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TITLE OF THE DOCTORAL THESIS:

The prediction, prevention, and mitigation of pancreatic fistula:

Recent advancements in the era of risk stratification and personalized management.

S.S.D MED/18

Coordinator: Prof. Vincenzo Corbo

Tutor: Prof. Giuseppe Malleo

PhD Student: Giampaolo Perri

SUMMARY

SYNOPSIS

This doctoral thesis is a collection of original papers sharing the theme of pancreatic fistula after pancreatic surgery. The common thread is the surgeon's will to address proactively this potentially catastrophic event during all perioperative phases, following a risk-based, dynamic, and personalized management. It therefore starts with a proposal for preoperative fistula risk stratification, tailored to the individual patient, aimed at improving preoperative counseling & decision-making, but also influencing actionable factors (e.g., BMI). It continues with an attempt to renovate current intraoperative risk scores, generally based on macroscopic features of the pancreatic gland, proposing a microscopic quantification of the acinar cell content in the residual parenchyma, ultimately responsible for the secretion of the dangerous enzymes that characterize postoperative fistula. This novel tool, developed in collaboration with San Raffaele Hospital, seems capable of dichotomizing the fistula risk in either high or low, finally getting rid of 'grey areas' where the preferable strategy is unknown. Moreover, a multicentric collaboration with Karolinska Institute and Oslo University Hospital demonstrates how early postoperative predictors, such as serum amylase values, can be used during the first two postoperative days to drive a fast-track surgical management after distal pancreatectomy. For patients in the high-risk categories after pancreatoduodenectomy, trans-anastomotic stents result as the optimal mitigation strategies to avoid severe morbidity. While in patients without a stent an early drain removal policy is recommended, in patients with stents drain removal should be postponed until a later postoperative period, when postoperative fistula predictors reach acceptable diagnostic accuracy. Finally, in selected case with extremely high fistula risk, total pancreatectomy seems a valuable option to prevent pancreatic fistula, given better surgical outcomes and comparable quality of life, only in few selected cases and after adequate counselling due to life-long exocrine and endocrine insufficiency.

CHAPTER I – (PREOPERATIVE) PREDICTION

Preoperative risk Stratification of postoperative pancreatic fistula: a risk-tree predictive model for pancreatoduodenectomy.

INTRODUCTION

Despite the drop in 90-day mortality rate around 2% at high-volume centers in recent years, pancreatoduodenectomy (PD) remains a complex procedure associated with a relevant complication burden.[1 2] Most of this morbidity is attributable to postoperative pancreatic fistula (POPF), its most influential complication, which occurs in 5 to 30% of cases.[3 4] POPF also contributes to additional surgical and systemic complications, such as delayed gastric emptying (DGE), post-pancreatectomy hemorrhage (PPH), sepsis, organ failure, and death. In fact, POPF eventually results in a longer hospital stay (LOS) and high costs of patient management.[5] Since POPF is relatively common and often responsible for the deviation of patient recovery from the expected pattern and timeline, the perioperative care and pace of recovery of patients undergoing PD have been difficult to standardize. Although clinical pathways and enhanced recovery after surgery (ERAS) protocols have been designed and implemented for PD, the associated results have generally been less effective than those obtained with other operative procedures.[6 7] The main reason for this discrepancy is the nonuniform distribution of POPF incidence. Several prediction models have been developed in an attempt to identify risk factors for POPF and prevent its formation or at least mitigate its severity and personalize postoperative care according to each patient's risk. The fistula risk score (FRS), which uses the intraoperative variables of main pancreatic duct (MPD) diameter, gland texture, estimated blood loss (EBL) and presumed pathology, is the most widely accepted and used risk score.[8] Recently, other alternative scores have been proposed, exploring different predictors.[9 10] However, despite their extensive validation or clinical effectiveness, these scores rely on intraoperative (or a mix of pre- and intraoperative) parameters, substantially limiting their value in the preoperative setting. As intraoperative scoring allows tailored intra- and postoperative management, a purely preoperative risk assessment would improve preoperative patient counseling, decision-making for borderline malignant cases, surgical planning, the application of preoperative ERAS protocols, selection for pre-habilitation and inclusion in clinical trials.

The aim of the present study was to build and prospectively validate a predictive model to stratify patients according to their risk of developing POPF after PD using exclusively variables that are already available in the preoperative setting.

METHODS

Study design

Data from consecutive patients who underwent PD from July 2017 to December 2019 at the Pancreatic Surgery Unit of Verona University Hospital (training cohort) and San Raffaele Hospital of Milan (validation cohort) were prospectively obtained and analyzed. Approval for data collection and analysis in this study was previously obtained from the review boards of the two institutions. Preoperative risk factors for POPF were initially identified in the training cohort. The regression risk-tree model for preoperative POPF risk stratification was developed in the training cohort using the aforementioned factors and then tested prospectively in the validation cohort.

Data collection, surgical procedures and outcomes

Pre-, intra- and postoperative demographic data were recorded in a prospectively maintained database. The MPD diameter was measured at the pancreatic neck using preoperative cross-sectional imaging (CT scan or MRI). Preoperative imaging was reviewed by GP (Verona cohort) and LC (San Raffaele cohort). Open PD was conducted in a standardized manner.[11] The risk of POPF was assessed intraoperatively according to the fistula risk score (FRS), and patients were stratified into negligible (FRS 0), low (FRS 1-2), moderate (FRS 3-6) and high (FRS 7-10) fistula risk zones (FRZ).[8] [12] Two drains were placed in the proximity of the pancreatic and biliary anastomoses in all patients in the high or intermediate risk zone or according to the surgeon's preference in patients in all other risk categories. A previously published protocol was used for drain management.[13] Postoperative outcomes were measured during hospitalization and/or after discharge up to 90 days after surgery and included POPF and biochemical leak, post-pancreatectomy hemorrhage (PPH), delayed gastric emptying (DGE), sepsis, postoperative length of stay (LOS), and in-hospital mortality.[3] [14] [15] [16]

Postoperative pneumonia was defined as parenchymal opacity at the chest x-ray associated with the use of antibiotics . Postoperative in-hospital major morbidity was defined as a Clavien-Dindo score ≥3.

Risk groups and statistical analysis

Continuous data are presented as medians and ranges and were compared using the independent sample t-test or the Mann-Whitney test, as appropriate. Categorical variables are presented as frequencies with percentages and were compared using the χ^2 test or Fisher's exact test in the case of small, expected frequencies. All the tests were 2-tailed. $P<0.05$ was considered statistically significant. Univariable and multivariable logistic regression models were used to assess preoperative variables that are potentially associated with POPF among the training cohort. Clinical factors with $P<0.2$ in the univariable analysis and potential clinical importance were included in the multivariable model.

A recursive partitioning regression tree analysis was performed to determine which variables best predicted the development of POPF. Variables included in the model were body mass index (BMI) and MPD diameter (as continuous variables) and American Society of Anesthesiologists (ASA) score, neoadjuvant treatment and presumptive pancreatic ductal adenocarcinoma (PDAC) diagnosis (as categorical variables). The classification tree internally selects the best cut-points for the continuous variables that are used in the model and it also selects which variables must be retained in the predictive model. After the first tree was generated, a second tree was generated for the validation cohort with the grouping variables created in the first tree. This approach was used to assess the external reproducibility of the original tree and its ability to identify similar risk groups. The goodness of both risk trees was evaluated by deriving the receiver operating characteristics (ROC) curve of the predicted probabilities obtained from the models and by computing the corresponding Area Under the Curve (AUC). Statistical analyses were performed using STATA14 for Windows, and the regression tree analysis was performed in R3.1.1 (The R Foundation for Statistical Computing) using the package Tree version 1.0–35.

RESULTS

Characteristics of the training cohort

A total of 566 patients underwent PD at Verona University Hospital during the study period and were included in the analysis. The clinical characteristics of the training cohort are reported in **Supplementary Table 1**.

Supplementary Table 1. Preoperative, Intraoperative and Postoperative Profiles of All Patients Who Underwent Pancreatoduodenectomy (n= 566) at Verona University Hospital (Training Cohort)

Characteristics		Total, n (%) (n= 566)
Preoperative		
Age, median (IQR), y		65 (14)
Female sex		247 (44)
BMI, median (IQR)		24.2 (4.4)
Smoker		125 (22)
Alcohol abuse		17 (3)
Diabetes		105 (19)
Weight loss		264 (47)
Ischemic cardiac disease		31 (5)
Hypertension		216 (38)
COPD		14 (2)
Chronic renal failure		11 (2)
ASA score		
	1-2	462 (82)
	3-4	104 (18)
Jaundice palliation		292 (52)
Preoperative MDR		58 (10)
Neoadjuvant therapy		148 (26)
Presumed diagnosis		
	PDAC/chronic pancreatitis	354 (63)
	Duodenal/ampullary/cystic/NET	212 (37)
MPD size, median (IQR), mm		4 (2)
Intraoperative		
Surgery type		
	Pylorus-preserving	501 (89)
	Whipple	65 (11)
Vascular resection		80 (14)
Intraoperative transfusion		79 (14)
Pancreatic anastomosis		
	PJ	522 (92)
	PG	44 (8)
Externalized pancreatic stent		252 (44)
Drain type		
	No Drain	43 (8)
	Open	505 (89)
	Closed	18 (3)
FRS zone		
	Negligible	31 (5)
	Low	122 (22)
	Moderate	300 (53)
	High	113 (20)
Postoperative		
POPF		112 (20)
POPF grade		
	BL	32 (6)
	B	94 (17)
	C	18 (3)
POAP		87 (15)
Fluid collection		206 (36)

Abscess		99 (17)
Biliary fistula		38 (7)
DJ/GJ fistula		16 (3)
Chyle leak		30 (5)
PPH		112 (20)
PPH grade		
	A	30 (5)
	B	63 (11)
	C	19 (3)
DGE		108 (19)
DGE grade		
	A	26 (6)
	B	61 (11)
	C	21 (4)
Sepsis		98 (17)
Pleural effusion		63 (11)
Postoperative pneumonia		95 (17)
Reintubation		39 (7)
Cardiac complication		30 (5)
UTI		23 (4)
Acute Kidney Injury		19 (3)
SSI		58 (10)
Percutaneous drainage		31 (5)
Enteral nutrition		181 (32)
TPN		172 (30)
Transfusions		135 (24)
Relaparotomy		51 (9)
ICU admission		69 (12)
Discharged with drains		34 (6)
Postoperative MDR		36 (6)
LOS, median (IQR), days		9 (15)
Readmission		27 (5)
Clavien-Dindo score		
	0	232 (41)
	1	38 (7)
	2	189 (33)
	3a	31 (5.5)
	3b	2 (0.5)
	4a	44 (8)
	4b	13 (2)
	5	17 (3)
Clavien-Dindo score ≥ 3		107 (19)
Mortality		17 (3)

Abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; ASA, American Society of Anesthesiologists; MDR, multidrug-resistant bacterial colonization; PDAC, pancreatic ductal adenocarcinoma; NET, neuroendocrine tumor; MPD, main pancreatic duct; PJ, pancreatico-jejunostomy; PG, pancreatico-gastrostomy; FRS, fistula risk score; POPF, postoperative pancreatic fistula; BL, biochemical leak; POAP, postoperative acute pancreatitis; DJ, duodeno-jejunal anastomosis; GJ, gastro-jejunal anastomosis; PPH, post pancreatectomy hemorrhage; DGE, delayed gastric emptying; UTI, urinary tract infection; SSI, surgical site infection; TPN, total parenteral nutrition; ICU, intensive care unit; LOS, length of hospital stay

The overall POPF rate was 20%, with a Clavien-Dindo morbidity rate ≥3 of 19% and a 90-day mortality rate of 3%. The median LOS was 9 days. In the univariable logistic regression model, BMI, ASA score ≥ 3, neoadjuvant therapy, presumptive diagnosis and MPD diameter were preoperative variables associated with the POPF risk. In the multivariable logistic regression model, BMI (OR 1.1, 95% CI 1-1.2; *P*<0.01), MPD diameter (OR

0.7, 95% CI 0.6-0.8; *P*<0.01) and ASA score ≥3 (OR 1.8, 95% CI 1.1-3; *P*=0.03) were confirmed to be independently associated with POPF (**Table 1**).

Table 1. Univariable and Multivariable Logistic Regression Analyses of Preoperative Predictors for POPF Among the Training Cohort

	Univariable			Multivariable		
Predictors	**OR**	**95% CI for OR**	***P***	**OR**	**95% CI for OR**	***P***
Female gender	1.3	(0.85-1.99)	0.8			
BMI, kg/m^2	1.1	(1.04-1.17)	**<0.001**	1.11	(1.04-1.17)	**0.001**
Age at diagnosis, years	1.007	(0.98-1.02)	0.4			
Smoker	1.08	(0.66-1.77)	0.7			
Alcohol abuse	2.27	(0.82-6.30)	0.1	NA		
Diabetes	0.68	(0.38-1.21)	0.1	NA		
Weight loss	1.03	(0.68-1.56)	0.8			
ASA score ≥ 3	1.87	(1.15-3-05)	**0.01**	1.78	(1.05-3)	**0.03**
Jaundice	1.0009	(0.66-1.51)	0.9			
Jaundice palliation	0.96	(0.63-1.46)	0.8			
Preoperative MDR	0.94	(0.47-1.88)	0.8			
Neoadjuvant therapy	0.47	(0.27-0.82)	**0.008**	NA		
Presumptive diagnosis						
PDAC/chronic pancreatitis	1 [Reference]					
Other	1.74	(1.14-2.64)	**0.009**	NA		
MPD diameter, mm	0.68	(0.60-0.78)	**<0.001**	0.70	(0.61-0.81)	**<0.001**

Abbreviations: BMI, body mass index; ASA, American Society of Anesthesiologists; MDR, multidrug-resistant bacterial colonization; PDAC, pancreatic ductal adenocarcinoma

Preoperative risk groups

The regression tree analysis allocated patients into three preoperative risk groups with an 8%, 21% and 32% risk of POPF (all *P*<0.01), respectively, based on the MPD diameter (≥ or < 5 mm) and BMI (≥ or < 25) **(Figure 1).**

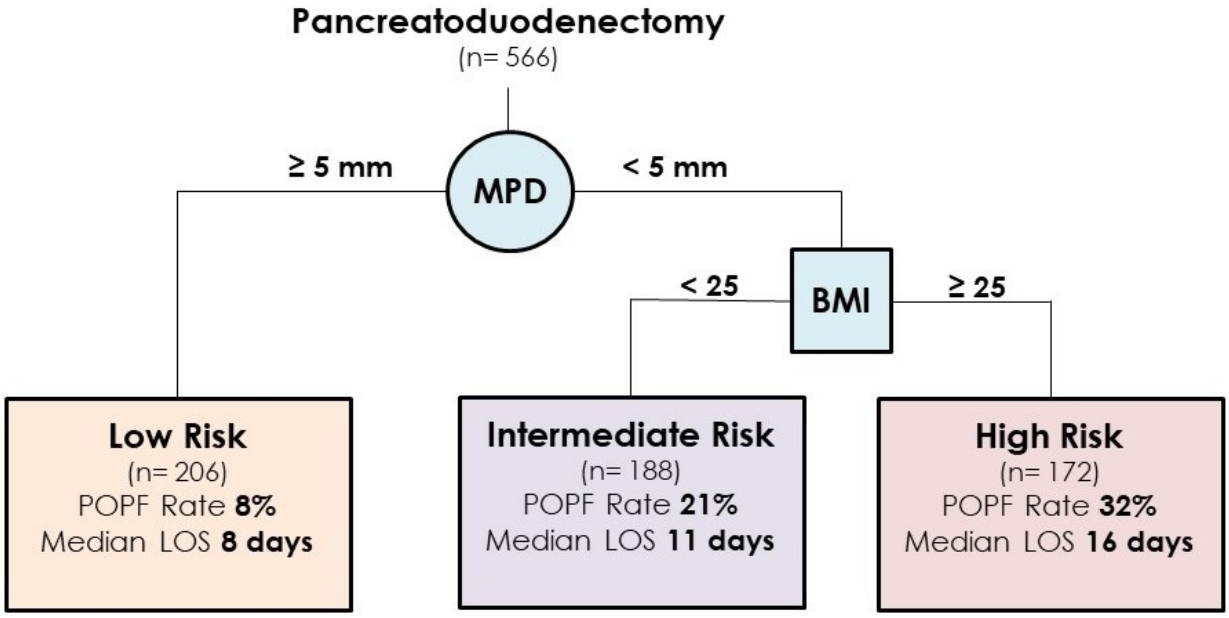


Fig 1. Regression tree to predict POPF (training cohort)

The three groups were labeled low, intermediate, and high risk and consisted of 206 (37%), 188 (33%) and 172 (30%) patients, respectively. The resulting median LOSs were 8, 11 and 16 days, respectively (all *P*< 0.01). The correspondence between preoperative risk groups and intraoperative FRZ is shown in **Figure 2**.

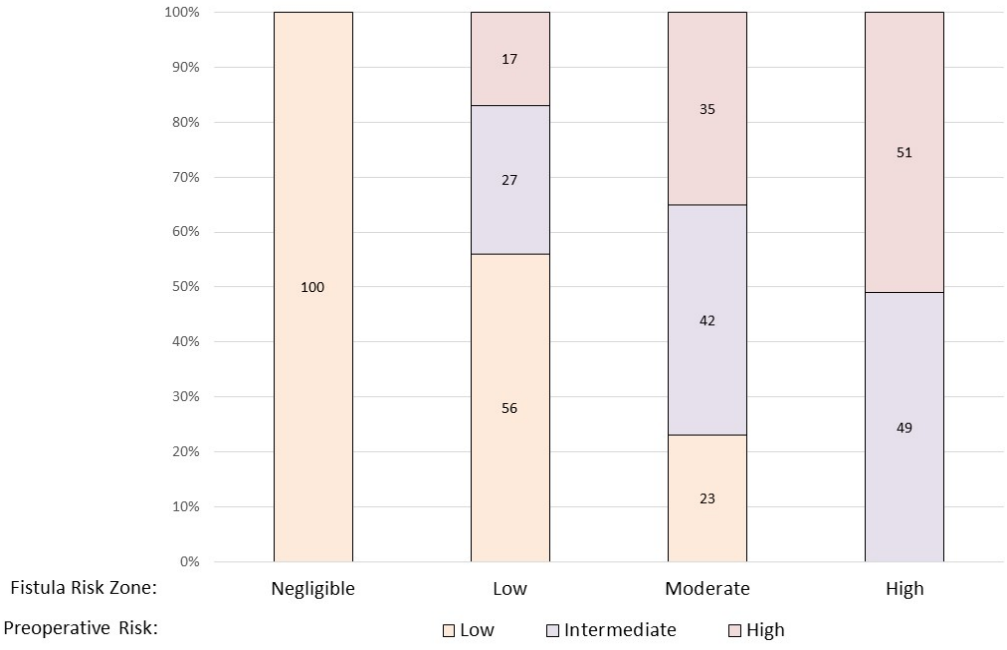


Fig 2. Correlation between preoperative risk groups and intraoperative fistula risk zone.

AUC for the classification tree was 0.70 (CI 95% 0.63-0.77) (**Figure 3**). The OR of POPF between the high- and low-risk groups was 5.2 (95% CI 2.9-9.4; *P*<0.01), with statistically significant differences in POPF risk detected between all risk groups (**Table 2**).

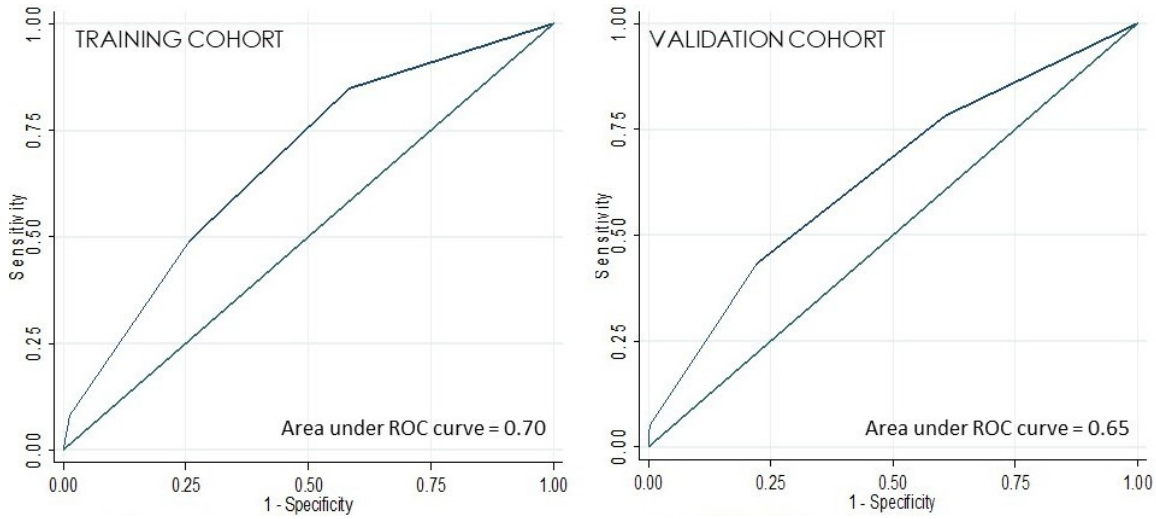


Fig 3. ROC Curve for regression tree predicting POPF in the training cohort.

