitus (DM) and pancreatic ductal adenocarcinoma is well recognized, its importance in intraductal papillary mucinous neoplasm of the pancreas (IPMN) is not well-defined. We sought to examine the associations of DM with degree of dysplasia and morphological subtypes in IPMN.

Methods: In 454 patients with resected IPMN, we evaluated associations of DM with high-grade dysplasia (HGD), invasive carcinoma, precursor epithelial subtype (gastric, intestinal, oncocytic, pancreatobiliary), and histological type of invasive carcinomas (tubular, colloid, oncocytic) using logistic regression. We performed multivariate analyses adjusting for worrisome features and high-risk stigmata of malignancy in a subset of 289 patients with annotated radiological characteristics.

Results: The prevalence of DM in our study was 34%. DM was significantly associated with HGD (OR 2.02, 95% CI 1.02–4.01, P ¼ 0.045) and invasive carcinoma (OR 2.05, 95% CI 1.08–3.87, P ¼ 0.027) after adjusting for worrisome features. Compared to patients without DM, those with recent-onset DM (○*5 years before surgery) had 6.9-fold (95% CI 2.38–19.92, P < 0.001) higher risk of invasive carcinoma. DM was associated with increased likelihood of intestinal-type precursor epithelium (OR 1.63, 95% CI 1.07–2.47, P ¼ 0.022) and colloid carcinomas (OR 2.46, 95% CI 1.01–5.99, P ¼ 0.047)

Conclusion: Preoperative DM was associated with significantly higher risk of HGD and invasive carcinoma in resected IPMN, and risk of invasive carcinoma was highest in patients with recent-onset DM. Patients with DM were more likely to harbor intestinal-type IPMN and colloid carcinomas. Our findings suggest that a diagnosis of DM in patients with IPMN may warrant more aggressive surveillance.

Introduction

Intraductal papillary mucinous neoplasms (IPMN) are mucin-producing cystic tumors arising from the epithelium of the pancreatic ductal system and are precursors to invasive carcinoma [1]. The management and diagnostic work-up of IPMN is complex and has evolved over recent years. The 2012 International Consensus Guidelines for the management of IPMN (2012 Fukuoka consensus) have defined clinical and radiological worrisome features and high-risk stigmata of malignancy that help guide clinical decision-making [2]. However, very limited attention has been paid to the role of metabolic risk factors in the malignant progression of IPMN.

Diabetes mellitus (DM) is a frequent finding in patients with pancreatic ductal adenocarcinoma (PDAC) and a wealth of epidemiological evidence indicates that new-onset DM is strongly associated with a PDAC diagnosis [3–10]. Whether DM is associated with malignant progression of IPMN is not well established, and only a few studies have reported associations between DM and

* Corresponding author. Department of Surgery, Massachusetts General Hospital, 55 Fruit Street, Wang Ambulatory Care Center 460. Boston, MA, 02114, USA.
E-mail address: cfernandez@partners.org (C. Ferna´ndez-del Castillo).

http://dx.doi.org/10.1016/j.pan.2017.08.073
1424-3903/

degree of dysplasia in IPMN with conflicting results [11–18]. Factors associated with increased risk of developing PDAC may or may not exert the same influence on the malignant progression of IPMN, since genomic and morphologic analyses of invasive carcinomas arising from IPMN have revealed critical distinguishing features from conventional PDAC [19–22]. These biological differences highlight the importance of evaluating risk factors for IPMN progression as a distinct entity.

The main goal of this study was to evaluate the association between preoperative DM and the degree of dysplasia in a series of resected IPMN from a single institution. We also explored whether DM was associated with the precursor epithelial subtype (gastric, intestinal, pancreatobiliary, or oncocytic) [22,23] and the histological type of invasive carcinomas arising from IPMN (tubular, colloid, or oncocytic) [22,23].

Methods

Study population

We evaluated 507 consecutive patients with pathologically-confirmed IPMN of the pancreas who underwent surgical resection at the Massachusetts General Hospital between March, 1990 and June, 2016. Demographic and clinical data including sex, age, and history of acute pancreatitis and jaundice were obtained from medical records. This study was approved by Institutional Review Board.

Assessment of preoperative diabetes mellitus

The primary criteria for diagnosis of DM were preoperative fasting plasma glucose ≥126 mg/dL or hemoglobin A1C ≥ 6.5%, based on the current American Diabetes Association guidelines [24]. We ascertained patient DM status by retrospective review of preoperative clinical laboratory chemistries from medical records. Additionally, self-report of a history of DM and the date it was diagnosed were recorded from preoperative surgical consultation notes and were used for secondary analyses.

Radiological evaluation

A radiologist blinded to the final pathological diagnosis evaluated preoperative computed tomography and magnetic resonance imaging studies. The most recent imaging study prior to the date of surgery was selected. Consistent with the 2012 Fukuoka consensus, we evaluated the presence of worrisome features and high-risk stigmata by assessing the main pancreatic duct (MPD) largest diameter, evidence of enhancing solid components within the cyst, size of the largest cyst, thickened/enhancing cyst walls, non-enhancing mural nodules, abrupt change in caliber of the MPD with distal pancreatic atrophy, and lymphadenopathy [2]. Radiological IPMN type was defined as follows: IPMN with MPD dilatation ≥5 mm were classified as main duct IPMN (MD-IPMN); lesions with cysts >5 mm showing communication with the MPD were classified as branch-duct IPMN (BD-IPMN); tumors that met criteria for both BD- and MD-IPMN were classified as mixed-type IPMN [2].

Pathological evaluation

Comprehensive gross and microscopic evaluation of the resection specimens was performed. The highest degree of cellular atypia was classified as low-, high-grade dysplasia (formerly carcinoma in situ), or invasive carcinoma [25]. High-grade dysplasia was defined as IPMN with severe architectural complexity (cribriforming and/or micropapillary structures), nuclear atypia (loss of polarity and/or nuclear pleomorphysm), and significant mitotic activity without evidence of invasion. Invasive carcinoma arising from IPMN was defined as microscopic and/or macroscopic invasion into the stroma beyond the basal membrane in direct continuity with areas of high-grade dysplasia. The predominant precursor epithelial subtype was classified as gastric, intestinal, pancreatobiliary, oncocytic, or mixed in cases where a single predominant subtype could not be identified [22,23]. The histological type of invasive carcinomas arising from IPMN was classified as tubular, colloid, or oncocytic [22].

Outcome measures

The main outcomes of interest were high-grade dysplasia and invasive carcinoma. Analysis of high-grade dysplasia used patients with low-grade dysplasia as the reference group, excluding patients with invasive carcinoma from the analysis. Analysis of invasive carcinoma used patients with low- and high-grade dysplasia as reference group. Secondary outcomes were the predominant precursor epithelial subtype and the histological type of invasive carcinomas.

Case selection

We first excluded patients with distinct concomitant PDAC or ampullary cancer arising separately from the IPMN (n = 27). We next excluded patients whose DM status could not be ascertained due to lack of preoperative clinical chemistry (n = 26), leaving 454 eligible patients for unadjusted (univariate) analyses. Of those, 289 patients had preoperative imaging available for complete radiological evaluation in our institution's Electronic Medical Record system and they comprised the study population for multivariable-adjusted analyses. Imaging for the remaining 165 patients was reviewed for clinical care prior to surgery, but their studies were not available for re-evaluation for research purposes when this study was conducted.

Statistical analysis

Associations of DM with high-grade dysplasia and invasive carcinoma were evaluated using unadjusted and multivariable-adjusted binomial logistic regressions calculating odds ratios (OR) and 95% confidence intervals (CI). Two multivariable-adjusted models were used based on known clinical and radiological predictors of malignant transformation from the 2012 Fukuoka consensus [2]: the first model adjusted for history of acute pancreatitis, cyst size ≥3 cm, thickened/enhancing cyst walls, abrupt change in caliber of the MPD with distal pancreatic atrophy, lymphadenopathy, and MPD diameter ≥5 mm (worrisome features); the second model adjusted for history of jaundice, enhancing solid component, and MPD diameter ≥10 mm (high-risk stigmata). Further, in stratified analyses, we examined the association of DM with high-grade dysplasia and invasive carcinoma among patients with MPD size <10 mm and in those with MPD size ≥10 mm.

We next evaluated whether the association of DM with high-grade dysplasia and invasive carcinoma differed by the duration of DM prior to surgery. The date of DM diagnosis was available only by patient self-report; therefore, this analysis was restricted to

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patients whose self-reported DM status was confirmed by direct laboratory measurements (n ¼ 351, of which 222 had preoperative images available and were eligible for multivariable-adjusted analysis). DM was classified based on the date of diagnosis as new-onset (diagnosed ≤1 year before surgery), recent-onset (diagnosed ≤5 years before surgery), or longstanding (diagnosed >5 years before surgery) based on a recent meta-analysis of the association between DM and PDAC [8].

In secondary analyses, we assessed the relationship between DM and predominant precursor epithelial subtype and the histological type of invasive carcinomas using binomial logistic regression. All hypothesis tests were two-sided and statistical significance was set at P ≤ 0.05. Statistical analyses were performed using SAS software (version 9.4; SAS Institute, Cary, NC).

Results

Baseline characteristics of the patients in our study population are shown in Table 1. Criteria for diagnosis of DM were met by 154 (34%) patients. Patients with DM were predominantly male and older, and were more likely to exhibit jaundice, MPD dilation, and an enhancing solid component.