Table 2. Comparison of the POPF Risk Between Groups (Odds Ratio and Observed/Expected Ratio)

	Training Cohort		Validation Cohort	
Risk Group	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***
Intermediate vs Low	3.0 (1.6-5.5)	**<0.001**	1.6 (0.9-2.8)	0.08
High vs Low	5.2 (2.9-9.4)	**<0.001**	3.5 (2.0-6.1)	**<0.001**
High vs Intermediate	1.7 (1-1-2.8)	**0.02**	2.2 (1.3-3.6)	**0.003**

Risk Group	**O/E (95% CI)**
Low	2.07 (1.39-2.97)
Intermediate	1.09 (0.76-1.52)
High	1.25 (0.93-1.65)

Characteristics of the validation cohort

A total of 456 patients underwent PD at San Raffaele Hospital during the study period and were considered for external validation. A comparison between the training and validation cohorts is shown in Supplementary Table 2. Preoperatively, patients in the validation cohort were older (median age 68 vs 65 years) and a higher percentage had an ASA score ≥ 3 (42 vs 18%), while intraoperatively, they were less frequently categorized into a high FRZ (3% vs 20%). Postoperatively, patients from the validation cohort had a higher rate of POPF (25% vs 20%) and lower rates of PPH (7% vs 20%).

Supplementary Table 2. Clinical Comparison of the Training Cohort and Validation Cohort

		Cohort, n (%)	
Characteristics	**Total, n (%) (n= 1022)**	**Training (n= 566)**	**Validation (n= 456)**
Preoperative			
Age, median (IQR), y	66 (13)	65 (14)	68 (14)
Female sex	460 (45)	247 (44)	213 (47)
BMI, median (IQR)	24.1 (4.2)	24.2 (4.4)	23.9 (4)
Diabetes	182 (18)	105 (19)	77 (17)

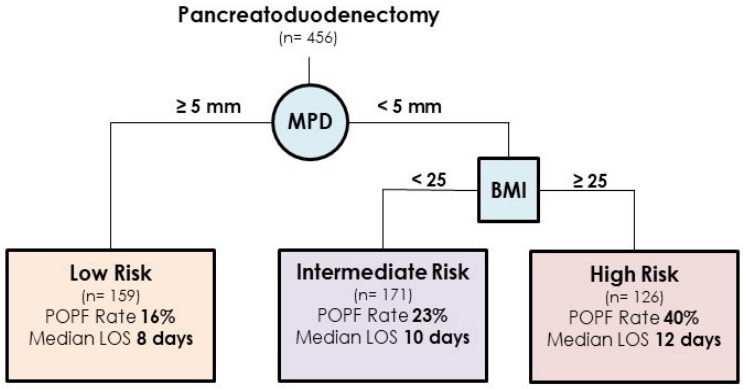
Ischemic cardiac disease	67 (7)	31 (5)	36 (8)
Hypertension	415 (41)	216 (38)	199 (44)
History of COPD/recent pneumonia	42 (4)	14 (2)	28 (6)
Chronic renal failure	23 (2)	11 (2)	12 (3)
ASA score			
1-2	727 (71)	462 (82)	265 (58)
3-4	295 (29)	104 (18)	191 (42)
Neoadjuvant therapy	275 (27)	148 (26)	127 (28)
Presumed diagnosis			
PDAC/chronic pancreatitis	617 (60)	354 (63)	263 (58)
Duodenal/ampullary/cystic/NET	405 (40)	212 (37)	193 (42)
MPD size, median (IQR), mm	4 (2)	4 (2)	4 (2)
Intraoperative			
Vascular resection	127 (12)	80 (14)	47 (10)
Intraoperative transfusion	111 (11)	79 (14)	32 (7)
FRS zone			
Negligible (0)	92 (9)	31 (5)	61 (13)
Low (1-2)	262 (26)	122 (22)	140 (31)
Moderate (3-6)	543 (53)	300 (53)	243 (53)
High (7-10)	125 (12)	113 (20)	12 (3)
Postoperative			
POPF	227 (22)	112 (20)	115 (25)
POPF grade			
BL	52 (5)	32 (6)	20 (4)
B	185 (18)	94 (17)	91 (20)
C	42 (4)	18 (3)	24 (5)
PPH	144 (14)	112 (20)	32 (7)
PPH grade			
A	36 (3)	30 (5)	6 (1)
B	68 (7)	63 (11)	5 (1)
C	40 (4)	19 (3)	21 (5)
DGE	186 (18)	108 (19)	78 (17)
DGE grade			
A	69 (7)	26 (5)	43 (9)
B	77 (8)	61 (11)	16 (4)
C	40 (4)	21 (4)	19 (4)
Sepsis, No. (%)	188 (18)	98 (17)	90 (20)
LOS, median (IQR), days	10 (13)	9 (15)	10 (11)
Mortality, No. (%)	31 (3)	17 (3)	14 (3)

Abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; ASA, American Society of Anesthesiologists; PDAC, pancreatic ductal adenocarcinoma; NET, neuroendocrine tumor; MPD, main pancreatic duct; FRS, fistula risk score; POPF, postoperative pancreatic fistula; BL, biochemical leak; PPH, post pancreatectomy hemorrhage; DGE, delayed gastric emptying; LOS, length of hospital stay

External validation

The risk-tree generated for the training cohort was applied to the validation cohort using the same variables (MPD diameter and BMI) and cutoffs. Patients in the validation cohort were stratified into three groups of 159 (35%), 171 (37%) and 126 (28%) patients with a resulting 16%, 23% and 40% risk of POPF (all P<0.01),

respectively. The resulting median LOSs were 8, 10 and 12 days (all *P*<0.01), respectively (**Supplementary Figure 1**).



Supplementary Fig 1. Regression tree to predict POPF (validation cohort)

POPF rates and the median LOSs in the training and validation cohorts based on risk groups determined by the regression tree analysis are shown in **Figure 4**.

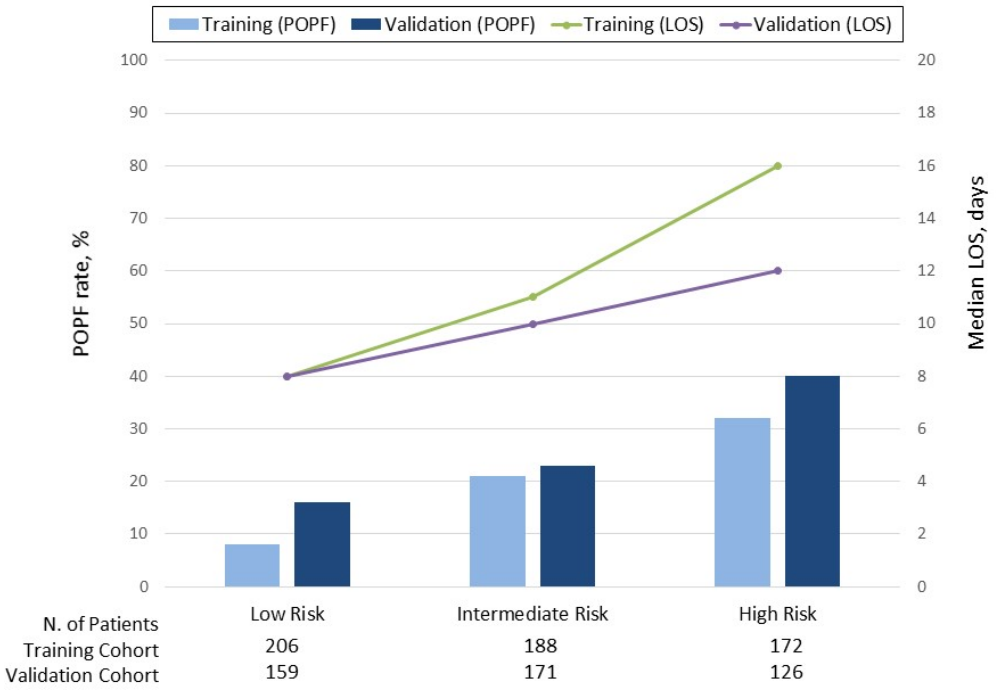


Fig 4. POPF rates and median LOSs in the training and validation cohorts stratified based on preoperative risk

The OR of POPF between the high- and low-risk groups was 3.5 (95% CI 2-6.1; *P*<0.01), with statistically significant differences in POPF risk detected between all risk groups except for intermediate- vs low-risk groups (**Table 2**). The observed/expected ratio for POPF incidence in the validation cohort were 2.07 (CI 1.39-

2.97), 1.09 (CI 0.76-1.52) and 1.25 (CI 0.93-1.65) for the low-, intermediate- and high-risk group, respectively. AUC for the validation cohort was 0.65 (CI 95% 0.59-0.71) (**Figure 3**).

DISCUSSION

This study proposes an easy and reproducible tool to preoperatively stratify the risk of POPF in patients undergoing PD according to BMI and the presence of MPD dilation on preoperative imaging. The risk-tree analysis identified three discrete classes of risk in the training cohort, with significantly different POPF rates and associated median LOSs, which were successfully reproduced in the validation cohort from an external institution. The incorporation of such a tool in clinical practice may enable better selection of surgical candidates, counseling implementation, and the establishment of definite intra- and postoperative protocols for personalized patient care.

Various strategies for estimating the risk of POPF have already been developed, most of which identify only intra- and postoperative risk factors.[8 9 10 17 18 19] The most widely used and extensively validated tool is the fistula risk score (FRS), which uses both endogenous and intraoperative variables such as the MPD diameter, gland texture, EBL and presumed pathology to stratify POPF risk. Intraoperative stratification surely allows the early identification of patients at highest risk, in which fistula prevention and mitigation strategies have the potential to positively affect surgical outcomes.[20] However, the intraoperative assessment has several major inherent limitations. A preoperative risk assessment would add crucial value, while its development has been mostly limited to either small, single-institution series or national registry data, with a lack of external validation or significant clinical discrimination ability.[21 22 23] In some cases, the previous ISGPF definition of pancreatic fistula including “grade A” was used, which is currently obsolete. The MD Anderson Cancer Center group implemented risk-stratified clinical pathways after pancreatectomy in 2017 based on a preoperative risk-tree able to stratify patients undergoing PD into two different risk groups according to PDAC diagnosis, BMI and MPD diameter.[24 25] Notably, this system has been practice changing once fully incorporated in preoperative evaluation.[26] The parameters included in the present study are similar, but our model was able to identify three different and clinically relevant risk groups after PD requiring only two

preoperative variables, namely, MPD diameter and BMI. The regression tree identified a 5 mm cutoff for the MPD diameter, which is easy to apply in clinical practice, as it indicates substantial and pathological dilation on preoperative imaging. A BMI of 25 or more also has precise clinical significance, as it indicates "overweight".

While a PDAC diagnosis, neoadjuvant therapy or diabetes are indirect indicators of a firm pancreas, the two variables included in the present model (BMI and MPD diameter) are not preoperative surrogates for more traditional intraoperative fistula risk variables. In particular, neoadjuvant therapy is an indirect indicator of a fibrotic pancreatic texture (and lower POPF risk)[27], but was not selected by the final tree model, probably because 'outweighed' by the importance of MPD diameter. Despite not being included by the present model, indirect indicators of pancreatic texture/function remain useful in the preoperative evaluation of risk and should be always considered. As MPD diameter was an independent predictor of POPF, additional subgroups could be identified based on additional MPD diameter cutoffs, but we favored a more simplified approach, considering the purpose of this risk-tree. Notably, our preoperative risk stratification model is intended to be an integration of, rather than an alternative to, existing intra- and postoperative POPF predictors such as FRS or postoperative drain fluid amylase values. The POPF risk may change over time, and all available information should be dynamically registered during the perioperative course to promptly apply prevention and mitigation strategies. However, the preoperative risk stratification showed a strong correlation with the subsequent intraoperative FRZ allocation, with an acceptable "stage migration" effect from high preoperative risk to low FRZ (17%) and from low preoperative risk to moderate FRZ (23%) (Figure 2). The overall POPF rates presented (20% in the training cohort; 25% in the validation cohort) appear high when compared to other recent multicentric international registries, where they ranges from 12% to 14%.[10 28] While to dissect the profound reasons of such discrepancy (i.e. diversity of patients characteristics/management policies/reporting; prospective vs retrospective collection of data) is beyond the means of the present discussion, it is important to notice how a POPF rate around 22% after PD was confirmed by a recent review of the last 20 years (and nearly 3000 PD) of pancreatic surgery in Verona Pancreas Institute (and interestingly, this rate did not change over time).[29] Wide differences in POPF rates

between Institutions may limit the applicability of the present model, which may need international validation in other countries. However, it is important to notice that its purpose was not to set a benchmark for POPF incidence for each group, but to part a population of patients undergoing PD into discriminate groups with significantly different relative risks. This concept also applies to other existent risk scores, such as the FRS. Notably, despite some major differences between the training and validation cohorts (including 5% POPF rate), as expected from patients treated at different institutions (Supplementary Table 2), the model was still able to identify three significantly different risk classes in the external validation cohort (Table 2). The preoperative stratification of POPF risk and resulting duration of hospitalization have the potential to improve patient selection and counseling, to allow selection for the application of preoperative ERAS protocols and to drive tailored inclusion into pre-habilitation programs or clinical trials. For example, the ability to predict with reasonable accuracy an LOS ≤ 8 days in half of patients with dilated MPD has important implications, allowing the selection of patients who will fully benefit from fast-track approaches before surgery. On the other hand, the prediction of POPF (with an LOS >15 days) in over one-third of overweight patients with nondilated MPD may be useful to improve preoperative patient counseling and preparation for surgery and even to orient actual surgical decision-making in patients who present borderline surgical fitness or lesions of uncertain pathological behavior (e.g., cystic and neuroendocrine neoplasms).

This study has several limitations. First, a preoperative score predictive of POPF clearly does not include characteristics of the gland that are identified only intraoperatively, such as pancreatic texture, MPD diameter at the pancreatic transection level, or EBL. Despite some limitations (e.g., the exact assessment of EBL), these variables indeed play a major role in POPF formation. Moreover, although the model was validated prospectively, it is based on retrospective data, and its application and relevance in clinical practice are still only theoretical. Further studies should evaluate the effect of tailored preoperative interventions based on preoperative risk evaluation. International validation in different countries (i.e., North America and Asia) may also be required in order to assess the generalizability of the BMI cut-off, a parameter which is subject to wide regional variations. Other additional predictors might be needed to refine the preoperative risk evaluation in the future. For example, a preoperative assessment of fecal elastase 1, a reliable and

inexpensive test to evaluate pancreatic exocrine function, might play a major role in risk prediction and might add value to the predictive capability of the present score when available.[30] Finally, other potential patient-related (i.e., frailty and sarcopenia) or case-related (i.e., surgical complexity) predictors of a complicated postoperative period should be prospectively evaluated in the subset of patients with a low preoperative fistula risk. In conclusion, in candidates for PD, the risk of POPF and related length of hospitalization can be quickly and accurately determined in the preoperative setting using BMI and MPD diameter measured radiographically. Using these two variables, patients can be stratified into three groups at low, intermediate and high risk for POPF. The early risk evaluation is complementary to the intra- and postoperative counterparts and might potentially guide clinical decision-making, improve patient counseling, and allow selection for pre-habilitation/ERAS protocols, risk reduction protocols or clinical trials.

CHAPTER II – (INTRAOPERATIVE) PREDICTION

Either high or low risk: the acinar score at the resection margin dichotomizes the risk spectrum of pancreas-specific complications after pancreatoduodenectomy.

INTRODUCTION

Postoperative pancreatic fistula (POPF) is the main driver of morbidity after pancreaticoduodenectomy (PD), with an overall incidence between 15-20%, and it can occur in up to 40% in high-risk individuals.[31 32 33] Risk stratification is crucial for POPF management, as the timely identification of elevated risk allows for the implementation of mitigation strategies and tailored pathways. [26 34] POPF prediction has been extensively tested in the intraoperative setting, the *time-zero* where most of the actionable strategies are applicable. [20 8] Several intraoperative scores, such as the Fistula Risk Score (FRS), are available based on different clinical variables expressing patient-, surgery-, and pancreas-related risk factors. [10 35] Gland texture and main pancreatic duct (MPD) diameter are the most relevant and widely accepted intraoperative factors, as recently established by a metanalysis from the International Study Group of Pancreatic Surgery (ISGPS). [36] In addition to offering significant technical challenges during pancreatic anastomosis, a soft glandular pancreas with a small MPD defines a "healthy" organ and is considered to be more prone to react to the biological trauma of surgery.[37] Surgical trauma can trigger a yet unknown chain of events in the high-risk pancreas, eventually leading to pancreas-specific complications such as POPF and the recently defined postoperative hyperamylasemia (POH) and/or acute pancreatitis (PPAP).[38] However, the surgeon's evaluation of macroscopic pancreatic features remains by definition subjective and may not always be conclusive, as most patients fall into "intermediate" risk categories (i.e., FRS 3-6; ISGPS class B-C).[8 39 40]

Despite expressing a definite area of the entire spectrum of risk stratification, an "intermediate" risk represents a gray area in which surgeons are challenged whether to adopt mitigation strategies. Moreover, there is no consistency regarding what measures should be adopted as the gold standard in these cases, as most evidence relies either on low- or high-risk categories.

Several attempts have been made to correlate pancreas-specific complications with more objective histopathological measures. [41 42 43] The proportion of pancreatic acinar content, fibrosis, and fat appears to be strongly correlated with POPF, but a real-time assessment of histopathologic characteristics while in the operating room presents technical challenges, which until now prevented its systematic implementation. A

recent study found that the acinar score, particularly an acinar content of ≥60% at the pancreatic resection margin, is an independent predictor of POPF and clinically relevant acute pancreatitis.[44]

The primary aim of this study is to integrate the analysis of histopathologic composition with current intraoperative risk stratification for pancreas-specific complications (POPF, POH, and PPAP as recently defined by the ISGPS[38]) in cases of uncertain/intermediate macroscopic features.

METHODS

Study design

Data from consecutive patients who underwent PD at the Pancreatic Surgery Unit of Verona University Hospital (January 2019 - June 2021) (*validation cohort*) were prospectively obtained and analyzed to validate findings from a previously published series[44] of consecutive PDs performed at San Raffaele Hospital of Milan (January 2018 - December 2019) (*training cohort*). Data from both the training and validation cohorts were eventually merged to build risk-stratification algorithms based on intraoperative (macroscopic) and histopathologic (microscopic) features. Approval for data collection and analysis in this study was obtained by the local ethics committee (1101CESC) and performed according to the recommendations of the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE).

Surgical procedures

In the validation cohort, PD was conducted in a standardized fashion.[20] Division of the pancreatic neck was typically performed using a cold scalpel and hemostatic sutures. The pancreas was transected with cautery only anecdotally in cases of hard/low-risk pancreas. Pancreaticojejunostomy with or without duct-to-mucosa (depending on MPD caliber) was the standard pancreatic anastomosis in the validation group and was performed by 8 expert pancreatic surgeons (>100 pancreatic resections) according to homogeneous institutional standards. Senior residents and junior attendings typically performed low-risk anastomosis and were always under supervision of an expert surgeon. An externalized pancreatic stent (PankreaPlus™ polyvinyl catheter) was placed in all high-risk patients and in selected patients at moderate risk. [20] The suture

materials were 3/0 TiCron for the pancreatic capsule and 5/0 Prolene for the pancreatic duct in all patients, while in the case of stent positioning, the stent was anchored using 3/0 VycrilRapid (only on the mucosa side). Two drains were placed in the proximity of the pancreatic and biliary anastomoses in all patients at high or moderate risk (or according to the surgeon's preference, in other risk categories). A previously published protocol was used for drain management, applying an early removal policy.[13 45] Serum pancreatic amylase levels were assessed routinely after surgery and from postoperative day (POD) 1 to 3.

Intraoperative risk (macroscopic features)

The risk of pancreas-specific complications was assessed intraoperatively according to macroscopic pancreatic features, including pancreatic texture based on manual palpation and MPD diameter measured at the pancreatic transection line. Estimated blood loss was reported using a direct estimation method.[46] Patients were stratified for POPF risk according to the FRS (*negligible/low/moderate/high* risk zones) and to the ISGPS (*A/B/C/D* classes) by combining pancreatic texture (soft vs. hard) and MPD diameter at transection line (≤3 vs. >3 mm). [8 36 12]

Histopathologic risk (acinar score)

In the validation cohort, histopathologic assessment of pancreatic resection margins was blindly performed on formalin-fixed, paraffin-embedded tissue after frozen section analysis by two expert pancreatic pathologists (C.L. and E.Bar.), without access to clinical information. Any inconsistency was solved by sharing the slide at a multiheaded microscope. All sections were assessed for acinar (Ac), fibrosis (Fc), and fat contents as a proportion of the total area of the surface.[41] The morphologic analysis was performed according to Partelli et al. [44] The cutoffs for Ac (≥60%) and Fc (≤10%, rounded from previous 15%) were maintained from this previous study and further validated in the new cohort.

Data collection and outcomes

Pre, intra, and postoperative demographic data were recorded prospectively. Postoperative outcomes were measured during hospitalization and/or after discharge up to 90 days after surgery and included pancreas-

specific complications graded and defined according to the ISGPS: biochemical leak (BL) and POPF, POH and PPAP, post pancreatectomy hemorrhage (PPH), delayed gastric emptying (DGE), sepsis, postoperative length of stay (LOS), and in-hospital mortality.[38 47 48 49 16] Postoperative complications were scored according to the Clavien–Dindo classification, and postoperative in-hospital major morbidity was defined as a Clavien–Dindo score ≥3. [50]

Statistical analysis

Continuous variables are expressed as the means and (SD) values or as median values with interquartile ranges (IQR) and were compared using the independent samples t test or the Mann–Whitney test, as appropriate. Categorical variables are expressed as frequencies with percentages and were compared using the $\chi 2$ test or Fisher's exact test in the case of expected small frequencies. The goodness of different risk trees was evaluated by deriving the receiver operating characteristic (ROC) curve of the predicted probabilities obtained from the models and by computing the corresponding area under the curve (AUC). The Youden index was calculated and used to select the most appropriate cutoff combined with clinical relevance. For each cutoff, sensitivity (SENS), specificity (SPEC), positive predictive value (PPV), and negative predictive value (NPV) were calculated. All the tests were 2-tailed. $P < 0.05$ was considered statistically significant. Statistical analyses were performed using STATA14 for Windows. Figures were created or modified using BioRender.com.

RESULTS

A total of 373 patients underwent PD at Verona University Hospital during the study period and were included in the validation cohort. The training cohort consisted of 388 patients who underwent PD at San Raffaele Hospital and were included in a previous study[44].

Clinical characteristics

The clinical characteristics of both cohorts are reported in **Supplementary Table 1**.

Supplementary Table 1. Clinical comparison of training cohort (n= 388) and validation cohort (n= 373)

Characteristics	Total, No. (%) (N= 761)	Cohort, No. (%)	
		Training= 388 (51)	Validation= 373 (49)
Preoperative			
Age, median (IQR), y	66 (14)	68 (13)	65 (15)
Female sex, No. (%)	358 (47)	180 (46)	178 (48)
BMI, median (IQR)	24 (4.3)	23.9 (4.1)	24 (4.4)
Diabetes, No. (%)	134 (18)	66 (17)	68 (18)
ASA score, No. (%)			
1-2	522 (69)	232 (60)	290 (78)
3-4	239 (31)	156 (40)	83 (22)
Preoperative therapy, No. (%)	281 (37)	114 (29)	167 (45)
Presumed diagnosis			
Duodenal/ampullary/cystic/NET	290 (38)	159 (41)	131 (35)
PDAC/chronic pancreatitis	471 (62)	229 (59)	242 (65)
Intraoperative			
Vascular resection, No. (%)	101 (13)	39 (10)	62 (17)
Intraoperative blood loss, median (IQR), ml	350 (400)	250 (150)	570 (550)
Operative time, median (IQR), min	346 (137)	312 (79)	412 (144)
Pancreatic texture, No. (%)			
Hard	392 (52)	205 (53)	187 (50)
Soft	369 (48)	183 (47)	186 (50)
MPD diameter, median (IQR), mm	4 (2)	3 (2)	4 (2)
MPD ≤3 mm, No. (%)	338 (44)	206 (53)	132 (35)
FRS zone, No. (%)			
Negligible (0)	76 (10)	53 (14)	23 (6)
Low (1-2)	206 (27)	113 (29)	93 (25)
Moderate (3-6)	406 (53)	211 (54)	195 (52)
High (7-10)	73 (10)	11 (3)	62 (17)
ISGPS Class, No. (%)			
A	270 (35)	119 (31)	151 (40)
B	122 (16)	86 (22)	36 (10)
C	153 (20)	63 (16)	90 (24)
D	216 (28)	120 (31)	96 (26)
Histopathologic component			
Acinar, median (IQR), %		60 (55)	40 (75)
Fibrosis, median (IQR), %		15 (35)	20 (60)
Fat, median (IQR), %		20 (20)	15 (20)
Postoperative			
POPF (including BL), No. (%)	201 (26)	119 (31)	82 (22)
POPF grade, No. (%)			
BL	30 (4)	19 (5)	11 (3)
B	138 (18)	78 (20)	60 (16)
C	33 (4)	22 (6)	11 (3)
B/C POPF, No. (%)	170 (22)	100 (26)	70 (19)
POH, No. (%)	232 (30)	143 (37)	89 (24)
PPAP, No. (%)	42 (6)	20 (5)	22 (6)
B/C PPH, No. (%)	73 (10)	24 (6)	49 (13)
B/C DGE, No. (%)	75 (10)	31 (8)	44 (12)
Sepsis, No. (%)	149 (20)	82 (21)	67 (18)
LOS, median (IQR), days	10 (12)	10 (10)	9 (14)
Mortality, No. (%)	22 (3)	13 (3)	9 (2)
Clavien–Dindo ≥3, No. (%)	160 (21)	92 (24)	68 (18)

Abbreviations: BMI, body mass index; ASA, American Society of Anesthesiologists; NET, neuroendocrine tumors; PDAC, pancreatic ductal adenocarcinoma; MPD, main pancreatic duct; FRS, fistula risk score; ISGPS, International Study Group for Pancreatic Surgery; POPF, postoperative pancreatic fistula; BL, biochemical leak; POH, postoperative hyperamylasemia; PPAP, post pancreatectomy acute pancreatitis; PPH, post pancreatectomy hemorrhage; DGE, delayed gastric emptying; LOS, length of hospital stay.

High-risk patients were more represented in the validation cohort according to FRS (17% vs. 3%), while the number of patients in the high-risk class (D) according to the ISGPS was similar (31% vs. 26%). The median Ac, Fc, and fat contents were 40%, 20% and 15%, respectively, in the validation cohort compared to 60%, 15%, and 20%, respectively, in the training cohort. In a sub analysis among PDAC patients, the median Ac and Fc were 20% and 40%, respectively, in patients who received preoperative treatment compared to 55% and 20%, respectively, in patients who did not (both p< 0.001). The overall rates of POH and POPF (including BL) were 24% and 22% in the validation cohort and 37% and 31% in the training cohort, respectively. The rates of PPAP and B/C POPF were 6% and 19% in the validation cohort and 5% and 26% in the training cohort, respectively.

Validation of the acinar score

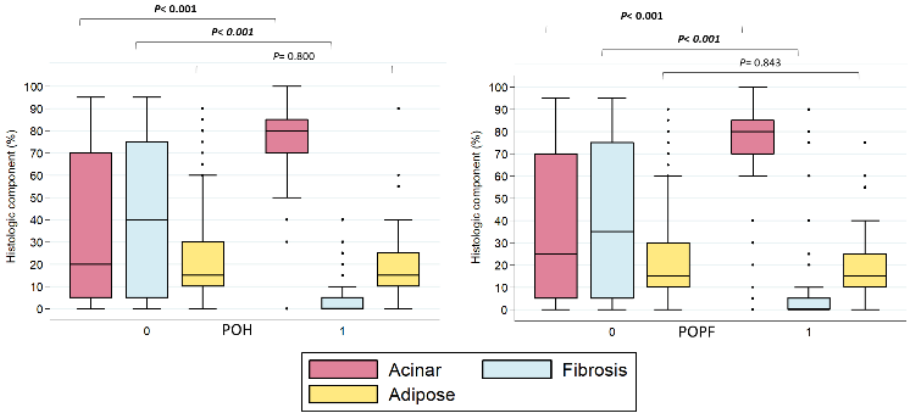
The relationship between histopathologic composition and pancreas-specific complications was tested in the validation cohort, as reported in **Table 1** and **Figure 1**.

Table 1. Relationships Between Histopathologic Composition and Pancreas-specific complications in the Validation Cohort (N= 373)

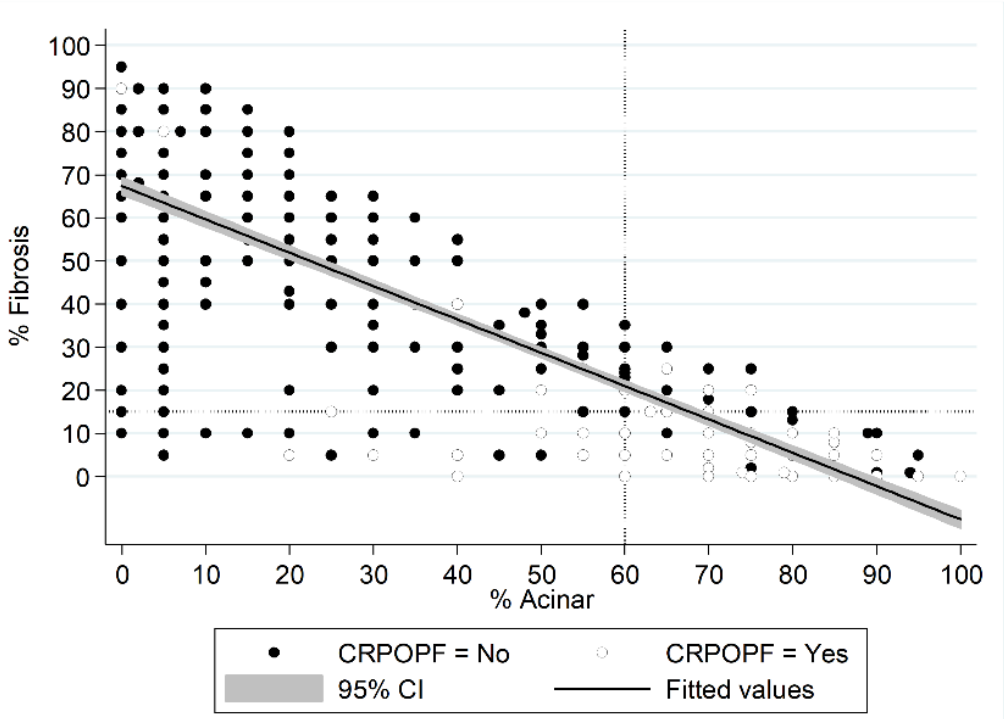
		Histopathologic Component, median (IQR), %		
Clinical Features		**Acinar**	**Fibrosis**	**Fat**
BL + POPF				
	Yes	80 (15)	0 (5)	15 (20)
	No	25 (65)	35 (70)	15 (15)
	P value	<0.001	<0.001	0.843
	AUC	0.80	0.84	0.50
B/C POPF				
	Yes	80 (15)	0 (5)	15 (20)
	No	30 (70)	30 (70)	15 (15)
	P value	<0.001	<0.001	0.659
	AUC	0.79	0.83	0.51
POH				
	Yes	80 (15)	0 (5)	15 (20)
	No	20 (65)	40 (70)	15 (15)
	P value	<0.001	<0.001	0.800
	AUC	0.82	0.84	0.49
PPAP				
	Yes	80 (15)	0 (5)	15 (20)
	No	40 (75)	20 (60)	18 (20)
	P value	<0.001	<0.001	0.437
	AUC	0.75	0.80	0.54
Soft Pancreas				
	Yes	75 (25)	5 (10)	12 (25)
	No	10 (35)	60 (55)	15 (20)
	P value	<0.001	<0.001	0.159
	AUC	0.86	0.89	0.54

Abbreviations: POPF, postoperative pancreatic fistula; BL, biochemical leak; POH, postoperative hyperamylasemia; PPAP, post pancreatectomy acute pancreatitis.

A


P< 0.001
P< 0.001
P= 0.800
P< 0.001
P< 0.001
P= 0.843
Histologic component (%)
POH
POPF
0
1
Acinar
Fibrosis
Adipose

B


% Fibrosis
% Acinar
CRPOPF = No
CRPOPF = Yes
95% CI
Fitted values

C

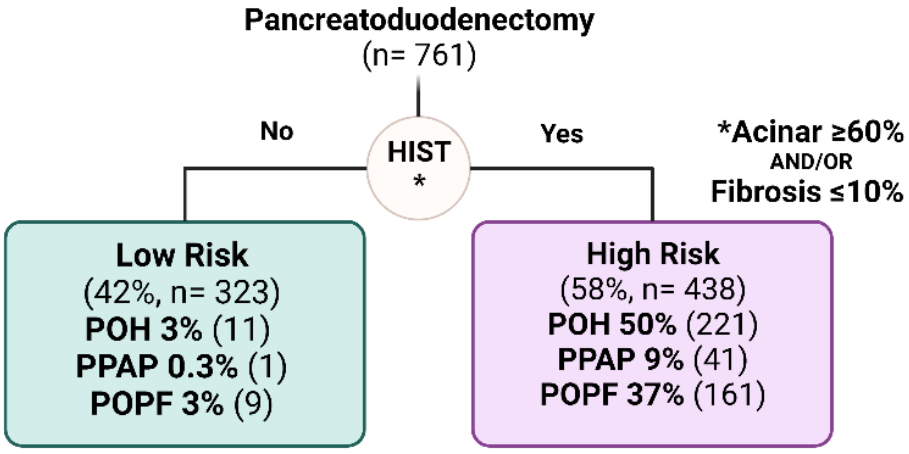

Pancreatoduodenectomy
(n= 761)
No
HIST
*
Yes
*Acinar ≥60%
AND/OR
Fibrosis ≤10%
Low Risk
(42%, n= 323)
POH 3% (11)
PPAP 0.3% (1)
POPF 3% (9)
High Risk
(58%, n= 438)
POH 50% (221)
PPAP 9% (41)
POPF 37% (161)

Figure 1. A) Histopathologic composition and pancreas-specific complications (POH, POPF) in the validation cohort. **B)** Scatterplot with simple linear regression showing the relationship between Ac/Fc and POPF. **C)** Risk stratification of the entire cohort (training + validation) using histopathologic characteristics only.

The median Ac and Fc were higher (80% vs. 20%; p<0.001) and lower (0% vs. 35%; p<0.001), respectively, in patients who developed POH, as in those who developed PPAP. The median Ac was also confirmed to be significantly higher (80% vs. 30%; p<0.001) in patients who developed POPF than in those who did not, while the median Fc was lower (0% vs. 30%; p<0.001). Fat content alone was not associated with any of the explored outcomes (**Figure 1A**). The ROC curves for Ac and Fc are shown in **Figure 2**.

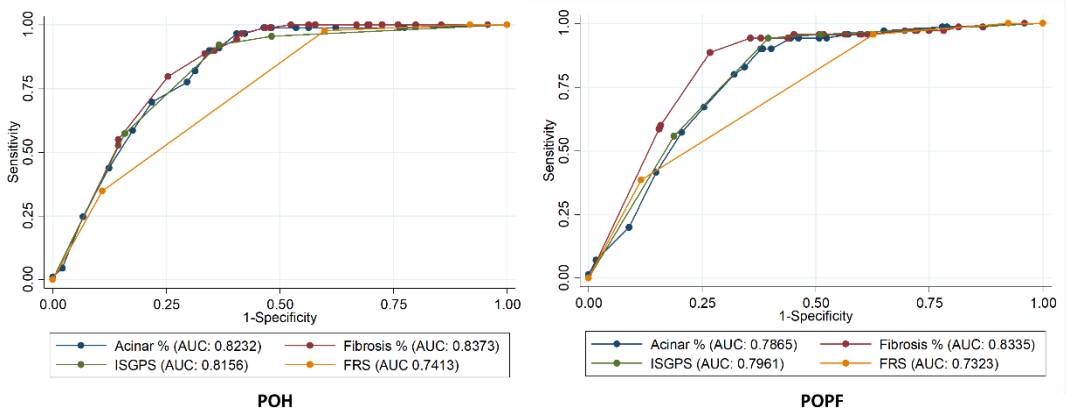


Figure 2. ROC curves and AUC for Ac, Fc, ISGPS, or FRS classification and pancreas-specific complications (POH/POPF).

The AUC of Ac was 0.82 for POH and 0.79 for POPF. A cutoff of Ac ≥60% predicted POH with a sensitivity of 90% and specificity of 65% and POPF with a sensitivity of 88% and specificity of 64%. The AUCs of Fc were 0.84 for POH and 0.83 for POPF. A cutoff of Fc ≤10% predicted POH with a sensitivity of 89% and specificity of 67% and POPF with a sensitivity of 91% and specificity of 66%. **Figure 1 B** shows the relationship between histopathologic components and POPF, with a high POPF incidence inside the Ac and Fc cutoffs. Using only histopathologic cutoffs (Ac ≥60% and/or Fc ≤10%), the whole cohort (training + validation, n= 761) could be divided into a low-risk (POH 3%/PPAP 0.3%/POPF 3%) group of 323 (42%) patients and a high-risk (POH 50%/PPAP 9%/POPF 37%) group of 438 (52%) patients (P<0.001 for all complication rates) (**Figure 1C**).

Acinar score and intraoperative risk stratification

The ISGPS classification, according to intraoperative features such as pancreatic texture (soft vs. hard) and MPD diameter (> or ≤ 3 mm), identified 270 (36%) patients at low risk (A= POH 4%/PPAP 1%/POPF 4%), 216 patients (28%) at high risk (D= POH 58%/PPAP10%/POPF 41%), and two intermediate risk classes (B= POH 32%/PPAP3%/POPF 17%; C= POH 36%/PPAP 9%/POPF 33%) of 122 (16%) and 153 (20%) patients, respectively (**Figure 3A**).

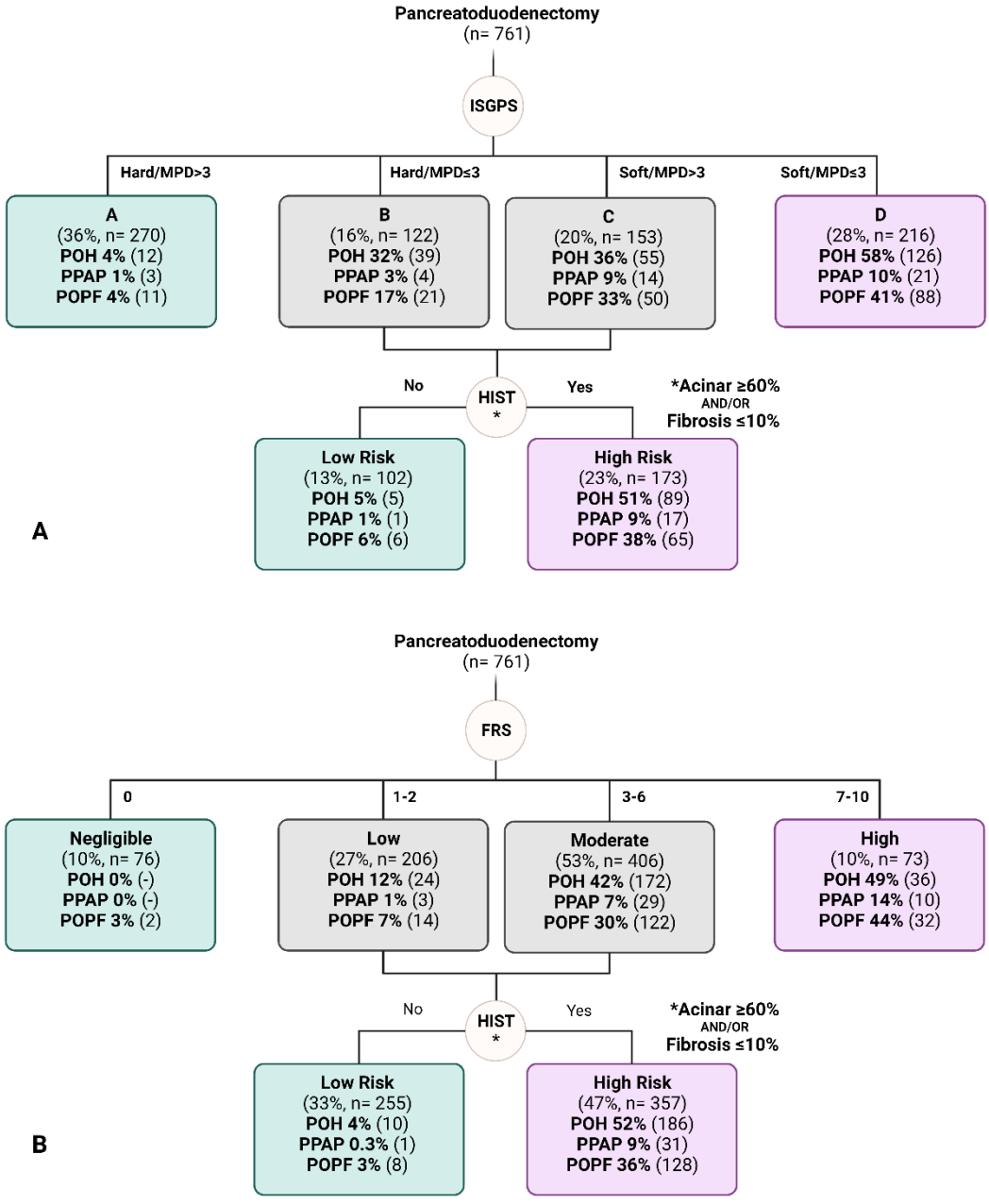


Figure 3. A) Integrated risk stratification: ISGPS classification and histopathologic characteristics. **B)** Integrated risk stratification: FRS and histopathologic characteristics.

Applying the same histopathologic cutoffs (Ac ≥60% and/or Fc ≤10%) to patients in the intermediate-risk classes only (B + C: n= 275, 36%), they could be effectively reclassified into a low-risk (POH 5%/PPAP 1%/POPF 6%) group of 102 (13%) patients and a high-risk (POH 51%/PPAP 9%/POPF 38%) group of 173 (23%) patients (P<0.001 for all complication rates). Similarly, 206 (27%) patients in the low-risk (POH 12%/PPAP 1%/POPF 7%) and 406 patients (53%) in the moderate-risk (POH 42%/PPAP 7%/POPF 30%) zone according to the FRS were reclassified into a low-risk (POH 4%/PPAP 0.3%/POPF 3%) and a high-risk (POH 52%/PPAP 9%/POPF 36%) group of 255 (33%) and 357 (47%) patients, respectively, using histopathologic cutoffs (**Figure 3B**).

The AUC of the histopathologic cutoffs for the diagnosis of POPF was 0.70 in the ISGPS intermediate-risk categories and 0.73 in the FRS low- and moderate-risk zones (**Table 2**).

Table 2. POPF prediction ability of different cutoffs for different risk-categories

Cut offs	Sens	Spec	PPV	NPV	AUC	Youden
In all patients						
Ac ≥60% and/or Fc ≤10%	95%	53%	37%	97%	0.74	0.478
High risk ISGPS (D)	52%	78%	41%	85%	0.65	0.301
High-risk FRS (7-10)	19%	93%	44%	80%	0.56	0.119
In ISGPS B-C						
Ac ≥60% and/or Fc ≤10%	92%	47%	38%	94%	0.70	0.386
In FRS 1-6						
Ac ≥60% and/or Fc ≤10%	94%	52%	36%	97%	0.73	0.460

The different histopathologic composition of low-, intermediate- and high-risk classes, before and after applying the acinar cutoffs, is shown in **Figure 4**.