Diabetes mellitus, high-grade dysplasia, and invasive carcinoma

We first evaluated the association of DM with high-grade dysplasia and invasive carcinoma (Table 2). DM was associated with a 1.6-fold (95% CI 1.00–2.62, P ¼ 0.051) higher risk of high-grade dysplasia in unadjusted analysis. After adjusting for worrisome features, DM was associated with a 2-fold (95% CI 1.02–4.01, P ¼ 0.045) higher risk of high-grade dysplasia. There was no significant association between DM and high-grade dysplasia after adjusting for high-risk stigmata (Table 2). DM was significantly associated with higher risk of invasive carcinoma in unadjusted analysis (OR 2.49, 95% CI 1.59–3.90, P < 0.001) and after adjusting for worrisome features (OR 2.05, 95% CI 1.08–3.87, P ¼ 0.027). We

Table 1
Baseline characteristics of 454 resected IPMN based on Diabetes Mellitus status.

	Overall	Diabetes Mellitus		
		Yes	No	P[a]
No. patients for clinical and pathological features	454	154 (34%)	300 (66%)	
Men, n (%)	231 (51%)	95 (62%)	136 (45%)	0.001
Age (years), mean (standard deviation)	66.7 (10.2)	69.1 (9.8)	65.5 (10.3)	<0.001
Jaundice, n (%)				
Yes	33 (7%)	21 (14%)	12 (4%)	<0.001
No	415 (92%)	130 (84%)	285 (95%)	
Unknown	6 (1%)	3 (2%)	3 (1%)	
Acute pancreatitis, n (%)				
Yes	102 (23%)	27 (18%)	75 (25%)	0.079
No	346 (76%)	124 (80%)	222 (74%)	
Unknown	6 (1%)	3 (2%)	3 (1%)	
Predominant precursor epithelial subtype, n (%)				
Gastric	259 (57%)	73 (47%)	186 (62%)	0.021
Intestinal	135 (30%)	57 (37%)	78 (26%)	
Pancreatobiliary	15 (3%)	7 (5%)	8 (2.7%)	
Oncocytic	20 (4%)	8 (5%)	12 (4%)	
Mixed	16 (4%)	8 (5%)	8 (2.7%)	
Unknown	9 (2%)	1 (1%)	8 (2.7%)	
Highest grade of dysplasia, n (%)				
Low grade	240 (53%)	62 (40%)	178 (59%)	<0.001
High grade	111 (24%)	40 (26%)	71 (24%)	
Invasive carcinoma	103 (23%)	52 (34%)	51 (17%)	
Histological type of invasive carcinoma, n (%)				
Tubular	51 (49%)	23 (44%)	28 (55%)	0.133
Colloid	33 (32%)	22 (42%)	11 (21%)	
Oncocytic	7 (7%)	3 (6%)	4 (8%)	
Unknown	12 (12%)	4 (8%)	8 (16%)	
No. of patients for radiological characteristics	289	111 (38%)	178 (62%)	
IPMN type, n (%)				
Branch duct	134 (46%)	39 (35%)	95 (53%)	0.003
Mixed-type	114 (40%)	57 (51%)	57 (32%)	
Main duct	41 (14%)	15 (14%)	26 (15%)	
MPD size, n (%)				
MPD < 5 mm	134 (46%)	39 (35%)	95 (53%)	0.004
5 mm ≤ MPD < 10 mm	87 (30%)	36 (32.5%)	51 (29%)	
MPD ≥ 10 mm	68 (24%)	36 (32.5%)	32 (18%)	
Enhancing solid component, n (%)				
Yes	40 (14%)	22 (20%)	18 (10%)	0.020
No	249 (86%)	89 (80%)	160 (90%)	
Thickened/enhancing cyst walls, n (%)				
Yes	38 (13%)	19 (17%)	19 (11%)	0.115
No	251 (87%)	92 (83%)	159 (89%)	
Cyst size (mm), mean (standard deviation)	30.3 (±15.2)	33.2 (±16.7)	28.4 (±13.9)	0.003
Lymphadenopathy, n (%)				
Yes	14 (5%)	9 (8%)	5 (3%)	0.041
No	275 (95%)	102 (92%)	173 (97%)	

Abbreviations: IPMN, intraductal papillary mucinous neoplasm; MPD, main pancreatic duct.
[a] Global chi-square or Fisher exact test for categorical variables; Wilcoxon rank-sum test for continuous variables.

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Table 2
Odds ratios for high-grade dysplasia and invasive carcinoma by Diabetes Mellitus status.

	High-Grade Dysplasia			Invasive Carcinoma		
	Number of patients	OR (95% CI)	P	Number of patients	OR (95% CI)	P
Diabetes Mellitus						
No	249	1.00 (reference)		300	1.00 (reference)	
Yes	102			154		
Unadjusted		1.62 (1.00–2.62)	0.051		2.49 (1.59–3.90)	<0.001
Adjusting for worrisome features		2.02 (1.02–4.01)	0.045		2.05 (1.08–3.87)	0.027
Adjusting for high-risk stigmata		1.57 (0.84–2.96)	0.161		1.46 (0.75–2.87)	0.270

Abbreviations: OR, odds ratio. Worrisome features: Acute pancreatitis, cyst size ○≥3 cm, thickened/enhancing cyst wall, abrupt change in caliber of main pancreatic duct with distal pancreatic atrophy, lymphadenopathy, and main pancreatic duct diameter ○≥5 mm.
High-risk stigmata: Jaundice, enhancing solid component, and main pancreatic duct diameter ○≥10 mm.

did not find a significant association between DM and invasive carcinoma after adjusting for high-risk stigmata (Table 2).

Stratified analysis by main pancreatic duct size

Based on the 2012 Fukuoka consensus, dilation of the MPD between 5 and 9 mm is a worrisome feature of malignancy in IPMN and warrants further work-up with endoscopic ultrasound (EUS) but it does not represent a direct indication for surgery by itself [2]. In turn, MPD dilation ○≥10 mm is a high-risk stigma and is an indication to consider surgery. Hence, the relevance of novel risk factors for malignant progression of IPMN is highest for patients with MPD size <10 mm.

We therefore examined whether the association between DM and malignant progression was present in patients with MPD <10 mm, and found that a diagnosis of DM was significantly associated with a 2.1-fold (95% CI 1.01–4.46, P ¼ 0.047) higher risk of high-grade dysplasia in these patients, independent of other worrisome features of malignancy. We found no statistically significant association between DM and high-grade dysplasia in patients with MPD dilation ○≥10 mm (P ¼ 0.273), although this analysis was limited by sample size (Table 3). DM was not significantly associated with invasive carcinoma in patients with MPD <10 mm but it was associated with a 3.3-fold (95% CI 1.03–10.30) higher risk of invasive carcinoma among patients with MPD ○≥10 mm (Table 3).

New-onset, recent-onset, and longstanding diabetes mellitus

Epidemiological evidence shows that risk of developing PDAC is higher in patients diagnosed with DM in the five years prior to cancer diagnosis. DM diagnosed five to nine years before cancer diagnosis is also associated with increased PDAC risk albeit to a lower extent [8]. Therefore, we assessed the duration of DM prior to surgery for IPMN in the subset of patients whose self-reported DM status was confirmed by direct laboratory measurements. The median duration of DM before surgical resection for IPMN was 45 months (range 1 month–47.2 years). The majority of IPMN from patients with new- and recent-onset DM harbored invasive carcinomas (63% and 59%, respectively), while this proportion was lower in patients with longstanding DM (41%).

After adjusting for worrisome features in patients with available preoperative imaging (n ¼ 222), neither recent-onset nor longstanding DM was significantly associated with high-grade dysplasia (P ¼ 0.264 and P ¼ 0.638, respectively). In contrast, recent-onset DM was associated with a 6.9-fold (95% CI 2.38–19.92, P < 0.001) higher risk of invasive carcinoma compared to patients without DM. Longstanding DM was not associated with increased risk of invasive carcinoma compared to patients without DM (P ¼ 0.224).

Diabetes mellitus, predominant precursor epithelial subtype, and histological type of invasive carcinoma

IPMN exhibit significant morphological heterogeneity in the epithelia of precursor lesions as well as in invasive carcinomas arising from them [22,23]. These morphological differences correlate with distinct genomic alterations and are associated with clinical presentation and prognosis [22,23,26–30]. We therefore sought to determine whether DM was associated with specific precursor epithelial subtypes and with the histological type of

Table 3
Odds ratios for high-grade dysplasia and invasive carcinoma by Diabetes Mellitus status stratified by main pancreatic duct diameter, adjusting for worrisome features.

	High-Grade Dysplasia					
	MPD <10 mm			MPD ○≥10 mm		
	No. of patients	OR (95% CI)	P	No. of patients	OR (95% CI)	P
Diabetes Mellitus						
No	128	1.00 (reference)		25	1.00 (reference)	
Yes	60	2.12 (1.01–4.46)	0.047	18	2.58 (0.47–14.12)	0.273
	Invasive Carcinoma					
	MPD < 10 mm			MPD ○≥ 10 mm		
	No. of patients	OR (95% CI)	P	No. of patients	OR (95% CI)	P
Diabetes Mellitus						
No	146	1.00 (reference)		32	1.00 (reference)	
Yes	75	1.85 (0.84–4.06)	0.126	36	3.26 (1.03–10.30)	0.045

Abbreviations: OR, adjusted odds ratio; MPD, main pancreatic duct.

Worrisome features: Acute pancreatitis, cyst size ○≥3 cm, thickened/enhancing cyst wall, abrupt change in caliber of main pancreatic duct with distal pancreatic atrophy, and lymphadenopathy; main pancreatic duct diameter 5–9 mm is a worrisome feature but it was not used in this analysis given that it is the stratifying variable.

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invasive carcinomas arising from IPMN.

Overall, gastric- and intestinal-type epithelia were the most frequent precursor subtypes in our patient population (Table 1). DM was significantly associated with lower likelihood of gastric-type epithelium (OR 0.52, 95% CI 0.35–0.77, P ¼ 0.001) and higher likelihood of intestinal-type epithelium (OR 1.63, 95% CI 1.07–2.47, P ¼ 0.022) (Table 4). Among patients with invasive carcinomas arising from IPMN, half had tubular histology, one third were colloid carcinomas, and a minority were oncocytic carcinomas (Table 1). The prevalence of tubular carcinomas was lower in patients with DM compared to those without DM (44% vs. 55%) but the difference was not statistically significant (P ¼ 0.101). In contrast, DM was significantly associated with a 2.5-fold (95% CI 1.01–5.99, P ¼ 0.047) higher likelihood of colloid carcinoma compared to patients without DM (Table 4).

Discussion

In this series of resected IPMN, preoperative DM was significantly associated with a twofold higher risk of high-grade dysplasia and invasive carcinoma, which was independent of known worrisome features of malignancy such as cyst size and a dilated pancreatic duct. Furthermore, the risk of malignant progression differed based on the duration of DM prior to surgery, and patients whose DM was diagnosed within five years had a 6.9-fold increased risk of harboring invasive carcinoma compared to patients without DM, whereas those with longstanding DM did not have an elevated risk. A history of DM was also linked to IPMN morphological subtypes, and we found that tumors from patients with DM were more likely to be of the intestinal-type, and those with invasive carcinomas arising from IPMN were more frequently colloid.

The observed prevalence of DM in our patient population (34%) was higher than that of subjects age 65 and older in the general population of the United States (26%) [31]. Wide variations in the prevalence of DM among patients undergoing resection for IPMN have been reported (11%–42%). Such variations likely reflect differences in sample size, patient selection, and method of DM ascertainment [11–18]. Among patients with invasive carcinomas arising from IPMN in our study, half had a diagnosis of DM, which is similar to the reported prevalence of DM in patients with PDAC undergoing fasting plasma glucose testing [3–5].

Previous studies of predictors of malignancy in IPMN have described an association between DM and dysplasia in IPMN, but the results are conflicting and no study has concretely focused on DM as risk factor for progression [11–18]. While some reports have found that DM is associated with higher degrees of dysplasia, most analyses have not examined whether associations were independent of other known predictors of malignant progression (e.g. 2012 Fukuoka consensus worrisome features and high-risk stigmata of malignancy). A study of 82 patients with resected IPMN found that after adjusting for radiological IPMN type, patients with DM were at higher risk of "malignancy" defined as high-grade dysplasia and invasive carcinoma combined in a single category [13]. This definition of malignancy is an important limitation. Assessment of high-grade dysplasia separately from invasive carcinoma is crucial in studies of IPMN, as the two lead to markedly different outcomes. Patients undergoing complete resection for IPMN with no evidence of invasive carcinoma have an excellent prognosis (>95% disease-specific survival) [32], whereas patients with invasive carcinoma arising from IPMN have a five-year survival of approximately 40% [33]. Therefore, markers that help identify high-grade dysplasia before progression to invasive carcinoma are the most relevant for practicing clinicians.

In our study population, a diagnosis of DM was associated with a twofold higher risk of both high-grade dysplasia and invasive carcinoma when analyzed separately. Notably, the associations were independent of known worrisome features of malignancy defined by the 2012 Fukuoka consensus guidelines [2]. Based on these guidelines, patients who show worrisome features should undergo further diagnostic evaluation with EUS and closer monitoring. Our findings indicate that a diagnosis of DM in patients with IPMN may be helpful to identify patients with high-risk lesions who could benefit from further diagnostic work-up to detect high-grade dysplasia, which may include EUS and fine-needle aspiration biopsy. Moreover, our results suggest DM is of value for detecting high-grade dysplasia in patients with a MPD <10 mm. The risk of malignant progression in our study differed by the duration of DM before surgery. Similar to observations made in epidemiological studies of PDAC, the association between DM and malignant progression in IPMN was stronger in patients with recently-diagnosed DM, and we found that patients whose DM was diagnosed within five years before resection had a 6.9-fold higher risk of invasive carcinoma relative to patients without DM.

A diagnosis of DM was also associated with distinct precursor epithelial subtypes and with the histological type of invasive carcinomas arising from IPMN. Tumors from patients with DM were less likely to be gastric-type predominant and more likely to have intestinal-type precursor epithelium; in cases of invasive carcinoma arising from IPMN, those from patients with DM were more likely to be colloid type. It is becoming increasingly clear that IPMN are genetically and morphologically heterogeneous. Invasive carcinomas arising from gastric-type predominant IPMN are mostly tubular carcinomas with a high prevalence of KRASmutations and a prognosis similar to that of conventional PDAC [22,23,27].

Table 4
Odds ratios for predominant precursor epithelial subtype and histological type of invasive carcinomas by Diabetes Mellitus status.

Precursor Epithelial Subtype	Gastric-Predominant[a]			Intestinal-Predominant[a]		
	Number of patients	OR (95% CI)	P	Number of patients	OR (95% CI)	P
Diabetes Mellitus						
No	292	1.00 (reference)		292	1.00 (reference)	
Yes	153	0.52 (0.35–0.77)	0.001	153	1.63 (1.07–2.47)	0.022
Invasive Carcinoma Histological Type	**Tubular Carcinoma[b]**			**Colloid Carcinoma[b]**		
	Number of patients	OR (95% CI)	P	Number of patients	OR (95% CI)	P
Diabetes Mellitus						
No	43	1.00 (reference)		43	1.00 (reference)	
Yes	48	0.49 (0.21–1.15)	0.101	48	2.46 (1.01–5.99)	0.047

Abbreviations: OR, unadjusted odds ratio.
[a] Compared vs. other epithelial subtypes combined.
[b] Compared vs. other histological types combined.

Please cite this article in press as: Morales-Oyarvide V, et al., Diabetes mellitus in intraductal papillary mucinous neoplasm of the pancreas is associated with high-grade dysplasia and invasive carcinoma, Pancreatology (2017), http://dx.doi.org/10.1016/j.pan.2017.08.073

Conversely, colloid carcinomas frequently arise from intestinal-type IPMN and exhibit very high prevalence of GNAS mutations and more favorable outcomes than tubular carcinomas [22,23,26–28,34,35]. Intestinal-type IPMN and colloid carcinomas strongly express the intestinal lineage marker CDX2 and secrete MUC2-rich, thick, viscous mucin [36]. We and others previously reported that episodes of acute pancreatitis in patients with IPMN are associated with intestinal-type tumors [29,37]; however, it is unclear whether this same chronic obstruction is leading to pancreatic endocrine dysfunction or if the mechanism is entirely different.

The current study has several strengths. It is to our knowledge the largest study evaluating the association of DM with high-grade dysplasia and invasive carcinoma in resected IPMN. To define DM we used current American Diabetes Association guidelines, thus reducing the risk of misclassification bias. Since all the patients in our study underwent surgical resection, we had pathological confirmation of an IPMN diagnosis and detailed information about tumor morphological features. Lastly, a subset of our patients had highly annotated radiological data that allowed us to assess the value of DM after adjusting for known clinical and radiological predictors of malignant progression; while the ability to examine the independent role of DM in multivariable-adjusted models is of great value, we were not able to do it in the entire patient population due to unavailable preoperative imaging studies.

Our study also had limitations. Our patient population is derived from a high-volume pancreatic surgery reference center, introducing potential for referral bias [38] which may limit the generalizability of our findings to the general populations. Our study population consisted of patients who underwent resection over a 26-year period and indications for surgery in IPMN have varied over time. At our institution, we have mostly followed the recommendations put forth by the 2006 International Consensus Guidelines (Sendai) [2] and since 2012 the revised Fukuoka consensus [39], but prior to that indications for surgery were not well defined. Lastly, date of DM diagnosis was derived from patient self-report and may be subject to recall bias [40]. To reduce the likelihood of misclassification, we restricted analyses based on DM duration to patients whose self-reported DM status was confirmed by laboratory tests. Moreover, as mentioned in the previous paragraph, the sample size for these analyses was further restricted by lack of preoperative imaging in a fraction of patients.

In summary, a diagnosis of DM – especially of recent onset – in patients with IPMN may help to identify patients with high-risk lesions who could benefit from further diagnostic work-up, independent of other worrisome features of malignancy. The risk-stratification value of DM was present in patients with MPD <10 mm, who continue to pose a management challenge. Presence of DM may also be informative of the morphological subtypes of IPMN, both in precursor and invasive lesions. Prospective observational studies evaluating the role of DM in patients under surveillance are warranted, and if this association is confirmed, the presence of DM could be added to the management algorithm.

Acknowledgements

The authors have no sources of funding or conflicts of interest to report.