Overall, 239 patients (31% of the total) were relocated from A (24%), B (66%), or C (68%) ISGPS classes into the histopathologic high-risk group, and 371 (49% of the total) were relocated from a negligible- (8%), low- (33%), or moderate- (71%) FRS zone into the histopathologic high-risk group (**Figure 5**).

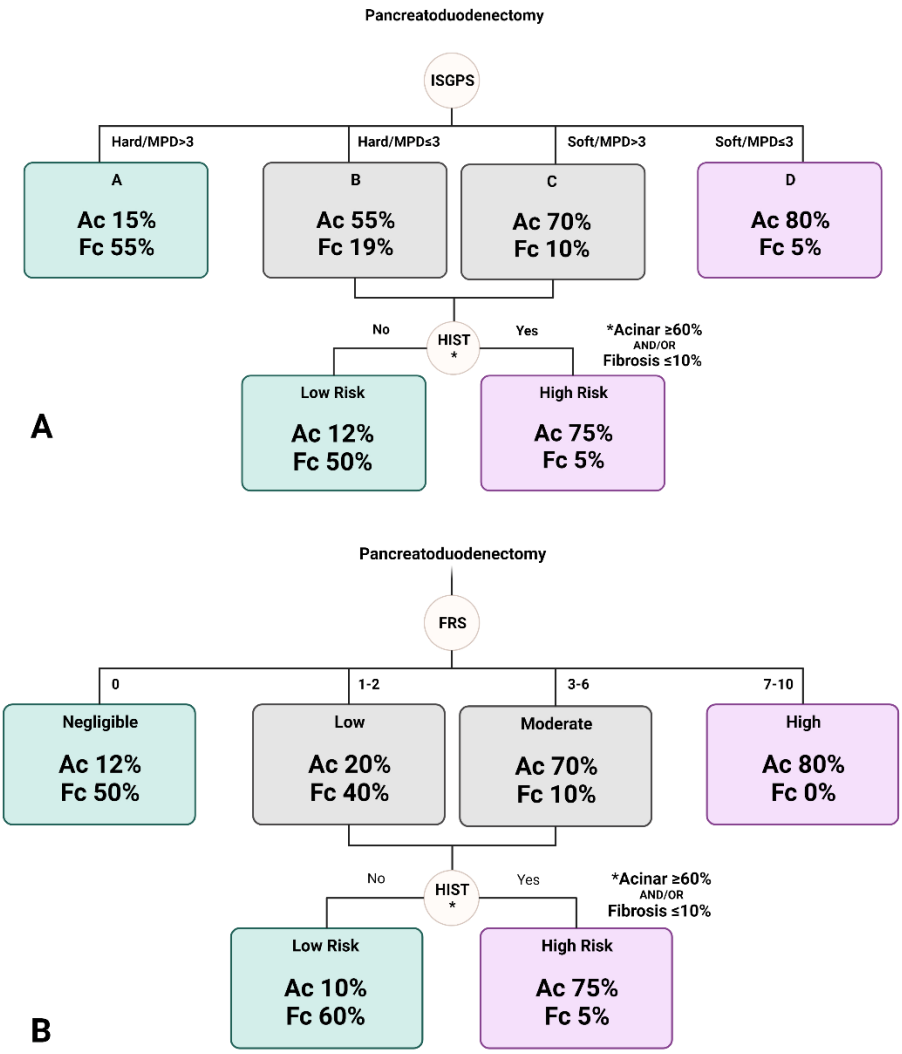


Figure 4. A) Median Ac/Fc content of ISGPS classes before and after the application of histopathologic cutoffs. **B)** Median Ac/Fc content of fistula risk zones before and after the application of histopathologic cutoffs.

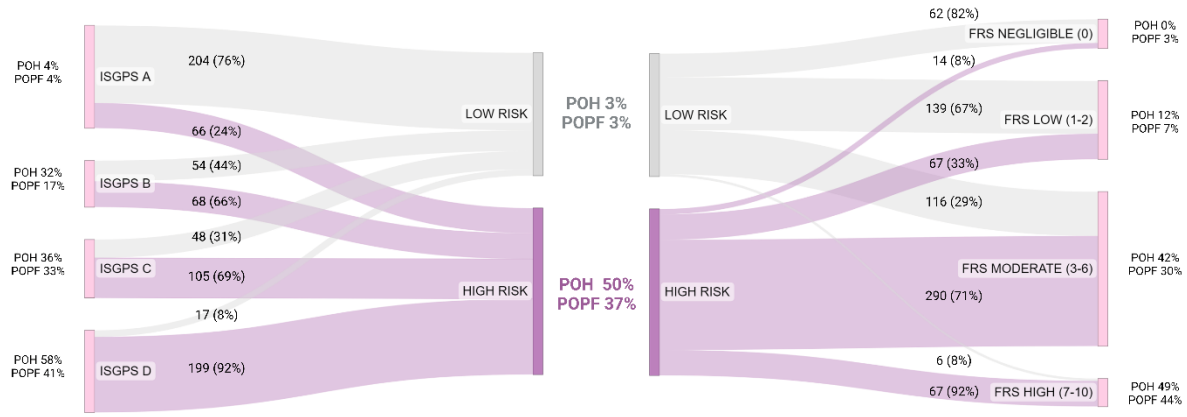


Figure 5. Sankey diagrams: reallocation of POH/POPF risk using histopathologic characteristics. ISGPS classification (left), Fistula Risk Score (right).

DISCUSSION

The present study aimed to ameliorate the predictive ability of existing intraoperative scores for pancreas-specific complications after PD by analyzing the pancreatic histopathologic composition (acinar score).

Acinar and fibrosis contents at the resection margin were confirmed to be strictly associated with POH, PPAP and POPF. The acinar score predicted the risk of pancreas-specific complications more accurately than currently available intraoperative scores. Moreover, the histopathologic risk appeared to be dichotomous - either high or low, questioning the existence of the "intermediate" risk categories to which most patients belong, according to the macroscopic pancreatic features. Therefore, selective use of intraoperative histologic assessment should be integrated with current risk scores in cases of inconclusive/intermediate macroscopic features to reallocate the risk into either low or high. Consequently, appropriate mitigation strategies can be selectively applied.

Pancreatic inflammation and pancreatic fistula are the most common complications after PD, and selective application of mitigation strategies in high-risk patients, such as trans anastomotic stenting and prophylactic drainage, is the current standard of care.[20 35] Despite still not being fully understood, the underlying mechanisms connecting pancreas-specific complications and soft, granular pancreas rely not only on the technical difficulty of pancreatic anastomosis but also on a higher susceptibility to surgical trauma.[37 51] Local complications have been found to be frequently preceded by postoperative pancreatic inflammation, an entity that used to be vaguely characterized but has been finally defined as POH and potentially PPAP – its later, dismal evolution – by the ISGPS.[38] The accepted risk factors for POH and POPF appear to be similar, consisting of the macroscopic surrogates of a "healthy" gland, such as a soft pancreatic texture and a small MPD diameter. These same features represent the common ground of those intraoperative risk scores, indicating the selective use of mitigation strategies. [8 36 52]

Many attempts have been made to correlate objective, histopathologic pancreatic measures such as acinar content, fat content, and fibrosis to postoperative complications. [41 42 53 54] Recently, in the largest series available, Partelli et al. found that POPF was associated with acinar content and inversely associated with

fibrosis but not fat, consistent with previous studies.[44] Moreover, postoperative pancreatitis and the intraoperative finding of a soft gland shared the same histopathologic indicators as POPF, best expressed as an acinar content ≥60% and fibrosis ≤15%. All these findings were validated by the present study, which also applied the latest ISGPS definitions for POH and PPAP, which were not yet available in the previous study.

However, the most relevant finding followed the application of the acinar and fibrosis score to the intermediate-risk cohort. Two well-definite risk categories emerged, with either very low or very high thresholds for both POPF and POH. By comparison with currently available intraoperative scores, this new histopathologic scoring allowed simpler and more accurate risk stratification. Moreover, it appeared clear how the existence of "intermediate" risk categories reflects the impossibility of correctly allocating some patients based on macroscopic features rather than a true intrinsic personalized risk. In addition to being surgeon-dependent and therefore subjective, currently available risk scores inevitably classify most patients into "intermediate" categories. A recent nationwide validation of the ISGPS risk classification for POPF showed how 37% of the 3900 included patients were allocated into B and C categories, with no significant difference in POPF risk between the two.[40] Similarly, in the present study, more than one-third (according to ISGPS) and up to 80% (according to FRS) of patients were categorized in the middle ground of moderate risk according to macroscopic features. Based on histopathologic scoring, it was possible to selectively dichotomize them into two definite groups, with either low- or high-risk. While patients with "extreme" pancreatic characteristics (i.e., soft pancreas + small MPD; hard pancreas + large MPD) can be correctly allocated most of the time based on those alone, patients with "undetermined" or "intermediate" intraoperative characteristics may extremely benefit from further histopathologic analysis to clarify the need for appropriate mitigation (i.e., not avoiding prophylactic drains and positioning of a trans anastomotic stent despite an MPD ≥3 mm/a hard gland). An historical limitation of histopathologic risk assessment is its lack of prompt availability in the intraoperative setting, which would jeopardize its clinical relevance. The intraoperative phase is indeed the first and foremost temporal window to prevent and mitigate future complications. Real-time, intraoperative frozen section analysis of all three components of tissue histology has been limited mainly due to artifacts intrinsic to the presence of the adipose component. However, the

role of fat has been suggested to be irrelevant for risk stratification. Intraoperative, real-time assessment exclusively of the acinar and fibrotic components could be performed in the frozen section margin at the same time as the oncologic margin assessment. Intraoperative assessment proved to be already technically feasible in several studies, including a clinical trial that used intraoperative assessment of acinar content as inclusion criteria for the perioperative administration of hydrocortisone.[43 55] According to the present results, intraoperative histopathologic analysis may be reserved for patients undergoing PD with intermediate macroscopic risk features, limiting the time and resources that would be necessary to perform it in every patient. The elimination of an intermediate risk class could be beneficial for further studies on the topic, improving the consistency between groups at risk for developing pancreas-specific complications.

This study has several limitations, the first being represented by its retrospective nature. As mentioned, resection margins were analyzed retrospectively on formalin-fixed, paraffin-embedded tissue after frozen section analysis. Therefore, despite hypothesis generation, the present findings did not represent an alternative to usual intraoperative stratification, and the predictive performance of the acinar score may decrease once applied prospectively in the intraoperative setting. However, real-time histopathologic risk assessment is possible and should be further explored by prospective studies with the aim of integrating it with current macroscopic risk stratification and improving mitigation of pancreas-specific complications in intermediate risk categories. Finally, considering surgery as the *time-zero*, many other factors and possible predictors add up and play a role during the early postoperative period, shaping the clinical path of complications. The further from time zero, the less accurate the prediction will be based solely on pancreatic gland features.[56] In conclusion, the histopathologic risk of pancreas-specific complications appears to be either high or low, and the presence of “intermediate” risk classes, in which most patients are intraoperatively categorized, probably reflects a coin flip based on undetermined macroscopic features rather than a quantification of their actual risk. Based on these results, future prospective studies should focus on the selective application of intraoperative histopathologic analysis in intermediate-risk patients to clarify the need for mitigation strategies.

CHAPTER III – (POSTOPERATIVE) PREDICTION

Postoperative hyperamylasemia is an early predictor of pancreatic fistula occurrence and severity after distal pancreatectomy: results from a European multicentric study.

INTRODUCTION

Distal pancreatectomy (DP) remains the standard of care for left-sided, resectable pancreatic cancers and benign or pre-malignant lesions. Currently it is mostly performed via minimal invasive techniques, decreasing time to functional recovery and hospitalization while maintaining comparable oncologic standards compared to open surgery[57 58 59 60 61]. Increasing evidence also supports the possibility of avoiding routine surgical drainage after DP, with the hypothesis of preventing drain-induced infections[62 63]. Shifting towards a fast-track postoperative approach, with reduced hospital stay and the omission of surgical drains, requires the ability of timely discriminating the subset of patients who will develop major morbidity. Despite a sensible decrease in perioperative mortality during the last decades, pancreatic surgery remains associated with remarkable postoperative morbidity.[64] The main driver of morbidity after pancreatectomy is pancreatic fistula (POPF), with an incidence ranging from 10% to 30%[31]. Pancreatic fistula after DP can have a higher incidence compared to pancreatoduodenectomy (PD)[65 66 67 68] but is usually less severe, due to a different physiopathology which includes the absence of anastomotic dehiscence. However, the occurrence of POPF after DP prolongs hospital stay, increases costs, and can lead a smaller subset of patients towards severe complications such as sepsis, post-pancreatectomy hemorrhage (PPH), and even death[65 69]. Early postoperative predictors of pancreas-specific morbidity after DP could be used as "red-flags", allowing a tailored approach during postoperative care mainly through mitigation strategies and decreasing failure to rescue and readmissions[70].

Postoperative hyperamylasemia (POH) was recently defined by the Internationals Study Group for Pancreatic Surgery (ISGPS) as an increase of serum amylase persisting for at least the first 48 hours after surgery, and as a necessary biochemical condition for the occurrence of the rarer (but clinically relevant) post-pancreatectomy acute pancreatitis (PPAP)[38]. The presence of POH has also been linked with the occurrence of additional pancreas specific morbidity including POPF after PD. Regardless of intraoperative scores, once POH is present, POPF takes place in up to 40-60% cases[52]. Moreover, despite representing a biochemical process with no standalone clinical relevance, POH predicts additional morbidity also in

absence of POPF and PPAP[71]. Previous evidence already suggested the relationship between pancreatic inflammation and POPF in the setting of DP[72]. However, the implications of POH (as defined by ISGPS[38]) and its relationship with POPF as its early predictor were explored only after PD. Similarly, there are no data about possible pre- and intra-operative factors associated with POH after DP.

The aim of this international multicentric study is to characterize POH after DP, with particular focus on its relationship with POPF occurrence and severity.

METHODS AND MATERIALS

Patients and study design

The study was designed as a retrospective, European multicentric registry. Data of consecutive patients who underwent DP between 2015 and 2021 were retrieved from the institutional prospectively maintained databases of the Pancreatic Surgery Department of Verona University Hospital (Italy), the Department of Hepato-pancreato-biliary Surgery of Oslo University (Norway), and the Department of Hepato-pancreato-biliary Surgery of Karolinska University Hospital (Sweden). All patients undergoing elective minimally invasive or open DP for all indications, with or without the administration of neoadjuvant therapy, were included. Patients with missing data on postoperative serum pancreatic amylase and/or drain fluid amylase (DFA) values in the first three postoperative days (POD) were excluded. The study was approved by the respective Institutional Review Boards.

Data and outcomes

Collected variables included: 1) patient's characteristics as age, gender, body mass index, comorbidities, presumptive diagnosis, neoadjuvant treatment, ASA score; 2) procedure details as minimally invasive vs. open approach, spleen preservation, vascular resection, pancreatic transection site (isthmic, pancreas-preserving/left to the isthmus, extended/right of the isthmus) and technique (stapler, ultrasonic dissector, hand sewed), operative time, estimated blood loss; 3) postoperative serum amylase activity (U/l) and C-reactive protein (C-RP) (mg/dl) on POD 1 to 3, drain amylase value (U/l) on POD 1 to 5 (when available).

Postoperative outcomes were measured during hospitalization and/or after discharging up to 90 days after surgery, and included pancreas-specific biochemical features & complications graded and defined according to the ISGPS: biochemical leak (BL) and POPF, POH and PPAP, PPH, delayed gastric emptying (DGE), postoperative length of stay (LOS), and in-hospital mortality. [38] [47] [48] [49] In particular, POH was defined as a sustained increase in serum amylase activity greater than the specific institutional upper limit (which were 65 U/L in Oslo and Stockholm, 52 U/L in Verona) persisting at least on PODs 1 and 2. Postoperative complications were scored according to the Clavien–Dindo classification and postoperative in-hospital major morbidity was defined as a Clavien–Dindo score ≥3.[50]

Statistical analysis

Categorical variables were reported as numbers with percentages and compared using the Chi-square test or Fisher's exact test when appropriate. Continuous variables were expressed as the means and standard deviation values or as median values with ranges and were compared using the Student T-test or the Mann-Whitney test as appropriate. Comparisons of continuous variables between multiple independent groups were performed using the Kruskal-Wallis' analysis of variance in the non-parametric or ANOVA in the case of parametric distribution. Testing for normal distribution was be performed using the Shapiro-Wilk test for normality. The analyses of POH and POPF predictors were carried out using standard logistic regression. Univariable and multivariable logistic regression models were used to assess pre- and intra-operative variables associated with POH, and early post-operative variables potentially associated with POPF (with two different models, including either categorical variables with cut-offs, or continuous variables). Clinical factors with $P<0.2$ in the univariable analysis and potential clinical importance were included in the multivariable model. The variables were assessed for multicollinearity and removed from the model when necessary. Moreover, the predictive ability of postoperative POPF predictors was evaluated at different PODs by deriving the receiver operating characteristics (ROC) curve of the predicted probabilities obtained from the models and by computing the corresponding Area Under the Curve (AUC). All tests were 2- tailed. P values < 0.05 were considered statistically significant. Figures were created with BioRender.com.

RESULTS

A total of 1192 patients underwent DP and were included in the study, and 210 patients (18%) developed POH. The characteristics of the study population are shown in **Table 1**.

Table 1. Clinical characteristics of all patients (N= 1192) according to the presence of POH

Characteristics	Total, No. (%) (N= 1192)	POH, No. (%)		*P*
		No (N= 982) (82%)	Yes (N= 210) (18%)	
Preoperative				
Age, median (IQR), y	65 (18)	66 (18)	61 (18)	**<0.001**
Female sex, No. (%)	630 (53)	517 (53)	113 (54)	0.759
BMI, median (IQR)	25 (6)	25 (6)	24 (5)	**<0.001**
Smoke, No. (%)	279 (23)	227 (23)	52 (25)	0.609
Diabetes, No. (%)	232 (19)	206 (21)	26 (12)	**0.004**
Cardiovascular disease, No. (%)	145 (12)	131 (13)	14 (7)	**0.007**
COPD, No. (%)	110 (9)	98 (10)	12 (6)	0.053
CKD, No. (%)	25 (2)	14 (1)	11 (5)	**<0.001**
ASA score 3-4, No. (%)	322 (27)	291 (30)	31 (15)	**<0.001**
Presumptive diagnosis				**0.002**
PDAC	495 (42)	428 (44)	67 (32)	
Cystic/NET/Other	697 (58)	554 (56)	143 (68)	
Neoadjuvant therapy, No. (%)	171 (14)	153 (16)	18 (9)	**0.009**
Intraoperative				
Surgery type, No. (%)				**<0.001**
Open	634 (53)	540 (55)	94 (45)	
Laparoscopic	447 (38)	366 (37)	81 (39)	
Robotic	111 (9)	76 (8)	15 (17)	
Spleen preservation, No. (%)	95 (8)	71 (7)	24 (11)	**0.041**
Vascular resection, No. (%)	64 (5)	54 (6)	10 (5)	0.667
Multiorgan, No. (%)	144 (12)	120 (12)	24 (11)	0.749
Transection site, No. (%)				**0.003**
Isthmus	799 (67)	678 (69)	121 (58)	
Regulated (to the left of isthmus)	361 (30)	277 (28)	84 (40)	
Extended (to the right of isthmus)	32 (3)	27 (3)	5 (2)	
Transection method, No. (%)				**0.001**
Stapler	953 (80)	797 (81)	156 (74)	
Ultrasonic scalpel	156 (13)	112 (11)	44 (21)	
Other	83 (7)	73 (8)	10 (5)	
Intraoperative blood loss, median (IQR), ml	150 (300)	150 (300)	200 (275)	**0.024**
Operative time, median (IQR), min	225 (133)	223 (137)	230 (114)	0.242
Postoperative				
B/C POPF, No. (%)	344 (29)	254 (26)	90 (43)	**<0.001**
POPF grade, No. (%)				**<0.001***
BL	240 (20)	195 (20)	45 (21)	
B	317 (27)	241 (25)	76 (36)	
C	27 (2)	13 (1)	14 (7)	
PPAP, No. (%)	44 (4)	_	44 (21)	
PPH, No. (%)	88 (7)	57 (6)	31 (15)	**<0.001**
PPH grade, No. (%)				**<0.001**
A	26 (2)	17 (2)	9 (4)	
B	48 (4)	32 (3)	16 (8)	
C	14 (1)	8 (1)	6 (3)	
DGE, No. (%)	69 (6)	55 (6)	14 (7)	0.548
DGE grade, No. (%)				0.692
A	42 (4)	35 (4)	7 (3)	
B	18 (2)	13 (1)	5 (2)	
C	9 (1)	7 (1)	2 (1)	
Fluid collection/abscess, No. (%)	457 (38)	341 (35)	116 (55)	**<0.001**
Sepsis, No. (%)	67 (6)	43 (4)	24 (11)	**<0.001**
Antibiotics, No. (%)	449 (38)	353 (36)	96 (46)	**0.008**
Percutaneous/endoscopic drainage, No. (%)	137 (24)	103 (24)	34 (25)	0.740
Pulmonary complications, No. (%)	141 (12)	88 (9)	53 (25)	**<0.001**
Cardiovascular complications, No. (%)	54 (5)	41 (4)	13 (6)	0.202
Nephrological complications, No. (%)	20 (2)	12 (1)	8 (4)	**0.008**
ICU admission, No. (%)	56 (5)	37 (4)	19 (9)	**0.001**
Re-operation, No. (%)	61 (5)	41 (4)	20 (10)	**0.001**

Drain removal, median (IQR), days	6 (14)	5 (11)	10 (24)	**<0.001**
LOS, median (IQR), days	8 (6)	8 (5)	10 (10)	**<0.001**
Readmission, No. (%)	171 (14)	129 (13)	42 (20)	**0.009**
Clavien-Dindo ≥2, No. (%)	558 (47)	436 (44)	122 (58)	**<0.001**
Clavien-Dindo ≥3, No. (%)	234 (20)	175 (18)	59 (28)	**0.001**
Mortality, No. (%)	7 (1)	3 (0.3)	4 (1.9)	**0.006**

Abbreviations: BMI, body mass index; ASA, American Society of Anesthesiologists; COPD, chronic obstructive pulmonary disease; CKD, chronic kidney disease; PDAC, pancreatic ductal adenocarcinoma; NET, neuroendocrine tumors; POPF, postoperative pancreatic fistula; BL, biochemical leak; POH, postoperative hyperamylasemia; PPAP, post-pancreatectomy acute pancreatitis; PPH, post-pancreatectomy hemorrhage; DGE, delayed gastric emptying; ICU, intensive care unit; LOS, length of hospital stay.