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Please cite this article in press as: Morales-Oyarvide V, et al., Diabetes mellitus in intraductal papillary mucinous neoplasm of the pancreas is associated with high-grade dysplasia and invasive carcinoma, Pancreatology (2017), http://dx.doi.org/10.1016/j.pan.2017.08.073

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Please cite this article in press as: Morales-Oyarvide V, et al., Diabetes mellitus in intraductal papillary mucinous neoplasm of the pancreas is associated with high-grade dysplasia and invasive carcinoma, Pancreatology (2017), http://dx.doi.org/10.1016/j.pan.2017.08.073

CANCER

Multiparametric plasma EV profiling facilitates diagnosis of pancreatic malignancy

Katherine S. Yang,[1,2]* Hyungsoon Im,[1,2]* Seonki Hong,[1,2]* Ilaria Pergolini,[3] Andres Fernandez del Castillo,[1] Rui Wang,[4,5] Susan Clardy,[1,2] Chen-Han Huang,[1,2] Craig Pille,[1,6] Soldano Ferrone,[3] Robert Yang,[1] Cesar M. Castro,[1,7] Hakho Lee,[1,2] Carlos Fernandez del Castillo,[3,7] Ralph Weissleder[1,2,8]†

Pancreatic ductal adenocarcinoma (PDAC) is usually detected late in the disease process. Clinical workup through imaging and tissue biopsies is often complex and expensive due to a paucity of reliable biomarkers. We used an advanced multiplexed plasmonic assay to analyze circulating tumor-derived extracellular vesicles (tEVs) in more than 100 clinical populations. Using EV-based protein marker profiling, we identified a signature of five markers ($PDAC^{EV}$ signature) for PDAC detection. In our prospective cohort, the accuracy for the $PDAC^{EV}$ signature was 84% [95% confidence interval (CI), 69 to 93%] but only 63 to 72% for single-marker screening. One of the best markers, GPC1 alone, had a sensitivity of 82% (CI, 60 to 95%) and a specificity of 52% (CI, 30 to 74%), whereas the $PDAC^{EV}$ signature showed a sensitivity of 86% (CI, 65 to 97%) and a specificity of 81% (CI, 58 to 95%). The $PDAC^{EV}$ signature of tEVs offered higher sensitivity, specificity, and accuracy than the existing serum marker (CA 19-9) or single–tEV marker analyses. This approach should improve the diagnosis of pancreatic cancer.

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer death in the United States, with a 5-year survival rate below 10%. Most newly diagnosed patients' (>80%) tumors are considered unresectable (1). Earlier detection could increase survival by an estimated 30 to 40% (2), and more reliable and real-time assessment of treatment effects could prolong survival and/or improve quality of life. Detecting serum concentrations of CA 19-9 is currently the best established blood test for PDAC and has a pooled sensitivity of 75.4% [95% confidence interval (CI), 73.4 to 77.4%] and a specificity of 77.6% (CI, 75.4 to 79.7%) (3). Although often used to follow treatment response, CA 19-9 is a poor biomarker for early detection, commonly rises late in the disease, and may be elevated in nonmalignant conditions such as biliary obstruction and pancreatitis (4). Recent modeling studies on assay performance cite a minimum sensitivity of 88% and a specificity of 85% to prolong patient survival and demonstrate cost effectiveness (2). Various approaches to achieving this are being explored (5, 6), including the use of CA242 (3), circulating tumor cells (7), circulating tumor-derived extracellular vesicles (tEVs), which include exosomes (8), metabolites (9) and proteomic analyses (10, 11), and circulating DNA (12). Beyond the inherent technical challenges of these advanced analyses, a key question is whether early reported findings can be validated independently in larger sets of patients.

tEVs offer an attractive approach to monitoring cancers using "liquid biopsies." tEVs are relatively more abundant than other circulating biomarkers, are structurally more stable, and contain protein and mRNA profiles that highly reflect parental cancer cells (13–15). Experimental studies have shed light on the composition and functional roles of tEVs (16, 17) for (i) diagnosis (13, 18, 19), (ii) long-range communication (16), and (iii) host cell interaction (20). Rapid and accurate analysis of tEVs in clinical samples is often hampered by three practical challenges: lengthy isolation procedures requiring ultracentrifugation to isolate tEVs, general unavailability of ultrasensitive assay systems to analyze large clinical cohorts for multiple markers, and specific cancer markers that separate tEVs from host cell EVs. Here, we developed an advanced plasmonic sensing system for higher-throughput analysis of clinical samples to directly address the shortcomings often found in translational clinical analyses. The basic operating principle relies on measuring spectral shifts of resonant light transmission through periodically arranged gold nanopores to which tEVs are captured by immunoaffinity. In proof-of-principle cancer experiments, we showed that tEVs can be detected by plasmonic sensors (14). However, the original prototype was manually operated and had limited throughput and chip production rates, thus preventing widespread clinical use. As a result, we devised a multiparametric system incorporating large numbers of sensing arrays (>100 sensing spots) and automatic operation to enable routine clinical sample analyses. Intrigued by recent reports (8, 21), we set out to determine key protein profiles of tEVs in 135 patients undergoing surgery for pancreatic pathologies. Motivated by clinical needs, we were particularly interested in defining practical and reliable tEV marker sets for PDAC diagnosis.

RESULTS

Nanoplasmonic sensors for high-throughput, sensitive analyses of tEVs

Figure 1 summarizes the working principle of the nanoplasmonic sensor (NPS) assay, specifically designed for clinical workflows, small clinical sample amounts, and high-throughput detection. The sensor chip contains periodically arranged nanopores (200 nm in diameter and 500 nm in periodicity) patterned in a 100-nm-thick gold film. The function of the pores is to transmit light shone onto the gold

[1]Center for Systems Biology, Massachusetts General Hospital, Boston, MA 02114, USA. [2]Department of Radiology, Massachusetts General Hospital, Boston, MA 02114, USA. [3]Department of Surgery, Massachusetts General Hospital, Boston, MA 02114, USA. [4]Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA 02115, USA. [5]Department of Population Medicine, Harvard Medical School and Harvard Pilgrim Health Care Institute, Boston, MA 02215, USA. [6]Department of Health Sciences, Northeastern University, Boston, MA 02115, USA. [7]Massachusetts General Hospital Cancer Center, Boston, MA 02114, USA. [8]Department of Systems Biology, Harvard Medical School, Boston, MA 02115, USA.
*These authors contributed equally to this work.
†Corresponding author. Email: rweissleder@mgh.harvard.edu

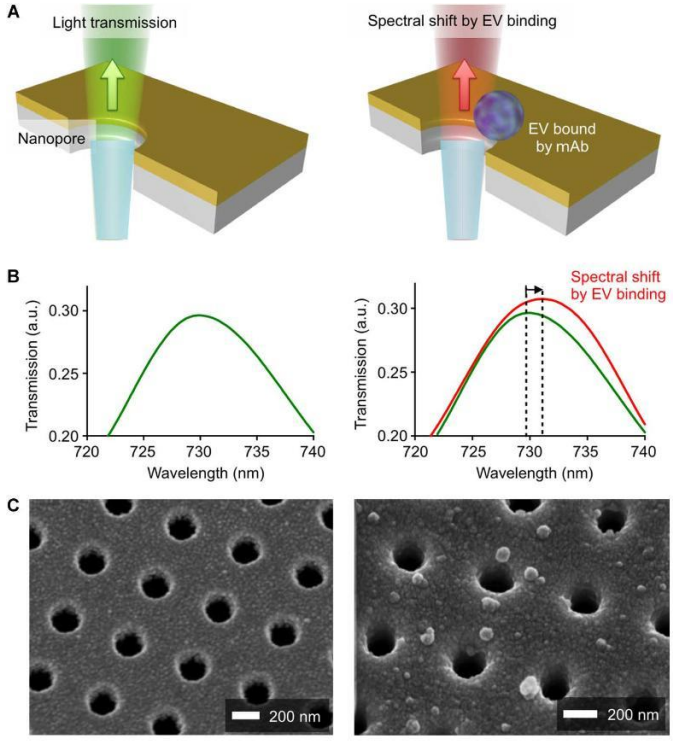

Fig. 1. Workingprincipleoftheplasmonsensorchip(NPS)fortEVs. (A) EVbinding to the nanopore surface via monoclonal antibody (mAb) immobilized on the gold surface causes a spectral shift of light transmitted through the nanopores. (B) The spectral shift of resonance peak in light transmission is measured to quantify the amount of EVscaptured on the nanopore surface. a.u., arbitrary unit. (C) Scanning electron micrographs show the periodically arranged nanopore array and EVscaptured on the surface. Each nanohole has a diameter of 200 nm and a periodicity of 500 nm.

surface (Fig. 1A). When EVs are bound in the vicinity of these pores via specific antibodies, the wavelength of the transited light shifts to red. It is this red shift that is detected by sensors and reflects the amount of bound EVs (Fig. 1, B and C).

The sensor chip is easily scalable to larger arrays of more than 1000 sensing sites; multiple sensor chips can be made through batch fabrication processes (see Materials and Methods for details). We prepared a customized chip with 100 detection sites to yield data for 25 antibodies/markers in quadruplicate. Antibody and EV solutions were printed on the sensor chip in spots as small as 100 nl through a molecularprintingmethod.Apiezoelectric microscope stage was incorporated into the system for scanning sensing arrays and collecting transmission spectra. Both printing and measurements are operated automaticallytoimproveassaythroughput and reduce variation among users. Overall, the smaller chip size, higher spot density, and smaller measurement volumes resulted in a 25× increase in sensitivity compared to a previous prototype (120 ml for 12 markers versus 10 ml for 25 markers). Figure S1 shows the NPS chip with 100 sensing arrays and the integrated setup optimized for processing clinical samples.