**Referred to 3x2 contingency table including different POPF grades*

Pre- and Intra-operative Characteristics

Patients who developed POH were younger (61 vs. 66 years; p< 0.001), had lower median BMI (24 vs. 25 kg/m2; p< 0.001), lower prevalence of ASA score of 3 or 4 (15% vs 30%; p< 0.001), lower rates of pre onset diabetes (12% vs 21%; p= 0.004) and cardiovascular diseases (7% vs 13%; p= 0.007), but higher rates of chronic kidney disease (5% vs 1%; p< 0.001) (**Table 1**). A presumptive diagnosis of PDAC, compared to others like cystic or neuroendocrine tumors, was less frequent in POH patients (32% vs 44%; p= 0.002). Similarly, fewer POH patients received neoadjuvant therapy (9% vs 16%; p= 0.009).

Patients who developed POH underwent more frequently minimally invasive surgery (55% vs 45%; p< 0.001) and underwent more frequently spleen preservation (11% vs 7%; p= 0.041), pancreatic transection to the left of the isthmus (40% vs 28%; p= 0.003), and pancreatic transection with ultrasonic scalpel (21% vs 11%; p= 0.001) (**Table 1**). The median intraoperative blood loss was higher in POH patients (200 vs 150ml; p= 0.024).

POH predictors

Pre- and intra-operative predictors for POH were analyzed with multivariable logistic regression analysis. Among pre-operative variables, age (OR= 0.98, 95% CI 0.97-0.99; p= 0.005) and neoadjuvant therapy (OR= 0.55, 95% CI 0.32-0.95; p=0.026) were independent predictors of POH (**Table 2**).

Table 2. Univariable and Multivariable Logistic Regression Analysis of Preoperative and Intraoperative Predictors for POH

Predictors	Univariable		*P*	Multivariable		*P*
	OR	95% CI for OR		OR	95% CI for OR	
Preoperative						
Age, years	0.97	(0.96-0.98)	**<0.001**	0.98	(0.97-0.99)	**0.005**
Female	1.04	(0.77.1.41)	0.760			
Smoker	1.09	(0.77.1.54)	0.609			
Obesity	0.57	(0.35-0.92)	**0.023**	_		
Diabetes	0.53	(0.34-0.82)	**0.005**	_		
Cardiovascular disease	0.46	(0.26-0.82)	**0.009**	_		
COPD	0.54	(0.29-1.01)	0.056	_		
CKD	3.82	(1.70-8.54)	**0.001**	_		
PDAC diagnosis	0.60	(0.44-0.83)	**0.002**	_		
Neoadjuvant therapy	0.50	(0.30-0.84)	**0.010**	0.55	(0.32-0.95)	**0.026**
Intraoperative						
Minimally invasive surgery	1.50	(1.11-2.03)	**0.007**	_		
Spleen preservation	1.65	(1.01-2.69)	**0.043**	_		
Vascular resection	0.85	(0.43-1.71)	0.667			
Multiorgan	0.92	(0.58-1.47)	0.749			
Regulated (left of isthmus)	1.69	(1.24-2.31)	**0.001**	1.68	(1.23-2.30)	**0.001**
Ultrasonic scalpel transection	2.05	(1.39-3.02)	**<0.001**	2.04	(1.38-3.01)	**<0.001**
Intraoperative blood loss, ml	1,0002	(0.9998-1.0005)	0.193			
Operative time, min	1,0002	(0.998-1.001)	0.695			

Abbreviations: COPD, chronic obstructive pulmonary disease; CKD, chronic kidney disease; PDAC, pancreatic ductal adenocarcinoma.

Among intra-operative variables, pancreatic transection to the left of isthmus (OR= 1.68, 95% CI 1.23-2.30; p= 0.001) and with ultrasonic scalpel compared to stapler (OR= 2.04, 95% CI 1.38-3.01; p< 0.001) were independent predictors of POH (**Table 2**).

Postoperative Outcomes

The median length of stay (10 vs. 5 days) and day of drain removal (10 vs. 8 days) were longer in POH patients (both p< 0.001) (**Table 1**). The overall rate of PPAP was 4% (n= 44), but 21% among POH patients. Patients with POH developed PPH more frequently (15% vs. 6%; p< 0.001), but not DGE (7% vs 6%; p= 0.548). Most postoperative complications and therapeutic interventions were also more frequent among POH patients (**Table 1**): fluid collections/abscesses (55% vs. 35%; p< 0.001), sepsis (11% vs. 4%; p< 0.001), antibiotic therapy (46% vs. 36%; p= 0.008), unplanned ICU admission (9% vs. 4%; p= 0.001), re-admission (20% vs. 13%; p= 0.009), and re-operation (10% vs. 4%; p= 0.001). Pulmonary (25% vs 9%; p< 0.001) and nephrological (4% vs 1%; p= 0.008) medical complications were also higher in POH patients. Finally, major morbidity (Clavien-

Dindo ≥3: 28% vs 18%; p< 0.001) and mortality (1.9% vs 0.3%; p= 0.006) were both significantly higher in patients with POH.

POH and POPF incidence

In total, 18% of patients (n= 210) developed POH and 29% of patients (n= 344) developed POPF. Among patients who developed POH, POPF rate was 43% (n= 90) compared to 26% (n= 254) among those without POH (p< 0.001) (**Table 1** and **Figure 1**).

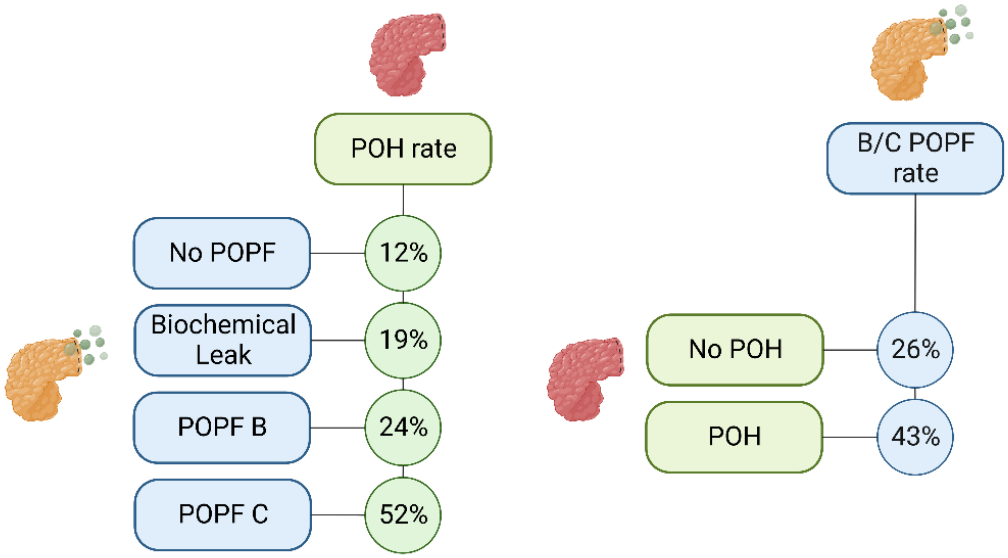


Figure 1. Relationship between POH and POPF incidence.

The rates of BL, B POPF and C POPF were 21%, 36% and 7%, respectively in patients with POH, while they were 20%, 25% and 1%, respectively in patients without POH (p< 0.001) (**Table 1**). Patients with higher POPF severity presented higher POH rates (namely no POPF= 12%; BL= 19%; POPF B= 24%; POPF C= 52%) (**Figure 1**).

POH and POPF severity

Among patients who developed POPF (n= 344), 90 (26%) experienced also POH (7% of the total) (**Table 3** and **Figure 2**).

Table 3. Postoperative clinical characteristics of patients with (N= 344) and without (N= 848) B/C POPF, according to the presence of POH

	Characteristics	Total, No. (%) (N= 344)	POH, No. (%)		*P*
			No (N= 254) (74%)	Yes (N= 90) (26%)	
B/C POPF	PPAP, No. (%)	31 (9)	_	31 (34)	
	PPH, No. (%)	43 (13)	23 (9)	20 (22)	**0.001**
	DGE, No. (%)	35 (10)	27 (11)	8 (9)	0.639
	Fluid collection/abscess, No. (%)	343 (99)	253 (99)	90 (100)	0.551
	Sepsis, No. (%)	55 (20)	33 (13)	22 (24)	**0.011**
	Antibiotics, No. (%)	268 (78)	197 (78)	71 (79)	0.794
	Percutaneous/endoscopic drainage, No. (%)	137 (40)	103 (41)	34 (38)	0.644
	Sphincterotomy, No. (%)	22 (6)	14 (6)	8 (9)	0.261
	ICU admission, No. (%)	40 (12)	23 (9)	17 (19)	**0.012**
	Re-operation, No. (%)	40 (12)	21 (8)	19 (21)	**0.001**
	POPF duration, median (IQR), days	26 (24)	25 (26)	27 (22)	0.238
	Drain removal, median (IQR), days	30 (22)	29 (24)	34 (26)	**0.034**
	LOS, median (IQR), days	12 (14)	11 (11)	18 (19)	**<0.001**
	Readmission, No. (%)	133 (39)	95 (38)	38 (43)	0.392
	Clavien-Dindo ≥3, No. (%)	185 (54)	132 (52)	53 (59)	0.258
	Mortality, No. (%)	4 (1)	1 (0.3)	3 (3)	**0.025**
	Characteristics	**Total, No. (%) (N= 848)**	**POH, No. (%)**		***P***
			No (N= 728) (86%)	**Yes (N= 120) (14%)**	
No B/C POPF	PPAP, No. (%)	13 (2)	_	13 (11)	
	PPH, No. (%)	45 (5)	34 (5)	11 (9)	**0.042**
	DGE, No. (%)	34 (4)	28 (4)	6 (5)	0.551
	Fluid collection/abscess, No. (%)	114 (13)	88 (12)	26 (22)	**0.004**
	Sepsis, No. (%)	12 (1)	10 (1)	2 (2)	0.801
	Antibiotics, No. (%)	181 (21)	156 (21)	25 (21)	0.883
	Pulmonary complications, No. (%)	63 (7)	48 (7)	15 (13)	**0.022**
	Cardiovascular complications, No. (%)	29 (3)	24 (3)	5 (4)	0.627
	Nephrological complications, No. (%)	5 (1)	5 (1)	_	0.363
	ICU admission, No. (%)	16 (2)	14 (2)	2 (2)	0.848
	Re-operation, No. (%)	21 (2)	20 (3)	1 (1)	0.211
	Drain removal, median (IQR), days	5 (3)	5 (3)	5 (4)	**0.001**
	LOS, median (IQR), days	7 (3)	7 (4)	8 (4)	**<0.001**
	Readmission, No. (%)	38 (4)	34 (5)	4 (3)	0.512
	Clavien-Dindo ≥2, No. (%)	243 (29)	206 (28)	37 (31)	0.569
	Clavien-Dindo ≥3, No. (%)	49 (6)	43 (6)	6 (5)	0.693
	Mortality, No. (%)	3 (0.3)	2 (0.3)	1 (0.8)	0.340

Abbreviations: POPF, postoperative pancreatic fistula; POH, postoperative hyperamylasemia; PPAP, post-pancreatectomy acute pancreatitis; PPH, post-pancreatectomy hemorrhage; DGE, delayed gastric emptying; ICU, intensive care unit; LOS, length of hospital stay.

The rate of PPAP in POPF patients was 9% (n= 31), 34% in patients with POPF and POH. Patients who had both POPF and POH had higher LOS (18 vs. 11 days; p< 0.001) compared to patients with POPF only, and considerably higher rates of PPH (22% vs. 9%; p= 0.001), sepsis (24% vs. 13%; p= 0.011), unplanned ICU admission (19% vs. 9%; p= 0.012), re-operation (21% vs. 8%; p= 0.001) and overall mortality (3% vs. 0.3%; p= 0.025).

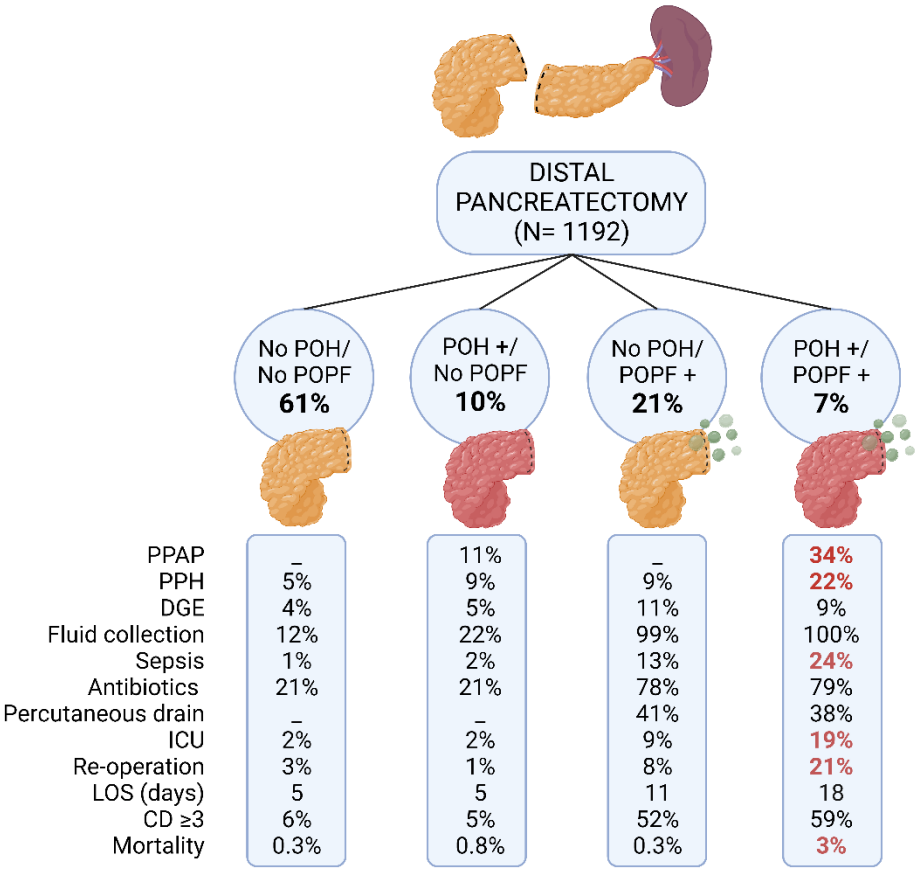


Figure 2. Relationship between POH, POPF severity, and other post-operative complications.

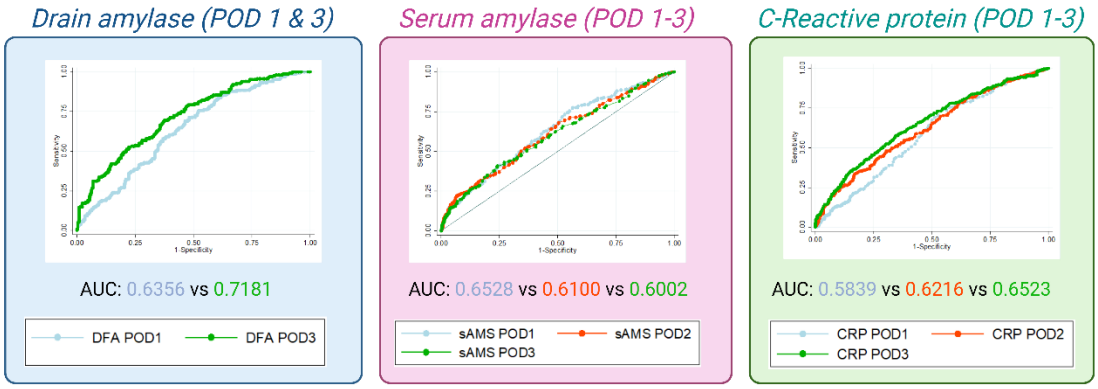
POH alone vs No POH/POPF

Among 848 patients who did not develop POPF, 120 (14%) experienced POH alone (10% of the total) (**Table 3** and **Figure 2**). The rate of PPAP among these patients was 11% (n= 13). Patients with POH alone had higher rates of PPH (9% vs 5%; p= 0.042), fluid collection/abscess (22% vs 12%; p= 0.004), and longer LOS (8 vs 7 days; p< 0.001) compared to patients who never developed POH or POPF.

Early Postoperative POPF predictors

The ROC curves of early postoperative POPF predictors at different PODs are represented in **Supplementary Figure 1**. The AUC of POD 1 DFA, POD 1 sAMS and POD 3 C-RP were 0.635, 0.652 and 0.652 respectively. The AUC for DFA and C-RP increased over time from POD 1 to POD 3, while the AUC of serum amylase (sAMS)

decreased. Of note, only 62% of patients had POD 3 DFA values available for analysis.



Supplementary Figure 1. A comparison of ROC curves for different POPF predictors between PODs 1 and 3. AUC= area under the curve.

A multivariable logistic regression analysis of early (≤ POD3) post-operative predictors for POPF identified POH (OR=1.58, 95% CI 1.14-2.19; p= 0.006), POD 1 DFA ≥ 2000 UI/L (OR=2.11, 95% CI 0.68-2.86; p< 0.001), and POD3 C-RP ≥200 mg/L (OR=2.19, 95% CI 1.68-2.86; p< 0.001) as independent predictors (**Table 4**). The POD 1 DFA, POD 1 serum amylase (sAMS), and POD3 C-RP, all expressed as continuous variables, were confirmed by multivariable analysis as independent predictors of POPF (**Table 4**).

Table 4. Multivariable Logistic Regression Analysis of Early (≤ POD 3) Postoperative Predictors for B/C POPF (cut-offs)

Predictors	Multivariable		*P*
	OR	95% CI for OR	
Cut-offs			
POD 1 DFA ≥ 2000 UI/L	2.11	(1.68-2.86)	**<0.001**
POH	1.58	(1.14-2.19)	**0.006**
POD 3 C-RP ≥ 200 mg/L	2.19	(1.68-2.86)	**<0.001**
Continuous			
POD 1 DFA	1.00007*	(1.00003-1.00012)	**0.001**
POD 1 sAMS	1.002*	(1.0006-1.0049)	**0.011**
POD 3 C-RP	1.005*	(1.003-1.006)	**<0.001**

Abbreviations: POD, postoperative day; DFA, drain fluid amylase; POH, postoperative hyperamylasemia; C-RP, C-reactive protein; sAMS, serum amylase.
**OR reflects 1 unit increase (UI/L or mg/L)*

A proposal for the integration of early predictors in clinical practice is depicted in **Figure 3**.

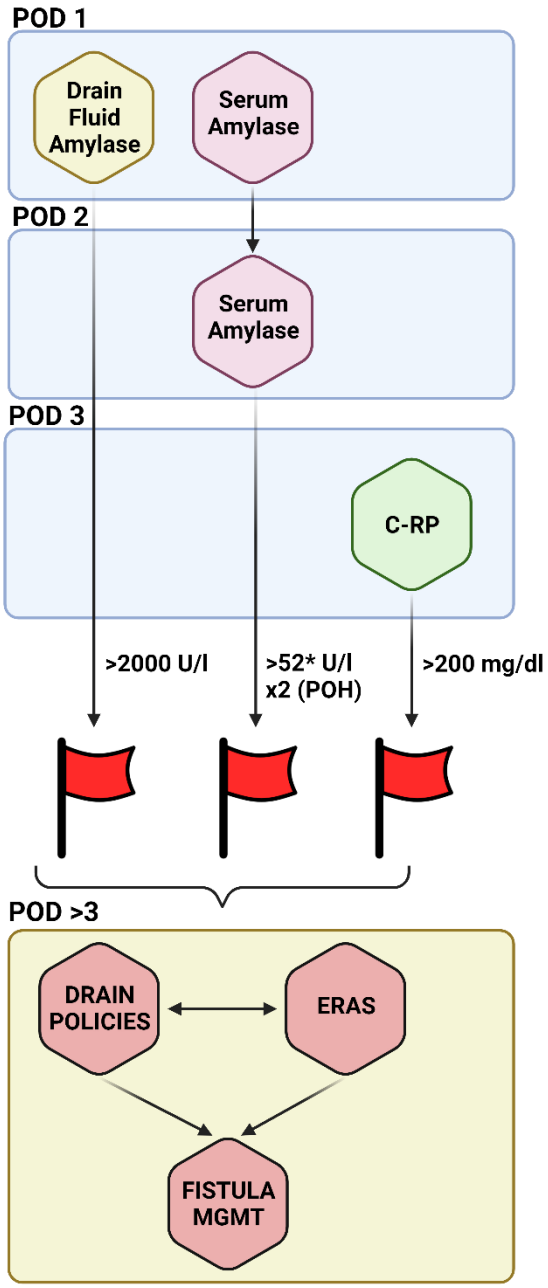


Figure3. The use of "red flags" in the early postoperative management to tailor drain policies (early removal), enhanced recovery/discharge, or early diagnosis/mitigation of pancreatic fistula.