Correlation between tEV composition in PDAC models and that of parental cells

A number of putative cell-associated PDAC markers have been described for

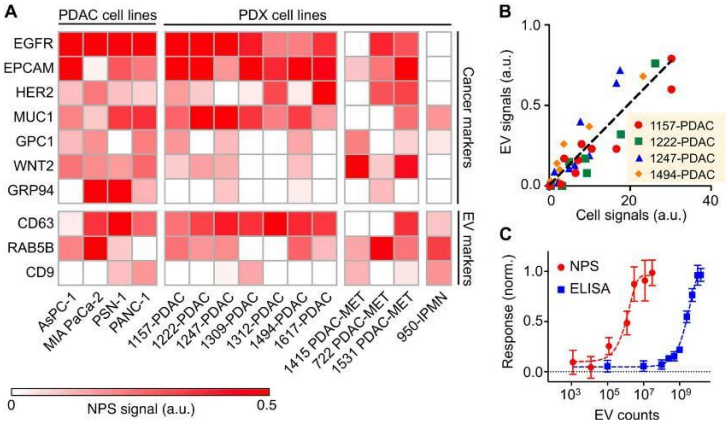

Fig. 2. In vitro profiling of tEV markers on cell line–derived EVs. (A) The molecular expression of cancer markers (EGFR,EPCAM,HER2, MUC1,GPC1,WNT2,and GRP94)and EVmarkers (CD63,RAB5B,and CD9)was characterized on EVs derived from 4 cancer cell lines and 11 PDXcell lines including PDAC,metastatic PDAC(PDAC-MET), and IPMN.(B) Correlation of protein expression between EVsand their parental cell lines (1157-PDAC, 1222-PDAC,1247-PDAC,and 1494-PDAC).(C) Sensitivity comparison between NPS and the gold standard ELISA.The responses were normalized against the values of highest concentrations.

individual patients, but using single markers in entire cohorts generally has insufficient sensitivity or specificity. Proteome analyses have identified soluble markers (11, 22–24), and profiling studies have identified cell surface (25–27) or exosomal markers (8, 28, 29). To calibrate and validate the new plasmonic sensing system, we investigated 15 putative cancer and EV markers (table S1) by performing flow cytometry

Table 1. Summary of patient cohorts.

Characteristic	Training cohort		Prospective cohort		Total
	Malignant	Benign	Malignant	Benign	
Total cases	22	10	82	21	135
Subtypes					
PDAC untreated	13	—	22	—	35
PDAC neoadjuvant tx	9	—	24	—	33
IPMN inter/high-grade	—	—	13	—	13
NET	—	—	12	—	12
Other cancer	—	—	11	—	11
Benign cystic tumor	—	—	—	5	5
Pancreatitis	—	—	—	8	8
Controls	—	10	—	8	18
Age (years)					
Median	68	48	65	57	63
Range	47–88	23–82	17–84	19–91	17–91
Sex					
Male	8	4	47	14	73
Female	14	1	40	14	69
CA 19-9					
PDAC untreated	1,148 (1–6,684)	—	1,006 (1–10,625)	—	1,064 (1–10,625)
PDAC neoadjuvant tx	2,258 (19–17,101)	—	657 (4–7,730)	—	1,175 (4–17,101)
IPMN	—	—	10.6 (1–26)	—	10.6 (1–26)
CEA					
PDAC untreated	7.0 (2–12)	—	3.4 (0.6–22.1)	—	5.0 (0.6–22.1)
PDAC neoadjuvant tx	11.0 (1–53)	—	56.4 (0.7–1,003)	—	40 (0.7–1,003)
IPMN	—	—	2.1 (0.7–3.3)	—	2.1 (0.7–3.3)
Stage					
I	0	—	10	—	10
II	1	—	42	—	43
III	5	—	5	—	10
IV	16	—	10	—	26
Co-therapies PDAC					
Folfirinox/XRT	4	—	21	—	25
Gemcitabine	5	—	1	—	6
Other	—	—	2	—	2

on whole pancreatic cancer cells (fig. S2). On the basis of the cell data, we eliminated some of the non–cancer-specific markers and performed NPS measurements on tEVs (Fig. 2A). Beyond the commonly used PDAC cell lines, we also investigated 11 patient-derived tumor xenograft (PDX) models of PDAC, metastatic PDAC, and intraductal papillary mucinous neoplasm (IPMN) (Fig. 2A). Our data show good correlation between expression patterns seen in whole cells and tEVs (Spearman correlation coefficient r = 0.86 for 1157-PDAC, 1222-PDAC, 1247-PDAC, and 1494-PDAC; Fig. 2B). The tEV assays by the NPS chip are on the order of 10^2 more sensitive than the gold standard enzyme-linked immunosorbent assay (ELISA) for this analysis (Fig. 2C).

Establishing a PDAC tEV panel

We next collected plasma from 32 patients enrolled in a training cohort involving 22 cases of PDAC and 10 healthy controls (Table 1). Figure 3A summarizes the chosen tEV markers for each patient, including pan-cancer markers (EGFR, EPCAM, HER2, and MUC1) and putative PDAC markers [GPC1, WNT2, and GRP94 (30)]. Using receiver operating characteristic (ROC) analyses, we determined sensitivity, specificity, and accuracy for each marker individually and also in combination (Fig. 3B and Table 2). We observed that no single marker achieved sufficiently high sensitivity and specificity. Therefore, we reasoned that a combination of multiple markers would be necessary. A previously identified generic quad marker cancer signature (31) (EGFR, EPCAM, HER2, and MUC1) had high sensitivity (91%), specificity (100%), and accuracy (94%). When we replaced HER2 with putative PDAC markers (GPC1 and WNT2), we further improved the sensitivity and specificity (Table 2). This $PDAC^{EV}$ signature, representing an unweighted sum of EGFR, EPCAM, MUC1, GPC1, and WNT2 signals, had an accuracy of 100% in this training cohort (Fig. 3, C and D). Because of the limited sample size (n = 32), we also tested all four and five marker combinations in the prospective cohort described below.

A number of observations were of particular interest. First, some of the chosen markers highly expressed in EVs did not provide diagnostic information (Rab5b, CD9, and CD63; fig. S3) and were thus eliminated from the ensuing prospective study. Second, GPC1 was not specific for PDAC in our cohort and had a lower accuracy as a single marker than marker combinations, similar to other markers tested. These findings did not change by using alternative commercially available GPC1 antibodies, all of which were validated before use (table S1). Third, other putative PDAC markers such as WNT2 showed better accuracy than GPC1.

Validation cohort

We next analyzed a prospective cohort of 43 patients undergoing surgery for pancreatic (n = 35) or other abdominal indications (n = 8, age-matched control group). In all 35 patients operated for pancreatic indications, tissue was available for clinical pathology interpretation (n = 22 for PDAC, n = 8 for pancreatitis, and n = 5 for benign cystic tumor). We obtained 2 to 10 ml of plasma from each patient on the day of or immediately preceding surgery, and NPS measurements were performed using identical markers from our training cohorts (see Materials and Methods for details).

Figure 4A summarizes the performance of the PDAC markers in differentiating PDAC from pancreatitis, benign, and control patient groups. Analyzing the heat map of EV markers once again demonstrated that no single patient had similar markers elevated. Rather, it was the combination of the five markers comprising the $PDAC^{EV}$ signature that resulted in an overall accuracy of 84%. In this prospective cohort, the $PDAC^{EV}$ signature (EGFR, EPCAM, MUC1, WNT2, and

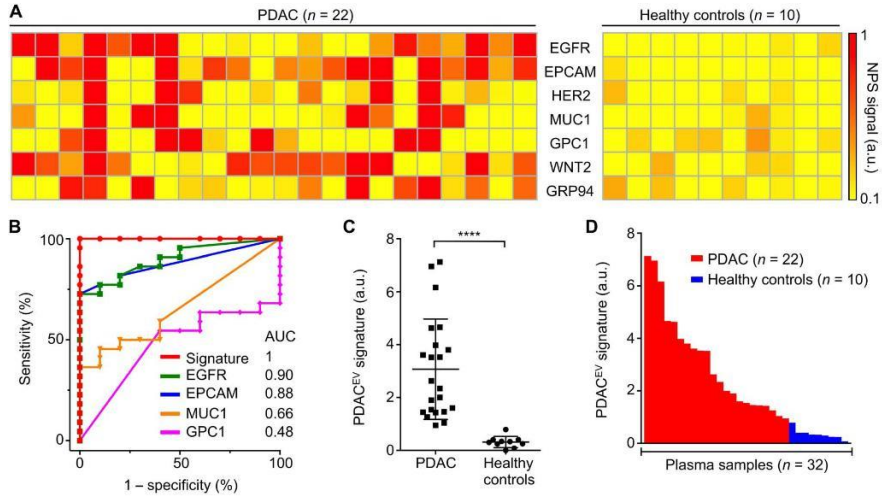

Fig. 3. Molecular profiling of plasma EV for a training cohort. (A) Putative cancer markers (EGFR, EPCAM, HER2, and MUC1) and PDAC markers (GPC1, WNT2 and GRP94) were profiled on EVs collected from 22 PDAC patients and 10 healthy controls. (B) ROC curves were calculated for single protein markers as well as for the $PDAC^{EV}$ signature combination to determine optimum EV threshold values. AUC, area under the curve. (C) A combined marker panel (EGFR, EPCAM, MUC1, GPC1, and WNT2) was established as a $PDAC^{EV}$ signature that showed 100% accuracy for the training cohort in distinguishing PDAC from healthy controls. P value was determined by Mann-Whitney test. ****P < 0.0001. (D) A waterfall plot shows the $PDAC^{EV}$ signature signals sorted from high (left) to low (right). Each column represents a different patient sample (red, malignant; blue, benign).

Table 2. Statistical analyses of EV markers for training and prospective cohorts. Ninety-five percent CIs are indicated in parentheses. NA, not applicable.

Biomarker(s)	n	Cutoff	AUC	Training cohort (n = 32)			Prospective cohort (n = 43)		
				Sensitivity (%)	Specificity (%)	Accuracy (%)	Sensitivity (%)	Specificity (%)	Accuracy (%)
EGFR	1	0.15 (0.01–0.24)	0.90 (0.79–1)	73	100	81	59 (36–79)	76 (53–92)	67 (51–81)
EPCAM	1	0.28 (0.01–0.34)	0.88 (0.77–0.99)	73	100	81	45 (24–68)	95 (76–100)	70 (54–83)
HER2	1	0.13 (0.03–0.32)	0.72 (0.55–0.89)	59	90	69	59 (36–79)	85 (64–97)	72 (56–85)
MUC1	1	0.34 (0.02–0.53)	0.66 (0.48–0.84)	36	100	56	36 (17–59)	90 (70–99)	63 (47–77)
GPC1	1	0.04 (0.04–0.68)	0.48 (0.28–0.67)	55	60	56	82 (60–95)	52 (30–74)	67 (51–81)
WNT2	1	0.18 (0.09–0.48)	0.84 (0.71–0.96)	77	90	81	64 (41–83)	76 (53–92)	70 (54–83)
GRP94	1	0.10 (0.02–0.46)	0.73 (0.55–0.90)	73	70	72	55 (32–76)	71 (48–89)	63 (47–77)
B7-H3	1	0.19 (0.02–0.23)	0.75 (0.58–0.93)	50	100	59	NA	NA	NA
EGFR+ EPCAM+ HER2+ MUC1	4	0.67 (0.29–0.68)	0.99 (0.97–1)	91	100	94	86 (65–97)	86 (64–97)	86 (72–95)
EGFR+ EPCAM+ GPC1+ WNT2	4	0.74 (0.65–0.84)	1.0	100	100	100	82 (60–95)	90 (70–99)	86 (72–95)
EGFR+ EPCAM+ MUC1+ GPC1+ WNT2	5	0.87 (0.68–1.00)	1.0	100	100	100	86 (65–97)	81 (58–95)	84 (69–93)
EGFR+ EPCAM+ HER2+ MUC1+ GPC1+ WNT2	6	0.89 (0.73–1.00)	1.0	100	100	100	95 (77–100)	81 (58–95)	88 (75–96)

GPC1) identified in the training cohort showed a sensitivity of 86% (CI, 65 to 97%) and a specificity of 81% (CI, 58 to 95%; Fig. 4B and Table 2), whereas total EV concentrations were not significantly different between the groups (Dunn's multiple comparisons test, P = 0.16 for PDAC and pancreatitis; P = 0.78 for PDAC and control) (Fig. 4C). Furthermore, the expression of GPC1 was not significantly different in PDAC relative to pancreatitis (P = 0.31) but was slightly higher in PDAC when compared to the control group (median values of 0.20 for PDAC and 0.02 for the control group; P = 0.018) (Fig. 4D). Figure 5 displays the experimental data of single markers and combinations as a waterfall plot. Table 2 summarizes the diagnostic accuracies of all markers and combinations in this prospective cohort.

We next correlated EV analyses to clinical gold standard serum biomarkers (CA 19-9 and CEA) in patients with PDAC. The $PDAC^{EV}$ signature was not correlated with either CA 19-9 (Spearman correlation coefficient r = −0.28; P = 0.26) or CEA (r < 0.001; P > 0.99) (Fig. 6A). In our cohort, only 61% of PDAC patients (11 of 18) showed an increased concentration of CA 19-9 (>37 U/ml, threshold value used in clinic), whereas 89% (16 of 18) had high $PDAC^{EV}$ values (>0.87 NPS signal; Table 2). For CEA, only 17% of PDAC patients (3 of 18) were positive (>5 ng/ml; Fig. 6B). Finally, we compared the $PDAC^{EV}$ signature against tumor size, showing a modest correlation for the signature (r = 0.58; P = 0.018) and little correlation (r = −0.09; P = 0.62) between EV counts and tumor size (Fig. 6C and fig. S4).

To further expand the clinical use of tEV analyses, we also studied several additional patient cohorts (n = 69; Table 1; Fig. 7 and figs. S5 and S6): (i) PDAC treated with neoadjuvant regimen (n = 24), (ii) IPMN (n = 14), (iii) other gastrointestinal (GI) cancers mimicking the symptoms of pancreaticoduodenal cancers (n = 11), (iv) pancreatic neuroendocrine tumors (NETs; n = 12), and (v) benign cystic tumors (n = 5). Patients with PDAC treated with neoadjuvant regimen had lower EV signatures (median values, 1.70 versus 0.86; Mann-Whitney test, P = 0.015) (fig. S7A) as a group compared to the untreated PDAC group, likely reflecting the smaller tumor mass and/or favorable treatment response.

We also studied a number of cases of IPMNs, which grow within the pancreatic ducts and are characterized by the production of thick mucinous fluid. IPMNs are important because some of them progress to invasive cancer and may therefore represent windows of opportunity to treat before aggressive and difficult-to-manage cancer develops. Our cohort contained 11 cases of intermediate and high-grade IPMN and 2 cases of low-grade IPMN (Fig. 7 and Table 1). As shown in fig. S7B, IPMN had an elevated $PDAC^{EV}$ signature compared to age-matched controls (Dunn's multiple comparisons test, P < 0.0001), but it was lower compared to PDAC (P = 0.022).

The validation cohort included a limited number of other pancreatic cancers or cancers that can mimic pancreatic symptomatology. These included NETs and gastroduodenal cancers. Again,

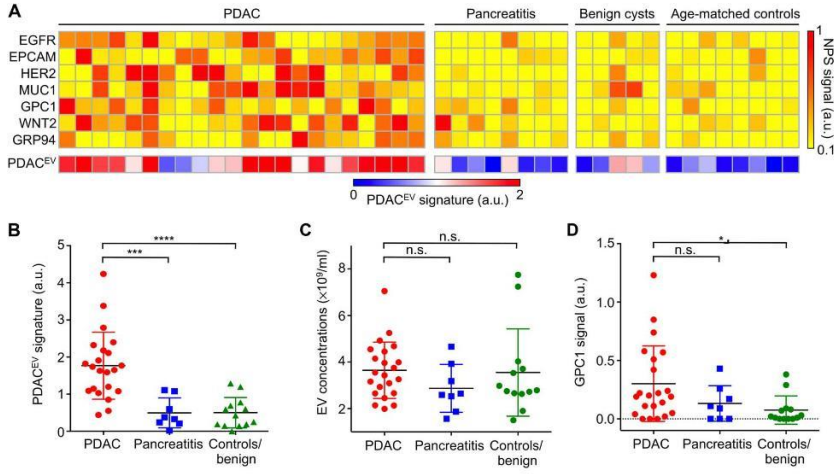

Fig. 4. The PDAC^{EV} signature differentiation of PDAC patients from pancreatitis and control patient groups. (A) Heatmap analysis of EV markers. The PDAC^{EV} signature is defined as a combined marker panel of EGFR, EPCAM, MUC1, GPC1, and WNT2. (B to D) The established PDAC^{EV} signature signals (B), EV concentrations (C), and single GPC1 signal (D) as measured for plasma EVs collected from 22 PDAC patients, 8 with pancreatitis, 5 with benign cystic tumors, and 8 age-matched controls. Pairwise comparison P values were determined by the Dunn's multiple comparisons test. *P < 0.05, ***P < 0.001, ****P < 0.0001. n.s., not significant.

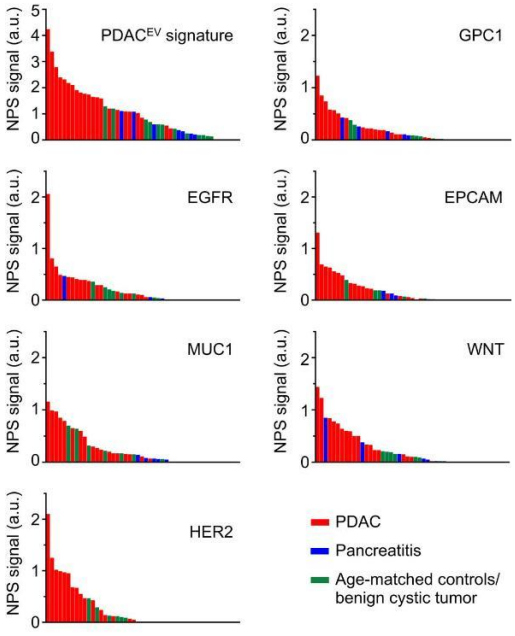

Fig. 5. Distribution of EV protein marker signals. Waterfall plots show EV protein content for each of the different biomarkers sorted from high (left) to low (right). Each column represents a different patient sample (red, PDAC, n = 22; blue, pancreatitis, n = 8; green, age-matched controls and benign cystic tumors, n = 13).

although the numbers are limited, most of the malignancies tested positive for some of the EV markers (for example, among 23 patients, 18 were positive for EGFR and/or EPCAM). These findings are in line with other observations. For example, EPCAM has been evaluated as a CTC detection marker in NET populations (32, 33). Of particular interest was the fact that 9 of 12 NETs tested positive for SSTR2 expression on EVs, whereas all PDAC patients (n = 22) and age-matched healthy controls (n = 8) were negative for SSTR2 with a threshold value of 0.15 (Dunn's multiple comparisons test, P < 0.0001 between PDAC and NET; P = 0.0018 between NET and control) (fig. S7C). Finally, we investigated a limited number of patients with benign mucinous tumors but no detectable malignancy. In these cases, we observed PDAC^{EV} signatures similar to the age-matched control group (Mann-Whitney test, P = 0.35; fig. S7D).