DISCUSSION

The aim of this study was to characterize POH after distal pancreatectomy and its possible relationship with POPF. We found that POH is relatively common after DP, and that patients with POH had higher POPF incidence and severity. Therefore, POH could be used as an early marker of a potentially complicated course after DP, due to its availability within the first 48 postoperative hours. The definition of POH as an elevated

serum amylase level for at least 48 hours after surgery is fairly recent[38]. Despite representing - by definition – a purely biochemical entity, POH seems indeed to harbor relevant clinical implications after PD. Bannone and colleagues found that once POH occurs after PD, POPF incidence may rise from 4% to 43%, 23% to 42%, and 48% to 59% in patients intraoperatively classified at low, moderate, and high risk of POPF, respectively[52]. The authors concluded that POH should be routinely implemented together with DFA and C-RP as a very early postoperative indicator of a complicated course after PD. Moreover, they showed that patients developing POH - but not POPF or PPAP - after PD have worse postoperative outcomes compared to uncomplicated patients[71], implying a possible stand-alone clinical significance of POH, irrespectively of the development of other pancreas-specific complications. Finally, despite using an outdated definition[73] different from the current one adopted by the ISGPS, another previous study from the Verona group identified "biochemical pancreatitis" as an independent predictor of POPF and severe morbidity after DP. The present multicenter series confirms the relationship between POH and POPF after a DP. While PPAP remains a rare event, one-fifth of patients will develop POH. In nearly half of them, POH will not probably represent just a biochemical finding, but the stigmata of a pathologic process of the pancreatic stump leading to POPF, increasing its severity, and causing additional morbidity. Despite the present study was not designed to give definitive answers on POH and PPAP physiopathology, different hypotheses are available. According to the ISGPS definition[38], emerging evidence defined PPAP as a local inflammatory/ischemic process of the pancreatic remnant related to the operative trauma. Such trauma may originate from a series of events ranging from manipulation, mobilization, alteration of blood supply, and/or stasis of pancreatic juice. All these triggers may cause a cascade involving acinar cell disruption, intracellular activation of proteolytic enzymes, pancreatic parenchymal edema, and peripancreatic inflammation, with local and/or systemic effects [38]. It is interesting to notice how some predictors of POH identified in the present study, such as parenchymal division with harmonic focus or left to the isthmus, could be involved in this process. Once established, pancreatic inflammation may impair the healing of the pancreatic remnant (even in the absence of anastomosis) with the subsequent development of POPF and worse POPF outcomes, triggering systemic inflammatory response syndrome and/or systemic sepsis. A word of caution should be used, as POH and

POPF do not always overlap, so it remains difficult to exactly characterize a cause-effect relationship between these two events. It also remains unknown whether the "clinically relevant" POH are misdiagnosed PPAP, in which an early abdominal imaging was not performed. Therefore, rather than a stand-alone complication, POH should be interpreted as a very early (≤48h) indicator of a possible postoperative complicated course. The morbidity and mortality burden of a DP is lower than that of a PD, favoring the implementation of enhanced recovery after surgery (ERAS) protocols. Moreover, minimally invasive DP has been fully implemented in worldwide clinical practice, aiming for earlier recovery and discharge compared to open surgery, and is currently considered the standard for resectable cases.[57] However, despite less severe, POPF after DP remains very common. Drain management is one of the most frequent reasons of delayed discharge after DP, as it is often difficult to discriminate in the early postoperative course if an amylase-rich drain fluid will evolve into a POPF or remain a biochemical leak. The ability to identify as soon as possible the subset of patients who should drop out from a fast-track based approach, or at least require special attention, is critical for the safe and successful implementation of early recovery after DP. As showed by a recent randomized controlled trial[70], the nationwide implementation of a postoperative algorithm based on early clinical parameter led to an impressive decrease of severe morbidity and mortality after pancreatectomy, through an earlier and more proactive use of abdominal imaging, antibiotics, and percutaneous drainages in selected patients. POH might represent such a label of caution, together with other established predictors such as DFA and C-RP. In addition to conventional predictors such as DFA and C-RP, POH could be used for early diagnosis of POPF, selecting patients to drop out from fast-track protocols and/or outpatient drain management. Moreover, POH can be used as a "red flag" among patients eventually developing POPF, suggesting a more proactive and cautious postoperative management, as these patients demonstrated to be at very high risk for severe complications and increased mortality. A possible clinical pathway would start with the observation of clinical predictors for the first 2-3 PODs, as showed in **Figure 3**, while keeping the surgical drain in place and applying ERAS protocols. If cut-offs are met, in POD 3 drain could be removed and enhanced recovery finalized within 24-48h, with possible early discharge. Patients not meeting the criteria would maintain the drain and, if required, start antibiotics/undergo CT scan if high suspect of POPF with

undrained collection/pancreatitis. Finally, increasing evidence suggests that prophylactic drainage during DP could be even avoided, due to a potential role in contaminating otherwise sterile fluid collections and contributing to POPF development[62 63]. Therefore, early and "pancreas-specific" predictors of morbidity not limited to the amylase content in the surgical drains, could be key to tailor the postoperative management after drainless DP. Serum amylase may play a major role in this setting, together with C-RP, to drive postoperative care in the absence of abdominal drains. The present study has several limitations, the first and foremost being represented by the retrospective design, the absence of risk stratification for POPF according to pancreatic characteristics, and the possible heterogeneity of treatments and outcomes between the participating institutions. The high rates of antibiotic administration and percutaneous drainage in this series (despite low rates of mortality, re-operation, and ICU admission) is probably resulting from a proactive approach, aiming at early detection and treatment of severe complications, but on the other hand obviously leading to a POPF "up-grading" and higher B POPF rates. Prospective studies are required for better understanding of POH/PPAP physiopathology, validation of their definitions, and possible incorporation of POH in clinical algorithms. Of note, some may consider protracted sAMS measurement redundant in case C-RP is already assessed daily. However, the overlap between POH and POD 3 C-RP >200 mg/dl was not complete among patients eventually developing POPF (18% of POPF patients with POD 3 C-RP <200 mg/ml had POH). Moreover, unlike C-RP, POH represents a "pancreas-specific" parameter, and its presence in combination with other available predictors reinforces the suspect of pancreas-related morbidity. Finally, despite being similarly effective for early POPF diagnosis, POD 3 C-RP was not as effective as POH to predict POPF severity (*not showed in the results*), as later C-RP measurements would probably be required. In conclusion, POH occurs in 20% of distal pancreatectomies and correlates with a worse postoperative outcome. Once POH has been diagnosed within 48 hours from surgery, clinicians should be aware of the increased risk of POPF occurrence and severity, eventually resulting in higher rates of sepsis, bleeding, re-operation, ICU admission, and mortality. Together with other available tools for risk assessment, POH should be used as an early predictor of pancreas specific complication development and severity.

CHAPTER IV – PREVENTION

High-risk pancreatic anastomosis vs. total pancreatectomy after pancreatoduodenectomy: postoperative outcomes and quality of life analysis.

INTRODUCTION

Total pancreatectomy (TP) was historically introduced to improve the survival of patients with pancreatic cancer, with the aim of avoiding anastomosis-related morbidity and mortality and reducing tumor recurrence rates. No benefit was recorded, as the relevant perioperative morbidity was associated with the difficulties of complete and lifelong pancreatic insufficiency, which severely affected surgical outcomes and patient quality of life (QoL).[74 75 76] Therefore, TP was abandoned in favor of standard head or tail resection and reserved only for diffuse/multifocal neoplastic disease, such as intraductal papillary mucinous neoplasms (IPMNs) in the main duct, multiple renal cell carcinoma metastases and neuroendocrine neoplasms (PanNETs).[77 78 79] However, both the indications and outcomes of TP have significantly changed during the last decade. Extensive use of neoadjuvant chemo- and radiotherapy for borderline resectable and locally advanced pancreatic cancers in recent years has led to a rehabilitation of extended pancreatic resections to achieve surgical radicality.[80 81 82] While the main indication for elective primary TP remains multifocal disease, the decision to perform a TP is often made intraoperatively to pursue a radical resection in patients with pancreatic cancer. Moreover, recent studies have reported improved perioperative outcomes and postoperative QoL after TP, presumably due to centralization at high-volume centers and development of long-acting insulin and modern pancreatic enzyme preparations.[83 84 85 86 87]

Postoperative pancreatic fistula (POPF) is the main cause of surgical morbidity after pancreatoduodenectomy (PD). Several risk score systems based on pre- and intraoperative parameters, such as the Fistula Risk Score (FRS) or the alternative Fistula Risk Score (a-FRS), have been proposed to predict the occurrence of POPF and stratify patients based on this risk.[88 89] Many authors have sought to mitigate the incidence and severity of POPF in high-risk anastomosis using different surgical techniques, stents, and medical treatments. However, both the morbidity and mortality rates remain extremely high after high-risk PD (HR-PD), even in the era of mitigation strategies.[20] Given the encouraging postoperative outcomes at high-volume centers, TP might be considered after intraoperative risk stratification as an alternative to HR-PD to avoid the occurrence of POPF. However, short- and long-term outcomes of these two surgical procedures have not yet been compared.

The aim of the present study was to evaluate TP with regards to postoperative outcomes as an alternative to HR-PD in patients at high risk for POPF development. The secondary outcomes were the incidence of exocrine and endocrine insufficiency and postoperative QoL.

METHODS

Study design

Approval for data collection and analysis for this study was obtained from our institutional review board. Prospectively collected data of consecutive patients who underwent open PD or TP from July 2017 to December 2019 were retrospectively reviewed. Patients who underwent PD were stratified into three subgroups according to the a-FRS: low-, intermediate-, and high-risk (HR-PD) (**Figure 1**). Patients who underwent TP were further stratified into two subgroups depending on whether the procedure was elective primary *en-bloc* TP or planned PD that was intraoperatively converted to TP during the same surgical session (C-TP). The a-FRS was also calculated in all patients undergoing C-TP.

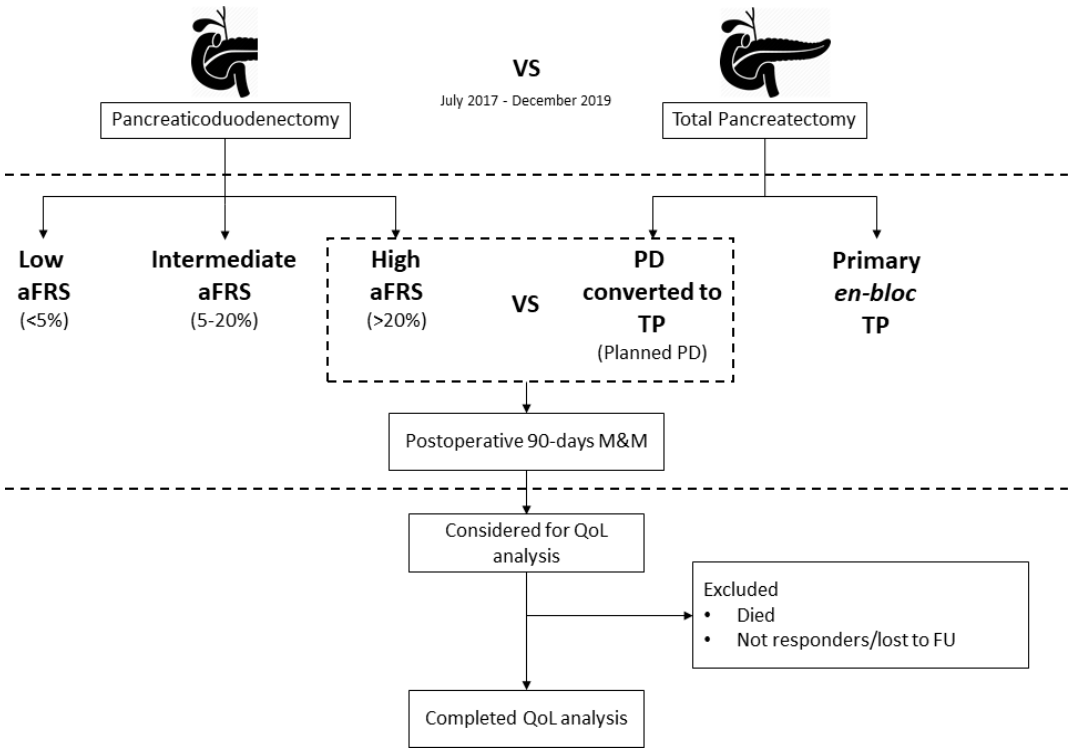


Figure 1. Study design

Surgical procedures

Open PD was carried out in a standardized fashion as previously described by our group.[11] A trans-anastomotic externalized stent and a feeding jejunostomy were placed in most patients in the high-risk zone according to the FRS score, in particular where a small main pancreatic duct diameter was present.[88] [20] Two drains were placed in the proximity of the pancreatic and biliary anastomoses in all patients in the high or intermediate risk zone or according to the surgeon's preference in all other risk categories. A previously published protocol was used for drain management.[13]

Elective primary TP was typically performed when multifocal or diffuse neoplasms were found on preoperative evaluation or in patients with chronic pancreatitis. C-TP was performed when high-grade dysplasia or invasive carcinoma were found to be present at the pancreatic resection margin upon intraoperative evaluation to achieve radical oncological resection. Other indications for C-TP were intraoperative macroscopic evidence of neoplastic disease affecting the whole gland, extended vascular resection, or technical issues (bleeding, non-reconstructable residual pancreas, evidence of intraoperative pancreatitis). In all cases of C-TP after preoperatively planned PD, the a-FRS was intraoperatively calculated.

Data collection and outcomes

Pre-, intra- and postoperative demographic data were recorded in a prospectively maintained database. Postoperative outcomes were measured during hospitalization and/or after discharge up to 90 days after surgery and included POPF and biochemical leak, post-pancreatectomy hemorrhage (PPH), delayed gastric emptying (DGE), sepsis, postoperative length of stay (LOS), and in-hospital mortality.[3] [14] [15] [16] Postoperative in-hospital major morbidity was defined as Clavien-Dindo ≥3.

Questionnaires

All the HR-PD and C-TP patients who were alive at the time of the study and had completed at least 12 months of follow-up (FU) were enrolled in the cross-sectional study of quality of life. All the eligible patients were interviewed by telephone. Five questionnaires were administered (**Supplementary Table 1**): (1) the EuroQoL

Group questionnaire (EQ-5D); (2) the European Organization for Research and Treatment of Cancer (EORTC) QLQ-C30 questionnaire; (3) the European Organization for Research and Treatment of Cancer (EORTC) QLQ-PAN26 questionnaire; (4) an original survey used to evaluate the occurrence and severity of exocrine and endocrine insufficiency; and (5) the Problem Areas in Diabetes (PAID) questionnaire. [90] [91] [92] [93] The comprehensive assessment consisted of a total of 116 questions.

Supplementary Table 1. QoL Questionnaires

Questionnaire	Area	Items	Scoring system
Euro QoL Group EQ-5D-3	Non disease-specific health states	(1) mobility; (2) self-care; (3) usual activities (e.g., work, study, housework, family, or leisure activities); (4) pain/discomfort; and (5) anxiety/depression	For each answer, there is a score from 1 to 3 in relation to the status of the patient. A lower score indicates a better quality of life. The final EQ-5D-3L score ranged from 0 to 1, where 1 was the best health status.
European Organization for Research and Treatment of Cancer (EORTC) QLQ-C30	Quality of life of cancer patients	(1) global health status (GHS); (2) five functioning scales; (3) nine cancer-specific symptoms	This questionnaire consists of 30 questions with a score from 1 to 4. A lower symptoms score indicates a better QoL, while a lower GHS and functioning score suggest poorer QoL. The scores were converted to a scale from 0 to 100 by linear transformation and are presented as the mean and standard deviation (SD).
European Organization for Research and Treatment of Cancer (EORTC) QLQ-PAN26	Severity of pancreas-related symptoms	Six pancreas-specific scales: (1) pancreatic pain; (2) digestive symptoms; (3) altered bowel habit; (4) hepatic function; (5) body image; (6) sexuality. One item about the satisfaction with healthcare	This questionnaire consists of 26 questions with a score from 1 to 4. A lower symptoms score indicates a better QoL, while a lower score of satisfaction with healthcare suggests a poorer QoL. The scores were converted to a scale from 0 to 100 by linear transformation and are presented as the mean and standard deviation (SD).
Original survey	Severity of exocrine and endocrine insufficiency	(1) postoperative weight; (2) presence of diabetes; (3) type of diabetes therapy; (4-5) use of pancreatic enzymes	
Problem Areas in Diabetes (PAID)	Psychosocial adjustment specific to diabetes	Twenty diabetes-specific questions	This questionnaire consists of 20 questions with a score from 0 to 4. A lower score indicates better acceptance of the disease. The scores were added and then multiplied by a coefficient (1.25) in order to obtain a final score (the PAID score).

Statistical analysis

Continuous variables are expressed as the means and (SD) values or as median values with ranges and were compared using the independent samples t-test or the Mann-Whitney test, as appropriate. Categorical

variables are expressed as frequencies with percentages and were compared using the $\chi 2$ test or Fisher's exact test in case of small expected frequencies. All the tests were 2-tailed. $P < 0.05$ was considered statistically significant. The scoring system and statistical analysis regarding the QoL questionnaires are described in **Supplementary Table 1**. Minimal clinically important difference (MCID) was calculated for (EORTC) QLQ-C30 and -PAN26. [94] Statistical analyses were performed using STATA14 for Windows.

RESULTS

Seven hundred two patients were included in the study: 566 (81%) patients who underwent PD and 136 (19%) patients who underwent TP. The baseline characteristics, clinical features, intra- and postoperative details of the patients are listed in **Supplementary Table 2**.

Supplementary Table 2. Preoperative, Intraoperative and Postoperative Profile of All Patients Who Underwent PD (N= 566) and TP (N= 136)

Characteristics	Total, No. (%) (N= 702)	Pancreatectomy, No. (%)		*P-value*
		PD= 566 (81)	TP= 136 (19)	
Preoperative				
Age, median (range), y	65 (16-87)	65 (16-87)	64 (29-83)	0.8
Female sex, No. (%)	312 (44)	247 (44)	65 (48)	0.3
BMI, median (range)	24 (15-40)	24 (15-40)	24 (17-40)	0.6
Smoker, No. (%)	153 (22)	125 (22)	28 (21)	0.7
Alcohol abuse, No. (%)	23 (3)	17 (3)	6 (4)	0.4
Diabetes, No. (%)	156 (22)	105 (19)	51 (37)	<0.01
Weight loss, No. (%)	326 (46)	264 (47)	62 (46)	0.8
Ischemic cardiac disease, No. (%)	42 (6)	31 (5)	11 (8)	0.2
Hypertension, No. (%)	290 (41)	216 (38)	74 (54)	<0.01
COPD, No. (%)	18 (3)	14 (2)	4 (3)	0.7
Chronic renal failure, No. (%)	17 (2)	11 (2)	6 (4)	0.09
ASA score, No. (%)				0.1
1-2	565 (80)	462 (82)	103 (76)	
3-4	137 (20)	104 (18)	33 (24)	
Jaundice palliation, No. (%)	338 (48)	292 (52)	46 (34)	<0.01
Preoperative MDR colonization, No. (%)	78 (11)	58 (10)	20 (15)	0.1
Neoadjuvant therapy, No. (%)	200 (28)	148 (26)	52 (38)	<0.01
Presumed diagnosis				0.8
PDAC/chronic pancreatitis	440 (63)	354 (63)	86 (63)	
Duodenal/ampullary/cystic/NET	262 (37)	212 (37)	50 (37)	
Intraoperative				
Surgery type, No. (%)				<0.01
Pylorus preserving	591 (84)	501 (89)	90 (66)	
Whipple	111 (16)	65 (11)	46 (34)	
Vascular resection, No. (%)	130 (18)	80 (14)	50 (37)	<0.01
Type of TP				
Elective primary TP			50 (37)	

Completion TP after planned PD			86 (63)	
Pancreatic anastomosis, No. (%)				
PJ		522 (92)		
PG		44 (8)		
Externalized pancreatic stent, No. (%)		252 (44)		
Blood loss, No. (%)				0.01
≤400 ml	267 (38)	206 (36)	61 (45)	
401-700 ml	209 (30)	181 (32)	28 (21)	
701-1000 ml	125 (18)	105 (19)	20 (15)	
> 1000 ml	101 (14)	74 (13)	27 (20)	
Intraoperative transfusion, No. (%)	114 (16)	79 (14)	35 (26)	<0.01
Drain type, No. (%)				<0.01
No Drain	43 (6)	43 (8)	0	
Open	613 (87)	505 (89)	108 (79)	
Closed	46 (7)	18 (3)	28 (21)	
a-FRS zone, No. (%)				0.04
0-5%	236 (34)	172 (30)	38 (44)*	
>5%-20%	347 (49)	293 (52)	35 (41)*	
>20%	119 (17)	101 (18)	13 (15)*	
Postoperative				
POPF, No. (%)		112 (20)		
POPF grade, No. (%)				
BL		32 (6)		
B		94 (17)		
C		18 (3)		
POAP, No. (%)		87 (15)		
Fluid collection, No. (%)	246 (35)	206 (36)	40 (29)	0.1
Abscess, No. (%)	114 (16)	99 (17)	15 (11)	0.06
Biliary fistula, No. (%)	51 (7)	38 (7)	13 (10)	0.2
DJ/GJ fistula, No. (%)	20 (3)	16 (3)	4 (3)	0.9
Chyle leak, No. (%)	34 (5)	30 (5)	4 (3)	0.2
PPH, No. (%)	131 (19)	112 (20)	19 (14)	0.1
PPH grade, No. (%)				0.2
A	33 (5)	30 (5)	3 (2)	
B	73 (10)	63 (11)	10 (7)	
C	25 84)	19 (3)	6 (4)	
DGE, No. (%)	126 (18)	108 (19)	18 (13)	0.1
DGE grade, No. (%)				0.2
A	31 (4)	26 (6)	5 (4)	
B	68 (10)	61 (11)	7 (5)	
C	27 (4)	21 (4)	6 (4)	
Sepsis, No. (%)	111 (16)	98 (17)	13 (10)	0.02
Pleural effusion, No. (%)	110 (16)	63 (11)	47 (35)	<0.01
Postoperative pneumonia, No. (%)	125 (18)	95 (17)	30 (22)	0.1
Reintubation, No. (%)	51 (7)	39 (7)	12 (9)	0.4
Cardiac complication, No. (%)	46 (7)	30 (5)	16 (12)	<0.01
UTI, No. (%)	31 (4)	23 (4)	8 (6)	0.3
Acute Kidney Injury, No. (%)	32 (5)	19 (3)	13 (10)	<0.01
SSI, No. (%)	70 (10)	58 (10)	12 (9)	0.6
Percutaneous drainage, No. (%)	42 (6)	31 (5)	11 (8)	0.2
Enteral nutrition, No. (%)	211 (30)	181 (32)	30 (22)	0.02
TPN, No. (%)	210 (30)	172 (30)	38 (28)	0.5
Transfusions, No. (%)	175 (25)	135 (24)	40 (30)	0.1
Relaparotomy, No. (%)	60 (9)	51 (9)	9 (7)	0.3