DISCUSSION

EVs are attractive as circulating biomarkers given their abundance, relative stability, and similar molecular makeup to parental cells (13–15). Despite these apparent advantages, it has been difficult to define single tumor-specific EV markers (mRNA, DNA, or protein) (8, 28, 34), validate purported malignancy biomarkers in larger patient cohorts (8, 13, 34), implement lengthy purification procedures (ultracentrifugation) into the clinical workflow (13), and commercialize cost-effective technologies. Here, we show in a sizable pancreatic data set that single EV protein biomarkers are unlikely to be sufficiently accurate to improve patient management. No individual putative protein tEV marker (EGFR, EPCAM, MUC1, GPC1, or WNT2) yielded sensitivities above 86% and specificities above 81% to be considered cost-effective (2). Many had much lower sensitivities/specificities, including GPC1, despite previous studies (5, 8); unfortunately, the previously used GPC1 antibody is no longer

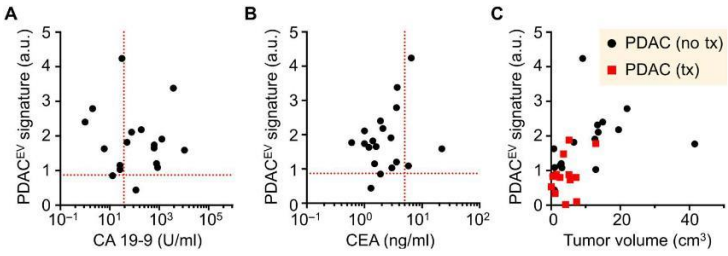

Fig. 6. Comparison of EV analyses with conventional clinical metrics. Correlation of the PDAC^{EV}signature values with serum biomarkers [CA19-9 (A) and CEA(B)] and the tumor diameter (C) for patients with PDAC.tx, treatment. The dashed red lines indicate the threshold values for positivity (CA19-9, 37 U/ml; CEA,5 ng/ml; PDAC^{EV}signature, 0.87).

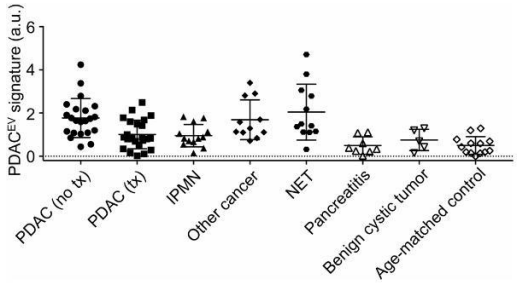

Fig. 7. EV analyses for patients with different types of pancreatic diseases. The PDAC^{EV}signature values were measured for patient cohorts (n = 103) including (i) PDACwithout treatment (n = 22), (ii) PDACtreated with neoadjuvant regimen (n = 24), (iii) IPMN (n = 13), (iv) other GI cancers mimicking the symptoms of pancreaticoduodenal cancers (n = 11), (v) pancreatic NET(n = 12), (vi) pancreatitis (n = 8), (vii) benign cystic tumors (n = 5), and (viii)age-matched controls (n = 18).

commercially available. Therefore, there is a possibility that the discrepancy could be attributed to the antibody used in the study.

On the basis of the hypothesis that tumoral heterogeneity will require multiplexed biomarkers for clinical use, we set out to define protein signatures representative of epithelial and pancreatic cancers (25). We initially surveyed about 50 proteins of potential interest and discarded all but 10 after feasibility studies in PDAC cell lines and PDX models. These 10 EV markers included 7 tEV markers (EGFR,EPCAM, MUC1, HER2, GPC1, WNT2, and GRP94) and 3 pan EV markers (CD63, CD9, and Rab5b). The pan EV markers were excluded from the tumor diagnostic marker panel and were solely used to confirm the presence of EVs in a given sample. From an initial training data set, we further refined the markers to five essential ones, which then constituted our PDAC^{EV} signature (EGFR, EPCAM, MUC1, GPC1, and WNT2). The signature was defined as the unweighted sum of each marker expression, with a score of >0.85 suggesting PDAC. It is conceivable to further improve on this panel by identifying additional molecular tumor markers present on the EV surface. A number of proteomic approaches have been used to identify putative markers, but validation work remains to be done. It would also be attractive to expand the panel to intravesicular markers such as mutant KRASprotein, but this would require EV lysis and further NPS assay optimization.

Applying the above PDAC^{EV} signature to 43 patients, we showed an overall sensitivity of 86% for detecting PDAC and a specificity of 81% for differentiating PDAC from other pancreatic diseases (Table 2). The accuracy was 84% (CI, 69 to 93%). The relatively high accuracy is most likely attributed to the selection of protein EV markers, the surgical patient cohorts enrolled, and ease of measurements, resulting in reduced analytical failures. The last point of particular interest is that existing EV analyses are cumbersome and often require large sample amounts. In contradistinction, we set out to develop a miniaturized sensing technology with an automated microarray spotter and scanning stage to perform measurements at scales that are clinically feasible and affordable. The NPS measurements performed here require ~10 min of measurement/analysis time and currently cost $60 (chip cost, $42; antibody cost, $18) per patient sample. Because the majority of current costs are driven by manual manufacture of chips and antibodies, it is expected that real costs will scale downward greatly with bulk fabrication. The current limits of the NPS technology as developed in this study are (i) the need for EV purification and concentration before measurement, (ii) lower sensitivity for intravesicular markers, (iii) the need for high-quality antibodies that are necessary for capture, and (iv) the composition of the marker panel. With further optimization and commercialization, all these points could be addressed and further improved.

A number of previous studies have investigated tEV as a diagnostic cancer marker by both protein and nucleic acid analyses (8, 13–15). Remaining questions include (i) whether these results hold up in larger patient cohorts and (ii) how cost-effectiveand practical are newer analytical techniques. For example, Melo et al. (8) explored the use of GPC1 as a single marker for detection of PDAC from EVs. Similarly, several studies have explored the serum proteome of PDAC (22–24) with the goal of providing more advanced diagnostic tools to guide clinicians. So far, measurement of tEV appears to be a promising venue for pancreatic diagnoses.

The current study was designed as a feasibility study to focus on some of the pressing questions in surgical oncology. Future studies should expand tEV analysis to assess treatment efficacy. Although the current study was not designed to investigate this, such work has been done for other cancers (14, 15, 35). For example, we have shown that longitudinal tEV profiling is feasible and can be informative in treatment assessment (14, 15, 35). Long-term efforts should also include longitudinally analyzing high-risk subjects for PDAC development, which will require larger data sets and multiyear follow-ups.

MATERIALSAND METHODS

Study design

We used data collected from a pilot study at Massachusetts General Hospital (MGH) to optimize use of the NPS technique for tEV detection in plasma and to identify useful biomarker combinations and their detection thresholds as a training data set. To more accurately assess the biomarkerperformance, we obtained an independent data set using de-identified specimens from patients with pancreatic-related diseases collected at MGH. Before processing clinical samples, we performed exhaustiveanalysisof known EV protein biomarkers relevantto PDAC in patient-derived cell lines. In addition, extensive correlation and optimization studies were performed to validate NPS measurements.

Cell lines

AsPC-1, MIA PaCa-2, PSN-1, and PANC-1 cell lines were purchased from the American Type Culture Collection. AsPC-1 and PSN-1 cells were maintained in RPMI 1640 medium. MIA PaCa-2 and PANC-1 cells were maintained in Dulbecco's modified Eagle's medium. All cell line media were supplemented with 10% fetal bovine serum, 100 IU of penicillin, and streptomycin (100 mg/ml). PDAC PDX cell lines were provided by C. Fernandez del Castillo and were all maintained in a 50:50 mix of Dulbecco's modified Eagle's and Ham's F-12 medium supplemented as above.

Selection of markers

Several PDAC proteomic studies have been described in the literature (10, 11, 22–24, 29) or are available online (http://wlab.ethz.ch/cspa/; https://www.proteomicsdb.org/#projects/4256; pancreaticcancerdatabase.org/publications.php). These literature sources were analyzed to define EV marker candidates. To derive the marker set, we surveyed these available databases, three vesicle databases (Vesiclepedia, EVpedia, and ExoCarta), and the literature for published markers. The putative "hits" were then screened using commercially available antibodies (see table S1). We eliminated targets that were not specific for cancer cells, yielded only duplicative information, or were primarily intravesicular proteins, which we were not able to capture efficiently. From the initial 50 candidate markers, we selected 10 after feasibility studies with PDAC cell lines and PDX models (fig. S2). We decided to take forward seven tumor markers, all of them vesicle surface markers that can be used for chip capture. In addition, we assayed for three ubiquitous EV markers: CD9, CD63, and Rab5b.

Antibody and biotinylation

All antibodies used in these studies are listed in table S1. For biotinylation, all antibodies [50 mg in 100 ml of phosphate-buffered saline (PBS)] were first passed through 0.5 ml 7K MWCO Zeba Spin Desalting columns (89882, ThermoFisher) to remove sodium azide. EZ-Link Sulfo-NHS-LC-Biotin (21327, ThermoFisher) was used for antibody biotinylation according to the manufacturers' instructions. Briefly, antibodies were mixed with a 20-fold molar excess of 10 mM biotin for 30 min at room temperature. Excess biotin was then removed using a second Zeba Desalting column. Antibody concentration was checked using a NanoDrop spectrophotometer (ThermoFisher).

Flow cytometry

Antibodies were tested and compared to EV signals from NPS using flow cytometry. On the day of EV collection from cell lines, a portion of the remaining cells were trypsinized for flow cytometry. Cells (500,000 to 1,000,000 per condition) were fixed in 4% paraformaldehyde in PBS (15710-S, Electron Microscopy Sciences) for 10 min at room temperature. Cells were washed twice with PBS plus 0.5% bovine serum albumin (BSA). Antibodies were diluted to 10 mg/ml in 100 ml of PBS plus 0.5% BSA and incubated with cells for 1 hour at room temperature. Cells were washed twice with PBS plus 0.5% BSA and then incubated with appropriate Alexa Fluor 488 secondary antibody diluted 1:1000 in PBS plus 0.5% BSA for 30 min at room temperature. Cells were washed twice with PBS plus 0.5% BSA. Fluorescent signal was measured using a FACSCalibur flow cytometer (BD Biosciences) and compared to appropriate isotype controls and secondary antibody–only signal using the following formula: (signal primary antibody − signal isotype control)/signal secondary antibody.