ICU admission, No. (%)		91 (13)	69 (12)	22 (16)	0.2
Drain removal, median (range), days		4 (1-90)	5 (1-90)	3 (2-35)	<0.01
Discharged with drains, No. (%)		35 (5)	34 (6)	1 (1)	0.01
Postoperative MDR colonization, No. (%)		67 (10)	36 (6)	31 (23)	<0.01
LOS, median (range), days		10 (4-289)	9 (4-289)	10 (5-62)	0.7
Readmission, No. (%)		30 (4)	27 (5)	3 (2)	0.1
Clavien-Dindo, No (%)					<0.01
	0	290 (41)	232 (41)	58 (43)	
	1	42 (6)	38 (7)	4 (3)	
	2	237 (34)	189 (33)	48 (35)	
	3a	38 (5)	31 (5.5)	7 (5)	
	3b	7 (1)	2 (0.5)	5 (4)	
	4a	53 (8)	44 (8)	9 (6)	
	4b	13 (2)	13 (2)	0	
	5	22 (3)	17 (3)	5 (4)	
Clavien-Dindo ≥3, No (%)		133 (19)	107 (19)	26 (19)	0.9
Mortality, No. (%)		22 (3)	17 (3)	5 (4)	0.6

Abbreviations: BMI: Body Mass Index; COPD: Chronic Obstructive Pulmonary Disease; ASA: American Society of Anesthesiologists; PDAC: Pancreatic Ductal Adenocarcinoma; NET: Neuro-Endocrine Tumor; PJ: Pancreatico-jejunostomy; PG: Pancreaticogastrostomy; POPF: Postoperative pancreatic fistula; BL: Biochemical Leak; POAP: Postoperative Acute Pancreatitis; DJ: Duodenal-jejunal anastomosis; GJ: Gastro-jejunal anastomosis; PPH: Postpancreatectomy Hemorrhage; DGE: Delayed Gastric Emptying; UTI: Urinary Tract Infection; SSI: Surgical Site Infection; TPN: Total Parenteral Nutrition; ICU: Intensive Care Unit; MDR: Multidrug-Resistant bacteria; LOS: Length of Hospital Stay.

TP versus PD

The patients who underwent TP exhibited higher rates of pre-existing diabetes (37% vs 19%; p< 0.01) and neoadjuvant treatment (38% vs 26%; p< 0.01) compared to the patients who underwent PD.

Intraoperatively, the patients who underwent TP presented higher rates of vascular resection (37% vs 14%; p< 0.01), with higher estimated blood loss (20% vs 13% of patients with > 1000 mL; p< 0.01).

Postoperatively, there was no difference between the patients who underwent TP and those who underwent PD in terms of overall mortality (4% vs 3%; p= 0.6), LOS (10 vs 9 days; p= 0.7) or readmission rate (2% vs 5%; p= 0.1). The patients who underwent PD had a 20% rate of POPF. The patients who underwent TP showed higher rates of cardiac complications (12% vs 5%), acute kidney injury (10% vs 3%) and pleural effusion (35% vs 11%) (all p< 0.01) but lower rates of postoperative sepsis (10% vs 17%; p= 0.02) compared to the patients who underwent PD. The incidence of Clavien-Dindo ≥3 morbidity was comparable between the two groups (19% vs 19%; p= 0.9).

HR-PD versus C-TP

One hundred one patients underwent HR-PD (18% of all PD patients), and 86 patients underwent C-TP (63% of all TP patients). The characteristics of the two subgroups are shown in **Table 1 and 2**.

Table 1. Preoperative and Intraoperative Profile of Patients Who Underwent HR-PD (N= 101) and C-TP (N= 86)

		Pancreatectomy, No. (%)		
Characteristics	**Total, No. (%) (N= 187)**	**HR-PD= 101**	**C-TP= 86**	***P-value***
Preoperative				
Age, median (range), y	65 (16-84)	65 (16-82)	65 (42-84)	0.3
Female sex, No. (%)	83 (44)	43 (43)	40 (47)	0.6
BMI, median (range)	25 (17-41)	26 (18-39)	25 (17-41)	**0.01**
Smoker, No. (%)	34 (18)	19 (19)	15 (17)	0.8
Alcohol abuse, No. (%)	11 (6)	5 (5)	6 (7)	0.5
Diabetes, No. (%)	46 (25)	10 (10)	36 (42)	**<0.01**
Weight loss, No. (%)	83 (44)	39 (39)	44 (51)	0.08
Ischemic cardiac disease, No. (%)	10 (5)	5 (5)	5 (6)	0.7
Hypertension, No. (%)	83 (44)	39 (39)	44 (51)	0.08
COPD, No. (%)	5 (3)	2 (2)	3 (4)	0.5
Chronic renal failure, No. (%)	5 (3)	1 (1)	4 (5)	0.1
ASA score, No. (%)				0.5
1-2	147 (79)	81 (80)	66 (77)	
3-4	40 (21)	20 (20)	20 (23)	
Jaundice palliation, No. (%)	73 (39)	34 (34)	39 (45)	0.1
Preoperative MDR colonization, No. (%)	27 (14)	9 (9)	18 (21)	**0.02**
Neoadjuvant therapy, No. (%)	52 (28)	16 (16)	36 (42)	**<0.01**
Presumed diagnosis				**<0.01**
PDAC	103 (55)	41 (41)	62 (72)	
Duodenal/ampullary/cystic/NET	84 (45)	60 (60)	24 (28)	
Intraoperative				
Surgery type, No. (%)				**<0.01**
Pylorus preserving	159 (85)	99 (98)	60 (70)	
Whipple	28 (15)	2 (2)	26 (30)	
Vascular resection, No. (%)	38 (20)	5 (5)	33 (38)	**<0.01**
Reason for completion TP, No (%)				
Positive pancreatic margin			42 (49)	
Technical issues			23 (27)	
Vascular resection/reconstruction			12 (14)	
Other			9 (10)	
Pancreatic anastomosis, No. (%)				
PJ		69 (68)		
PG		32 (32)		
Externalized pancreatic stent, No. (%)		93 (92)		
Blood loss, No. (%)				0.2
≤400 ml	60 (32)	29 (29)	31 (36)	
401-700 ml	54 (29)	35 (34)	19 (22)	
701-1000 ml	32 (17)	17 (17)	15 (17)	
> 1000 ml	41 (22)	20 (20)	21 (24)	
Intraoperative transfusion, No. (%)	49 (26)	19 (19)	30 (35)	**0.01**
Drain type, No. (%)				**<0.01**
No Drain	0	0	0	
Open	166 (89)	100 (99)	66 (77)	
Closed	21 (11)	1 (1)	20 (23)	

Abbreviations: BMI: Body Mass Index; COPD: Chronic Obstructive Pulmonary Disease; ASA: American Society of Anesthesiologists; PDAC: Pancreatic Ductal Adenocarcinoma; NET: Neuro-Endocrine Tumor; PJ: Pancreatico-jejunostomy; PG: Pancreaticogastrostomy.

Table 2. Postoperative Profile of Patients Who Underwent HR-PD (N= 101) and C-TP (N= 86)

Characteristics	Total, No. (%) (N= 187)	Pancreatectomy, No. (%)		*P-value*
		HR-PD= 101	C-TP= 86	
Postoperative				
POPF, No. (%)		39 (39)		
POPF grade, No. (%)				
BL		12 (12)		
B		34 (34)		
C		5 (5)		
POAP, No. (%)		39 (39)		
Fluid collection, No. (%)	91 (49)	61 (60)	30 (35)	**<0.01**
Abscess, No. (%)	39 (21)	28 (28)	11 (13)	**0.01**
Biliary fistula, No. (%)	16 (9)	7 (7)	9 (10)	0.3
DJ/GJ fistula, No. (%)	7 (4)	3 (3)	4 (5)	0.4
Chyle leak, No. (%)	14 (7)	12 (12)	2 (2)	**0.01**
PPH, No. (%)	41 (22)	28 (28)	13 (15)	**0.03**
PPH grade, No. (%)				0.08
A	9 (5)	6 (6)	3 (3)	
B	25 (13)	19 (19)	6 (7)	
C	7 (4)	3 (3)	4 (5)	
DGE, No. (%)	48 (26)	34 (34)	14 (16)	**<0.01**
DGE grade, No. (%)				**0.05**
A	12 (6)	8 (8)	4 (5)	
B	24 (13)	18 (18)	6 (7)	
C	12 (6)	8 (8)	4 (5)	
Sepsis, No. (%)	40 (21)	31 (31)	9 (10)	**<0.01**
Pleural effusion, No. (%)	61 (33)	31 (31)	30 (35)	0.5
Postoperative pneumonia, No. (%)	41 (22)	21 (21)	20 (23)	0.6
Reintubation, No. (%)	24 (13)	16 (16)	8 (9)	0.1
Cardiac complication, No. (%)	24 (13)	13 (13)	11 (13)	0.9
UTI, No. (%)	15 (8)	9 (9)	6 (7)	0.6
Acute Kidney Injury, No. (%)	18 (10)	11 (11)	7 (8)	0.5
SSI, No. (%)	31 (17)	23 (23)	8 (9)	**0.01**
Percutaneous drainage, No. (%)	13 (7)	6 (6)	7 (8)	0.5
Enteral nutrition, No. (%)	91 (49)	72 (71)	19 (22)	**<0.01**
TPN, No. (%)	78 (41)	53 (52)	25 (29)	**<0.01**
Transfusions, No. (%)	66 (35)	38 (38)	28 (33)	0.4
Relaparotomy, No. (%)	20 (11)	14 (14)	6 (7)	0.1
ICU admission, No. (%)	33 (18)	22 (22)	11 (13)	0.1
Drain removal, median (range), days	5 (2-75)	7 (3-75)	3 (2-35)	**<0.01**
Discharged with drains, No. (%)	13 (7)	12 (12)	1 (1)	**<0.01**
Postoperative MDR colonization, No. (%)	32 (17)	10 (10)	22 (26)	**<0.01**
LOS, median (range), days	16 (5-289)	21 (6-289)	10 (5-62)	**<0.01**
Readmission, No. (%)	6 (3)	4 (4)	2 (2)	0.5
Clavien-Dindo, No (%)				**<0.01**
0	52 (28)	16 (16)	36 (42)	
1	5 (3)	3 (3)	2 (2)	
2	83 (44)	51 (50)	32 (37)	
3a	12 (6)	7 (7)	5 (6)	
3b	5 (3)	1 (1)	4 (5)	
4a	18 (10)	14 (14)	4 (5)	
4b	5 (3)	5 (5)	0	
5	7 (4)	4 (4)	3 (3)	
Clavien-Dindo ≥3, No (%)	47 (25)	31 (31)	16 (19)	**0.05**
Mortality, No. (%)	7 (4)	4 (4)	3 (3)	0.6

Abbreviations: POPF: Postoperative pancreatic fistula; BL: Biochemical Leak; POAP: Postoperative Acute Pancreatitis; DJ: Duodenal-jejunal anastomosis; GJ: Gastro-jejunal anastomosis; PPH: Postpancreatectomy Hemorrhage; DGE: Delayed Gastric Emptying; UTI: Urinary Tract Infection; SSI: Surgical Site Infection; TPN: Total Parenteral Nutrition; ICU: Intensive Care Unit; MDR: Multidrug-Resistant bacteria; LOS: Length of Hospital Stay.

Preoperatively, the patients who underwent C-TP exhibited lower BMI (25 vs 26; p< 0.01), higher rates of pre-existing diabetes (42% vs 10%; p< 0.01) and more frequent multidrug-resistant bacterial colonization (21% vs 9%; p< 0.01)(**Table 1**). The presumed diagnosis of the C-TP patients was more frequently PDAC (72% vs 41%; p< 0.01). Consequently, the C-TP patients also underwent neoadjuvant therapy more frequently than the HR-PD patients (42% vs 16%; p< 0.01). The decision to perform C-TP was made intraoperatively due to the pancreatic margin being positive for malignancy (49%), technical issues (27%; including highly friable pancreatic parenchyma and microscopic/undetectable or posterior main pancreatic duct), the need for vascular resection/reconstruction (14%) or for other reasons (10%; including intraoperative pancreatitis, bleeding and iatrogenic splenic laceration).

Intraoperatively, the patients who underwent C-TP presented lower rates of pylorus preservation (70% vs 98%; p< 0.01), higher rates of vascular resection (38% vs 5%; p< 0.01) and more frequent needs for intraoperative transfusion (35% vs 19%; p= 0.01) compared to the patients who underwent HR-PD (**Table 1**).

Postoperatively, the patients in the C- TP group exhibited lower rates of postoperative fluid collection (35% vs 60%; p< 0.01), intra-abdominal abscess (13% vs 28%; p= 0.01), chyle leak (2% vs 12%; p= 0.01), PPH (15% vs 28%; p= 0.03), DGE (16% vs 34%; p< 0.01), sepsis (10% vs 31%; p< 0.01) and SSI (9% vs 23%; p= 0.01) compared to the patients in the HR-PD group (**Table 2**). The rates of POPF and POAP in the HR-PD group were both 39%. The patients who underwent C-TP had lower rates of Clavien-Dindo ≥3 morbidity (19% vs 31%; p= 0.05) and shorter median LOS (10 vs 21 days; p < 0.01) compared to the patients who underwent HR-PD. Mortality was comparable between the two groups (3% vs 4%; p= 0.6).

Quality of Life and Pancreatic Insufficiency

Of the 187 patients who underwent either HR-PD or C-TP, 61 (33%) died and 31 (17%) were lost to FU. The remaining 95 patients (62 in the HR-PD group, 33 in the C-TP group) were included in the QoL analysis, with a median FU of 30 months and a response rate of 100%. The results of the QoL and endocrine/exocrine insufficiency questionnaires are shown in **Table 3**.

Table 3. Quality of Life and Exocrine and Endocrine Insufficiency after HR-PD (N= 62) and C-TP (N= 33)

Characteristics	Total, Mean (SD) (N= 95)	Pancreatectomy, Mean (SD)		Clinically Relevant Difference (Δ score)	*P-value*
		HR-PD= 62	C-TP= 33		
EQ-5D					
EQ-5D-3L score	0.76 (0.21)	0.73 (0.22)	0.81 (0.19)		0.1
EORTC QLQ-C30					
Global Health Status	78.15 (17.91)	77.55 (19.24)	79.29 (15.32)	No change (-1.74)	0.8
Functional scales					
Physical Functioning	86.24 (17.25)	85.37 (19.57)	87.87 (11.83)	No change (-2.50)	0.9
Role Functioning	85.26 (26.30)	84.67 (24.94)	86.36 (20.17)	No change (-1.79)	0.9
Emotional Functioning	80.87 (20.83)	80.24 (21.61)	82.07 (19.55)	No change (-1.83)	0.8
Cognitive Functioning	87.71 (17.56)	86.29 (19.67)	90.40 (12.52)	No change (-4.11)	0.6
Social Functioning	86.31 (22.93)	85.48 (25.33)	87.87 (17.81)	No change (-2.39)	0.8
Symptom scales/items					
Fatigue	17.77 (22.00)	18.63 (23.44)	16.16 (19.26)	No change (+2.47)	0.8
Nausea and Vomiting	4.38 (10.92)	4.30 (9.02)	4.54 (13.98)	No change (-0.24)	0.3
Pain	9.29 (17.8)	8.06 (17.00)	11.61 (19.31)	No change (-3.55)	0.3
Dyspnea	5.61 (15.86)	6.45 (16.89)	4.04 (13.83)	No change (-2.41)	0.4
Insomnia	24.91 (29.56)	22.04 (29.53)	30.30 (29.30)	Little change (-8.26)	0.1
Appetite Loss	7.36 (17.65)	8.60 (19.95)	5.05 (12.13)	No change (+3.55)	0.6
Constipation	7.71 (18.48)	9.13 (21.05)	5.05 (12.13)	No change (+4.08)	0.5
Diarrhea	22.10 (27.33)	24.19 (27.77)	18.18 (26.47)	Little change (+6.01)	0.2
Financial Difficulties	10.17 (21.78)	10.21 (22.24)	10.10 (21.22)	No change (+0.11)	0.9
EORTC QLQ-PAN26					
Functional scales					
Satisfaction with healthcare	94.03 (17.68)	94.08 (17.36)	93.93 (18.94)	No change (-1.58)	0.8
Symptom scales/items					
Pancreatic pain	15.78 (17.25)	15.86 (18.40)	15.65 (15.13)	No change (+0.21)	0.6
Digestive symptoms	21.75 (22.29)	21.23 (22.81)	22.72 (21.57)	No change (-1.49)	0.6
Altered bowel habit	25.43 (26.61)	26.34 (26.91)	23.73 (26.36)	No change (+2.61)	0.6
Hepatic function	6.49 (13.59)	6.98 (14.00)	5.55 (12.95)	No change (+1.43)	0.6
Body image	17.89 (26.76)	18.01 (27.54)	17.67 (25.66)	No change (+0.35)	0.8
Sexuality	25.08 (26.28)	29.03 (27.14)	17.67 (23.17)	Moderate change (+11.36)	**0.045**
Ascites	34.38 (29.35)	37.63 (27.97)	28.28 (31.31)	Little change (+9.35)	0.09
Taste change	17.54 (24.23)	17.20 (24.69)	18.18 (23.70)	No change (-0.98)	0.7
Indigestion	8.77 (21.85)	7.52 (20.39)	11.11 (24.53)	No change (-3.59)	0.4
Flatulence	14.38 (24.14)	13.97 (24.55)	15.15 (23.70)	No change (-1.18)	0.7
Worry about low weight	21.75 (25.15)	19.89 (24.48)	25.25 (26.39)	Little change (-5.36)	0.3
Weakness arms/legs	21.75 (27.40)	22.58 (27.51)	20.20 (27.56)	No change (+2.38)	0.6
Dry mouth	8.07 (22.12)	6.98 (21.02)	10.10 (24.27)	No change (-3.12)	0.3
Treatment side effects	15.48 (25.17)	17.20 (26.81)	12.12 (21.75)	Little change (+5.08)	0.3
Worry about future health	40.70 (31.20)	40.86 (31.17)	40.40 (29.76)	No change (+0.46)	0.9
Limited activity planning	12.28 (24.81)	11.29 (22.53)	14.14 (28.90)	No change (-2.85)	0.9
Exocrine insufficiency					
Weight loss, No. (%)	77 (81)	49 (79)	28 (85)		0.4
Weight loss (kg), median (SD)	8 (7)	7 (7)	8 (5)		0.7
Pancreatic enzymes supplementation, No. (%)	72 (76)	39 (63)	33 (100)		**<0.01**
N. of capsules, median (SD)	10 (7)	6 (5)	13 (7)		**<0.01**
Endocrine insufficiency					
Diabetes, No. (%)	41 (43)	8 (13)	33 (100)		**<0.01**
Diabetes therapy, No. (%)					**<0.01**
Diet and exercise	0	0	0		
Oral antidiabetic agents	2 (5)	2 (3)	0		
Insulin	37 (90)	4 (6)	33 (100)		
Insulin + oral antidiabetic agents	2 (5)	2 (3)	0		
Total PAID score, mean (SD)	10.60 (11.89)	5.98 (12.52)	13.25 (10.83)		**<0.01**

According to the EQ-5D-3L, EORTC QLQ-C30 and EORTC QLQ-PAN26 questionnaires, QoL was comparable between the two groups (**Figure 2**).