EV isolation from cell culture

Cells were grown for 48 hours in normal growth medium supplemented with 5% EV-depleted fetal bovine serum (A2720801, ThermoFisher). Conditioned medium was collected in 50-ml tubes and centrifuged at 300g for 10 min. Medium was filtered through a 0.22-mm cellulose acetate vacuum filter (430767, Corning) and then aliquoted into ultracentrifuge tubes (344058, Beckman). Medium was centrifuged at 100,000g for 70 min to pellet EVs. The pellet was washed with PBS and repelleted by centrifugation at 100,000g for 70 min. EVs were resuspended in an appropriate volume of PBS and stored at −80°C until NPS measurement.

Sample collection

The current study was designed to prospectively obtain fresh samples and then correlate them with pathological and clinical information. All clinical data were entered into a unified database and used for blinded analyses by the biobank coordinator at MGH. The biospecimen collection was optimized for EV analysis and included the following steps: (i) collect whole blood into one 10-ml purple-top EDTA tube, (ii) mix blood by inverting the tube 10 times, (iii) store vacutainer tubes upright at 4°C and process within 1 hour of blood collection, (iv) centrifuge blood samples for 10 min at 400g at 4°C, (v) collect the plasma layer in a 15-ml conical tube with a pipette without disturbing the buffy coat, (vi) centrifuge the plasma layer for 10 min at 1100g at 4°C, (vii) pipette the plasma into a 15-ml labeled conical tube, and (viii) store at −80°C until processing.

EV isolation from plasma

Plasma was thawed, aliquoted into ultracentrifuge tubes, and diluted to 30- to 35-ml total volume in PBS. Plasma was initially centrifuged at 14,000g for 20 min to pellet cell debris. Cleared supernatant was passed through a 0.22-mm polyvinylidene difluoride (Millipore) syringe filter into an ultracentrifuge tube. EVs were then pelleted by ultracentrifugation at 100,000g for 70 min. The pellet was resuspended in PBS and centrifuged again at 100,000g for 70 min. The final EV pellets were resuspended in 300 ml of PBS and stored at −80°C until NPS measurement.

EV size measurements

Nanoparticle tracking analysis (Nanosight) was used to assess EV size and concentration. Measurements were done as reported in the literature (36). Briefly, samples were diluted in PBS (generally a 1:100 dilution). Five 30-s videos were recorded using the following settings for all measurements: threshold, 1482; gain, 680. Videos were processed, and the highest and lowest EV concentrations were excluded.

NPS fabrication

We used interference lithography to prepare NPS devices (fig. S1). First, periodic nanohole patterns were made on a double-polished 4-inch (~10 cm) Si wafer coated with a 125-nm silicon nitride (SiN) layer. The patterned wafer was dry-etched using reactive ion etching to create nanoholes in the SiN layer. In this step, only a partial layer was etched to protect the front Si surface from the subsequent silicon etching with potassium hydroxide (KOH). The opposite Si backside was lithographically patterned to define sensing sites and wet-etched with KOH at 80°C. Patterned wafers were diced into individual NPS chips, with each chip containing 100 (10 × 10) measurement sites. After removing the remaining SiN layer, a 100-nm Au film with a 2-nm Ti adhesion layer was directly deposited on the patterned SiN

side. After the EV assays, the metal films were removed by Au etchant and hydrogen fluoride (HF) solutions to regenerate chips. After cleaning the patterned Si templates, fresh metal films were deposited on the regenerated Si templates.

NPS measurement

The fabricated Au chip was first incubated with a 1:3 mixture of 10 mM linear polyethylene glycol (thiol-PEG-biotin, 1 kDa, Nanocs Inc., and methyl-PEG-thiol, 0.2 kDa, Fisher Scientific Inc.) overnight at room temperature. After washing in PBS, the chip was secondarily incubated with neutravidin (Thermo Scientific; 50 mg/ml in PBS with 0.2% BSA) for 40 min at room temperature. Finally, after washing, 0.5 ml of biotinylated antibodies (10 mg/ml in PBS with 0.2% BSA) was added to individual nanopore arrays by using a microarray spotter (DigiLab Inc.) and incubated for 40 min at room temperature with humidity. The antibody-conjugated chip was washed in PBS and then measured with a spectrometer (USB4000-UV-VIS-ES, Ocean Optics Inc.) to obtain a baseline spectrum.

For EV detection, EV samples (0.5 ml, in 1% BSA) were spotted onto individual sensor arrays using the microarray spotter and incubated in a humidity chamber for 50 min at room temperature. The chip was washed with PBS to remove unbound EVs, and light transmission of each nanopore array was measured. A custom-built software program (MATLAB R2015a, MathWorks Inc.) was used to analyze spectral shifts after EV binding. A set of control arrays with isotype control antibodies was used to measure signals due to nonspecific binding; these background signals were subtracted from the positive arrays.

Patients

Between 2015 and 2016, 135 patients underwent surgical resection of pancreatic neoplasms or other abdominal abnormalities. Through an Institutional Review Board–approved protocol at MGH (principal investigator: C.F.d.C.), blood samples were acquired. All samples were anonymized, and only age, gender, medical history, and final pathological diagnosis were recorded. All samples were processed by operators blinded to the sample type.

Statistics

The Spearman correlation coefficient was used to quantify the correlations between different variables. Group differences were tested using the nonparametric Mann-Whitney test for two groups and the Kruskal-Wallis test for more than two groups; P values for pairwise comparisons were obtained using the Dunn's multiple comparison test. ROC curves were constructed for individual markers and selected marker combinations to describe the accuracy of detecting cancer. The cutoff points were selected using Youden's index, which maximizes the sum of sensitivity and specificity. We used data from the training cohort (n = 32) to select the optimal cutoff points associated with individual markers and marker combinations and then evaluated the sensitivity, specificity, and accuracy of predicting tumor status associated with the optimal cutoff points using data from the prospective cohort (n = 43). Selection of marker combinations was informed by literature, biological information, and data-driven statistical procedures. One set of markers was selected through fitting the least absolute shrinkage and selection operator (lasso) paths for regularized logistic regression (37) to the training cohort, where the tuning parameter was selected through a 10-fold cross-validation (38). For marker combinations, the sums of selected markers were used to predict tumor status. Notably, although the lasso procedure suggested a linear combination of markers with the weights being the estimated coefficients, the uncertainty associated with these estimated coefficients was large. We therefore used the unweighted sums for all marker combinations for ease of implementation in practice. CIs for AUC were calculated using the DeLong method (39), and for the cut points, the stratified bootstrap percentile method was used. Exact CIs for sensitivity, specificity, and accuracy were obtained on the basis of binomial distributions. All tests were two-sided, and a P value of <0.05 was considered statistically significant. Analyses were performed using R version 3.3.2 and GraphPad Prism 7.

SUPPLEMENTARY MATERIALS

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Fig. S1. NPS setup.

Fig. S2. Comparison of PDAC and EV markers on cells and EVs.

Fig. S3. EV markers in a training cohort.

Fig. S4. Comparison of EV counts with conventional clinical metrics.

Fig. S5. NPS signal from validation cohort tEVs.

Fig. S6. EV counts for patients with different types of pancreatic diseases.

Fig. S7. Comparison of the $PDAC^{EV}$ signature and SSTR2 in different patient cohorts.

Table S1. Antibodies used in flow cytometry and NPS.

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Acknowledgments: We thank our clinical colleagues involved in the clinical care of the patients reported here. We also thank A. Roberts for help with screening antibodies before this clinical study. Funding: Part of this study was funded by a grant from the Lustgarten Foundation (R. Weissleder), NIH R01CA204019 (R. Weissleder), P01CA069246 (R. Weissleder), K99CA201248 (H.I.), R01HL113156 (H.L.), R21CA205322 (H.L.), and a pilot grant from the Andrew L. Warshaw, M.D. Institute for Pancreatic Cancer Research at MGH (K.S.Y. and H.I.). C.P. was supported by the CaNCUREprogram, Northeastern University, NIH (R25CA17174650). Author contributions: K.S.Y., H.I., S.H., and R. Weissleder designed the study and all experiments; K.S.Y., H.I., and S.H. performed all experiments; I.P., C.M.C., and C.F.d.C. collected patient samples; A.F.d.C., S.C., C.-H.H., and C.P. assisted with data collection; K.S.Y., H.I., S.H., R. Wang, R.Y., H.L., and R. Weissleder analyzed the data; S.F. provided new reagents; R. Weissleder, K.S.Y., H.I., C.P., and H.L. provided funding; K.S.Y., H.I., H.L., C.M.C., and R. Weissleder wrote the paper. Competing interests: Exosome Diagnostics Inc. licensed a patent application submitted by MGH that covers the nanoplasmonic sensing system used in the research. H.I., C.M.C., H.L., and R. Weissleder are inventors of the patent application. H.I. and H.L. serve as consultants for Exosome Diagnostics Inc. R. Weissleder is a cofounder of T2 Biosystems and Lumicell. He serves as a scientific advisor for ModeRNA Therapeutics, Tarveda Therapeutics, and Alivio Therapeutics. None of these activities are related to the manuscript. Data and materials availability: Data and materials are available upon request by contacting the corresponding author.

Submitted 1 November 2016
Accepted 29 March 2017
Published 24 May 2017
10.1126/scitranslmed.aal3226

Citation: K. S. Yang, H. Im, S. Hong, I. Pergolini, A. F. del Castillo, R. Wang, S. Clardy, C.-H. Huang, C. Pille, S. Ferrone, R. Yang, C. M. Castro, H. Lee, C. F. del Castillo, R. Weissleder, Multiparametric plasma EV profiling facilitates diagnosis of pancreatic malignancy. Sci. Transl. Med. 9, eaal3226 (2017).