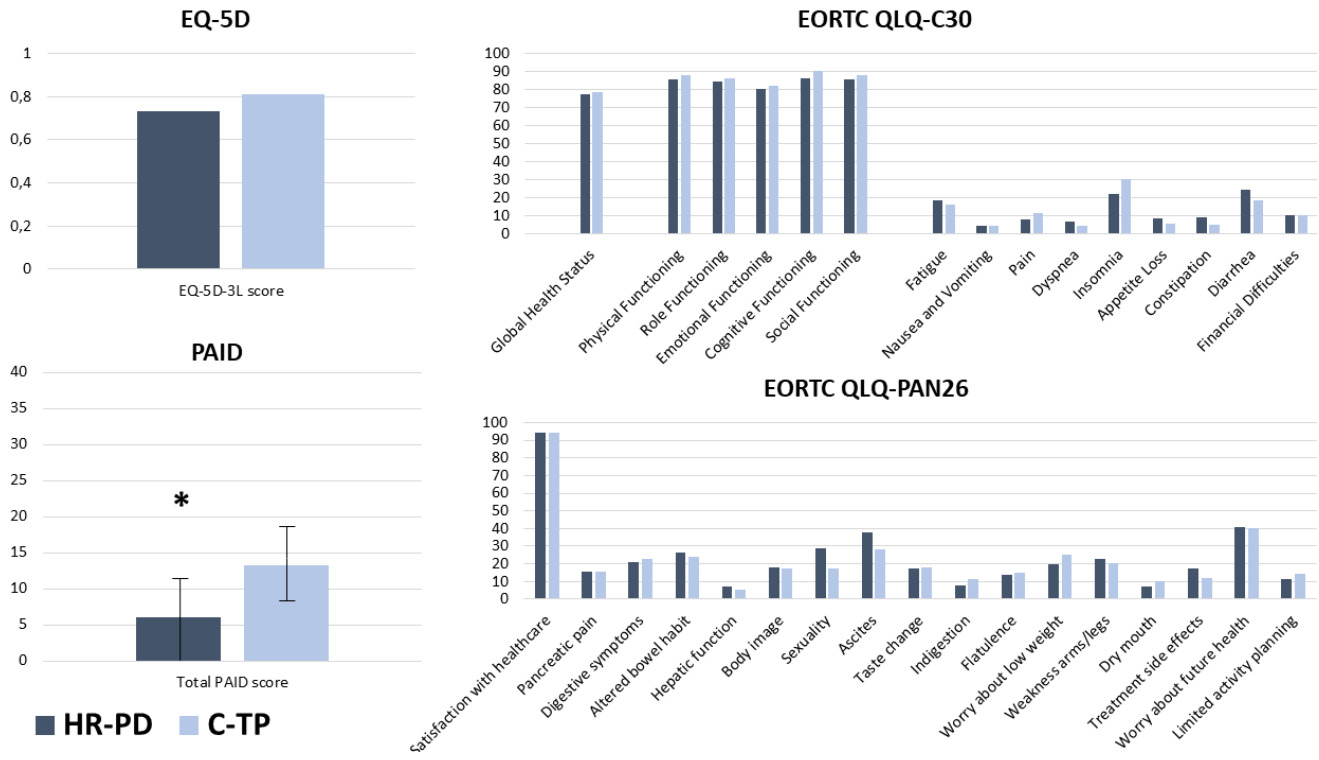


Figure 2. Analysis of QoL after HR-PD or C-TP

The EQ-5D-3L scores were 0.81 (SD 0.19) and 0.73 (SD 0.22) for the C-TP and HR-PD groups, respectively (p= 0.1). The EORTC QLQ-C30 questionnaire revealed GHS scores of 79.2 (SD 15.3) vs 77.5 (SD 19.2), respectively (p= 0.8). In general, patients primarily complained of fatigue, diarrhea and insomnia. The EORTC QLQ-PAN26 questionnaire showed digestive symptoms, altered bowel habits, abdominal tenderness (ascites), arm/leg weakness, low weight and worries about future health to be the most relevant clinical manifestations after HR-PD and C-TP, and no substantial differences were observed between the two groups; only symptoms related to sexuality were more common after HR-PD (p= 0.045; moderate change).

All the patients who underwent C-TP required pancreatic enzyme supplementation vs. 63% in the HR-PD group (p< 0.01), with a higher number of capsules needed per day (13 vs 6; p< 0.01). All the patients in the C-TP group exhibited postoperative diabetes (observed in 13% of patients in the HR-PD group (p< 0.01)),

which was always associated with insulin dependency (observed in 9% of patients in the HR-PD group (p< 0.01)) (**Figure 3**). Finally, according to the PAID questionnaire, the psychological burden of diabetes was heavier in patients who underwent C-TP (total PAID score: 13.2 vs 6; p<0.01) (**Figure 2**).

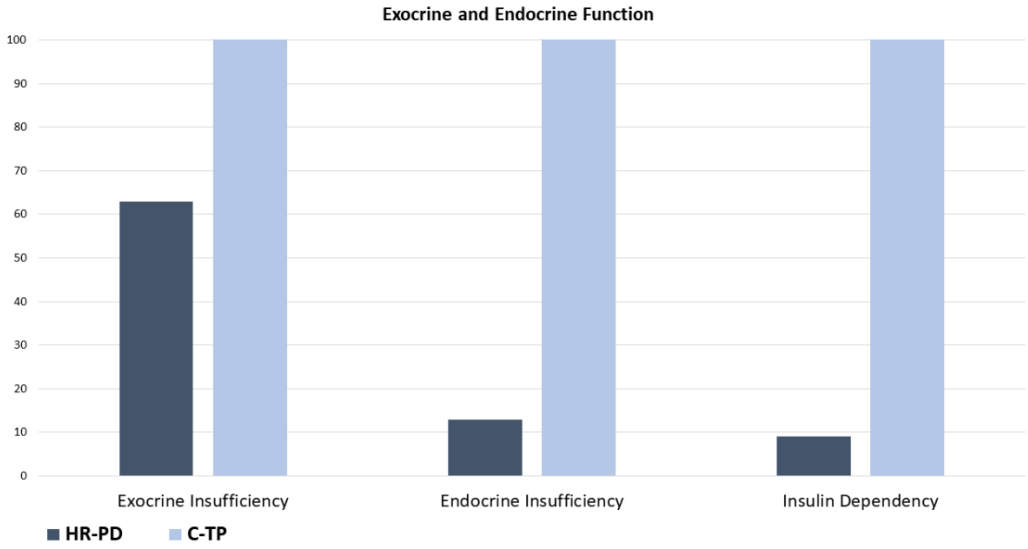


Figure 3. Exocrine and Endocrine Function after HR-PD or C-TP

A sub-analysis comparing QoL and pancreatic insufficiency in patients who underwent C-TP and in a subset of patients who developed POPF after HR-PD (n= 39; with 12 patients dead or lost to FU) showed similar results (**Supplementary Table 3**).

Supplementary Table 3. Quality of Life, Exocrine and Endocrine Insufficiency after High-risk PD who developed POPF (N= 27) and TP after planned PD (N= 33)

Characteristics	Total, Mean (SD) (N= 60)	Pancreatectomy, Mean (SD)		Clinically Relevant Difference (Δ score)	*P*
		HR PD + POPF= 27	TP= 33		
EQ-5D					
VAS score	0.76 (0.22)	0.71 (0.25)	0.81 (0.19)		0.1
EORTC QLQ-C30					
Global Health Status	76.66 (19.87)	73.45 (24.24)	79.29 (15.32)	Little change (5.84)	0.4
Functional scales					
Physical Functioning	86.44 (16.82)	84.69 (21.54)	87.87 (11.83)	No change (3.18)	0.8
Role Functioning	83.05 (24.04)	79.01 (27.96)	86.36 (20.17)	Little change (7.35)	0.3
Emotional Functioning	82.91 (20.02)	83.95 (20.91)	82.07 (19.55)	No change (1.88)	0.4
Cognitive Functioning	86.94 (17.65)	82.71 (21.91)	90.40 (12.52)	Little change (7.69)	0.3
Social Functioning	87.22 (19.49)	86.41 (21.69)	87.87 (17.81)	No change (1.46)	0.9
Symptom scales/items					
Fatigue	18.88 (22.89)	22.22 (26.68)	16.16 (19.26)	Little change (6.06)	0.5
Nausea and Vomiting	3.61 (11.10)	2.46 (6.03)	4.54 (13.98)	No change (2.08)	0.8
Pain	10 (20.40)	8.02 (21.86)	11.61 (19.31)	No change (3.59)	0.1
Dyspnea	5.55 (15.24)	7.40 (16.87)	4.04 (13.83)	No change (3.36)	0.3
Insomnia	25.55 (29.66)	19.75 (29.61)	30.30 (29.30)	Moderate change (10.55)	0.1
Appetite Loss	6.66 (16.00)	8.64 (19.81)	5.05 (12.13)	No change (3.59)	0.6
Constipation	6.11 (16.79)	7.40 (21.35)	5.05 (12.13)	No change (2.35)	0.9
Diarrhea	18.88 (24.82)	19.75 (23.12)	18.18 (26.47)	No change (1.57)	0.6
Financial Difficulties	11.66 (23.63)	13.58 (26.56)	10.10 (21.22)	No change (3.48)	0.6
EORTC QLQ-PAN26					
Functional scales					
Satisfaction with healthcare	95.27 (15.37)	96.91 (10.37)	93.93 (18.94)	No change (2.98)	0.4
Symptom scales/items					
Pancreatic pain	15.97 (18.55)	16.35 (22.34)	15.65 (15.13)	No change (0.70)	0.4
Digestive symptoms	23.05 (22.35)	23.45 (23.68)	22.72 (21.57)	No change (0.73)	0.9
Altered bowel habit	24.44 (27.69)	25.30 (29.73)	23.73 (26.36)	No change (1.57)	0.9
Hepatic function	6.94 (14.80)	8.64 (16.90)	5.55 (12.95)	No change (3.09)	0.5
Body image	15.55 (25.09)	12.96 (24.60)	17.67 (25.66)	No change (4.71)	0.3
Sexuality	22.22 (26.33)	27.77 (29.23)	17.67 (23.17)	Moderate change (10.10)	0.1
Ascites	30.55 (31.46)	33.33 (32.02)	28.28 (31.31)	Little change (5.05)	0.5
Taste change	18.88 (26.30)	19.75 (29.61)	18.18 (23.70)	No change (1.57)	0.8
Indigestion	10.00 (25.52)	8.64 (27.09)	11.11 (24.53)	No change (2.47)	0.3
Flatulence	14.44 (24.05)	13.58 (24.90)	15.15 (23.70)	No change (1.57)	0.7
Worry about low weight	23.33 (26.25)	20.98 (26.38)	25.25 (26.39)	No change (4.27)	0.4
Weakness arms/legs	22.77 (27.78)	25.92 (28.24)	20.20 (27.56)	Little change (5.72)	0.3
Dry mouth	8.88 (23.66)	7.40 (23.26)	10.10 (24.27)	No change (2.70)	0.4
Treatment side effects	13.88 (22.37)	16.04 (23.33)	12.12 (21.75)	No change (3.92)	0.4
Worry about future health	40.55 (31.94)	40.74 (35.00)	40.40 (29.76)	No change (0.34)	0.9
Limited activity planning	12.77 (26.10)	11.11 (22.64)	14.14 (28.90)	No change (3.03)	0.9
Endocrine insufficiency					
Weight loss, No.(%)	48 (80)	20 (74)	28 (85)		0.3
Weight loss (Kg), median (SD)	7.5 (6)	7 (7)	8 (5)		0.2
Pancreatic enzymes supplementation, No. (%)	50 (83)	17 (63)	33 (100)		**<0.01**
N. of capsules, median (SD)	11 (7)	5 (3)	13 (7)		**<0.01**
Exocrine insufficiency					
Diabetes, No. (%)	36 (60)	3 (11)	33 (100)		**<0.01**
Diabetes therapy, No. (%)					**<0.01**
Diet and exercise	0	0	0		
Oral hypoglicemic drugs	0	0	0		
Insuline	35 (97)	2 (7)	33 (100)		
Insuline + oral hypoglicemic drugs	1 (3)	1 (4)	0		
Total PAID, mean (SD)	10.98 (10.85)	2.63 (5.81)	13.25 (10.83)		**<0.01**

DISCUSSION

The postoperative morbidity of total pancreatectomy was lower compared to that of high-risk pancreaticoduodenectomy, while the mortality was similar. However, although cancer- and pancreas-specific QoL seemed to be comparable, exocrine insufficiency after HR-PD was less severe and endocrine insufficiency only occurred in a minority of patients, with improved diabetes-specific QoL. These findings suggest a possible role for C-TP after PD only in selected cases among patients at high-risk for POPF, after adequate counseling.

While the outcomes of PD have been widely standardized[95], TP remains defined by outdated series associated with increased postoperative burden. Recent studies have shown improved postoperative outcomes of TP at high-volume centers. Stoop et al. found an overall major postoperative morbidity of 34.5%, which decreased to 23.2% in the most recent years, similar to another bicentric study including patients from 2000 to 2014.[83] [86] A multicenter snapshot study, including TP performed at both high- and low- volume centers between 2018 and 2019, showed a major morbidity of 25% and an in-hospital mortality of 5%.[96] In the present study, major morbidity rates of patients undergoing TP were confirmed to be approximately 20%, which were similar to those of patients undergoing PD. Patients who underwent TP experienced higher rates of nonsurgical complications (cardiac complications, acute kidney injury and pleural effusion) compared to PD but reduced rates of sepsis and abdominal abscess. These results suggest that TP can be performed with an acceptable overall postoperative morbidity. POPF is the main cause of morbidity after PD, affecting around 1/3 of patients, despite available mitigation strategies and contributing to other associated major surgical complications and mortality.[20] [35] We hypothesized that in patients at high risk zone for POPF – especially with specific conditions of the pancreatic remnant, such as a small/eccentric pancreatic duct or a very soft pancreas – C-TP may represent a potential rescue strategy to avoid pancreatic anastomosis. To the best of our knowledge, this is the first study to directly compare the postoperative outcomes of C-TP to those of PD in high-risk patients. In the HR-PD subset, POPF and severe complication rates were 39% and 31%, respectively, similar to what is described in the current literature.[20] [35] Patients who underwent C-TP exhibited strikingly better postoperative outcomes. In the HR-PD group, the rates of abdominal fluid collection, PPH,

DGE, and LOS were nearly doubled, and the rate of sepsis was three times higher than those in the C-TP group. These findings must be interpreted while taking in account that currently recognized mitigation strategies, including intraoperative positioning of externalized pancreatic stent and jejunostomy, were extensively applied in the HR-PD group in this series. Despite acceptable postoperative outcomes, performing TP still raises important concerns due to the inevitable presence of its long-term sequelae, which are related to the complete absence of residual pancreatic function. The management of endocrine insufficiency after TP has improved over the past decades, leading to diabetes-specific outcomes that seem equivalent to other types of insulin-dependent diabetes.[97] However, QoL seems to be heavily impaired after TP and particularly affected by endocrine and exocrine insufficiency.[83] [84] [85] Conversely, there is no information regarding QoL and pancreatic insufficiency incidence or severity after PD in high-risk patients, in whom the soft pancreas and the nondilated pancreatic duct may imply a higher integrity of residual pancreatic function; on the other hand, the higher incidence of major morbidity may negatively affect QoL.[98] Of note, the results of nonspecific, cancer-specific and pancreas-specific QoL questionnaires were all similar between the C-TP and HR-PD patients in this study, and the EORTC QLQ-30 global health status score after TP was higher than that reported in recent literature.[84] [86] Diabetes was present in only 13% of the patients who underwent HR-PD, a percentage slightly inferior to that reported for patients undergoing PD, while exocrine insufficiency was present in more than half of HR-PD patients.[99] [100] [101] The psychosocial impact of diabetes, the need for insulin therapy and the severity of exocrine insufficiency were all significantly higher after C-TP, confirming a heavier burden related to sequelae of pancreatic insufficiency in these patients.

The present study has some limitations that warrant emphasis. First, it was not possible to fully evaluate and compare the long-term systemic effects of diabetes in both cohorts due to the short median follow-up time. Moreover, we decided to consider C-TP, excluding primary TP, to simulate a possible intraoperative scenario in which, after the demolitive phase, the surgeon has to choose whether to proceed with a high-risk pancreatic anastomosis or to complete a TP. However, pancreatico-jejunostomy with duct-to-mucosa and exteriorized pancreatic stent is the predominant practice for difficult/high-risk pancreas at our Institution, especially after the results of a recent randomized controlled trial.[20] For this reason, the main indication for

C-TP in our series was the need for radical oncological resection (63% of cases) rather than a technically challenging anastomosis (27%), possibly in patients who were already diabetic (42% of patients). This obviously suggests selection bias, as conversely, the indication for surgery in patients who underwent HR-PD was only in a minority of pancreatic cancer patients (41% vs 72%; p< 0.01). This differences in surgical indications and complexity of C-TP and HR-PD led to a disproportionate rate of pre- and intra- operative factors (higher rates of diabetes, pancreatic cancer, neoadjuvant treatment, vascular resections, blood loss, intraoperative transfusions) that may have negatively affected postoperative outcomes of C-TP. Despite this bias, postoperative outcomes were still significantly better after C-TP, highlighting the magnitude and severity of surgical morbidity related to HR-PD. Finally, the a-FRS stratification was heterogeneous in the two groups. The patients who underwent HR-PD were all in the high-risk group; conversely, in the C-TP group, all risks groups were represented. This limitation may be secondary in a retrospective study, as the pancreatic gland is completely removed during TP, and therefore, the fistula risk is purely theoretical and has no practical value. We acknowledge that a randomized controlled trial would be a more appropriate scenario to test our hypothesis. However, a word of caution must be given, as TP-related sequelae would make such a trial debatable by an ethics committee without a preliminary study exploring surgical outcomes of HR-PD and C-TP in a retrospective fashion. Hopefully the present study will lead the way for future prospective data.

Our results shed new light on an 'old' question, specifically whether C-TP can be considered a fallback option in patients at high risk of POPF after PD. While we do not advocate for C-TP as an alternative to PD in all clinical high-risk scenarios, it may be considered in highly selected patients for whom the short-term benefits in the postoperative setting may overcome the disadvantages due to long-term sequelae of pancreatic insufficiency. For example, C-TP may be considered in cancer patients with high-risk pancreases, for whom access to adjuvant chemotherapy, which is often delayed by the occurrence of POPF, is crucial.

In conclusion, C-TP is associated with improved postoperative outcomes compared to HR-PD. However, pancreatic insufficiency affects patients undergoing C-TP more severely, with impaired diabetes-related psychosocial functioning. The delicate ethical implications of such decision should always entail adequate preoperative counselling.

CHAPTER V – MITIGATION

Dynamic, risk-based reappraisal of drain fluid amylase after pancreatoduodenectomy with or without anastomotic stent: a prospective observational protocol for a personalized drain management.

INTRODUCTION

Early drain removal is a fundamental step of the enhanced recovery after surgery (ERAS) pathway in patients undergoing pancreatoduodenectomy (PD).[102] Its implementation in the post-operative care is associated with improved outcomes in terms of pancreatic fistula (POPF) and other pancreas specific morbidity development, intra-abdominal infections, and length of hospital stay. [103] [104] [105] The timely identification of patients who are at the lowest risk of developing a POPF is essential for the safe application of early drain removal policies.[106] Several protocols have been proposed to guide the decision making. Most of them are based on the assessment of drain fluid amylase (DFA) during the early post-operative days (PODs).[107] [108] [109] [110] [111] Each protocol is based on definite DFA thresholds, usually associated to a high negative predictive value (NPV). Unfortunately, a protocol based on a single DFA threshold is not suitable for all clinical scenarios. A "one-size-fits-all" approach is indeed inadequate, given the significant effect of patient-specific risk factors and the dynamic nature of POPF prediction and diagnosis during the early postoperative course.[109] [110] [112] [113] [114] A risk-based strategy for POPF prediction and mitigation is in fact the current standard for PD. Moreover, other variables besides DFA are commonly in use, to guide drain removal but also to predict POPF severity and indicate its appropriate management.[115] [116] [70]

Trans-anastomotic stents (internal or external) are considered optimal mitigation strategies for patients in the high-risk categories, lowering POPF severity and complication burden.[117] [118] [119] [120] [121] However, such mitigation strategies may also alter the value and the meaning of usual post-operative POPF predictors, in addition to patient-factors. Intuitively, the presence of a stent may have relevant implications on DFA predictive ability, reducing their diagnostic performance. When an externalized trans-anastomotic stent (ETS) is placed, causing the almost complete diversion of pancreatic juice outside the abdominal cavity, low DFA levels may not exclude the eventual occurrence of POPF. [114] In case of high-risk PD with ETS, early drain removal based solely on DFA could be detrimental, but to date no other reliable predictors are available to guide the decision making in this critical subset of patients. Other factors, like post-operative

hyperamylasemia (POH), C-reactive protein (C-RP) or the volume of pancreatic juice production, may help to stratify these patients for early drain removal.

The aim of this prospective study was to dynamically monitor DFA values and other possible clinical predictors during the entire early post-operative period, correlating them with the onset of POPF and establishing a new risk-based protocol for postoperative drain management in patient undergoing PD with or without ETS.

METHODS

Study design

All patients undergoing pancreaticoduodenectomy (PD) at the General and Pancreatic Surgery Department of the University of Verona Hospital Trust from January 1st, 2021, to December 31st, 2022, were considered eligible and enrolled in this prospective observational protocol (DAV-PR). The study was designed to last 27 months, 24 for patients' recruitment and 3 for follow-up for the last patient included. The follow-up referred to the 90-days post-operative recovery time. Approval for data collection and analysis in this study was obtained by the local ethics committee (CESC2566) and performed according to the recommendations of the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE).

Procedures

Pancreatoduodenectomy was performed according to institutional standards.[118] During surgery, presumed preoperative pathology, estimated blood loss (EBL), intraoperative pancreatic stump texture and main pancreatic duct (MPD) size were combined to assess the fistula risk score (FRS).[122] All patients underwent double-layered duct-to-mucosa pancreatico-jejunostomy (PJ) or pancreatico-gastrostomy (PG) (in few selected cases with high POPF risk). An ETS (PankreaPlus™ polyvinyl catheter) was employed as a specific mitigation strategy in all patients at high risk for POPF and in selected cases when the risk was estimated as moderate (i.e., presence of a MPD ≤3mm) (**Figure 1**), with a previously described surgical technique[120]. According to institutional policies, both selective drain placement and early removal policy were followed.[104]

[123] [114] Two open, passive drains were placed in the proximity of the pancreatic and biliary anastomoses in all patients in the high or intermediate risk zone. In case of negligible to low risk of POPF (FRS 0 – 2), surgical drains were omitted in selected patients, which were excluded from the following analysis. Early drain removal was defined as a removal on POD 3. In case of drain placement, early removal was promoted at the surgeon's discretion based on the POD1 DFA value.

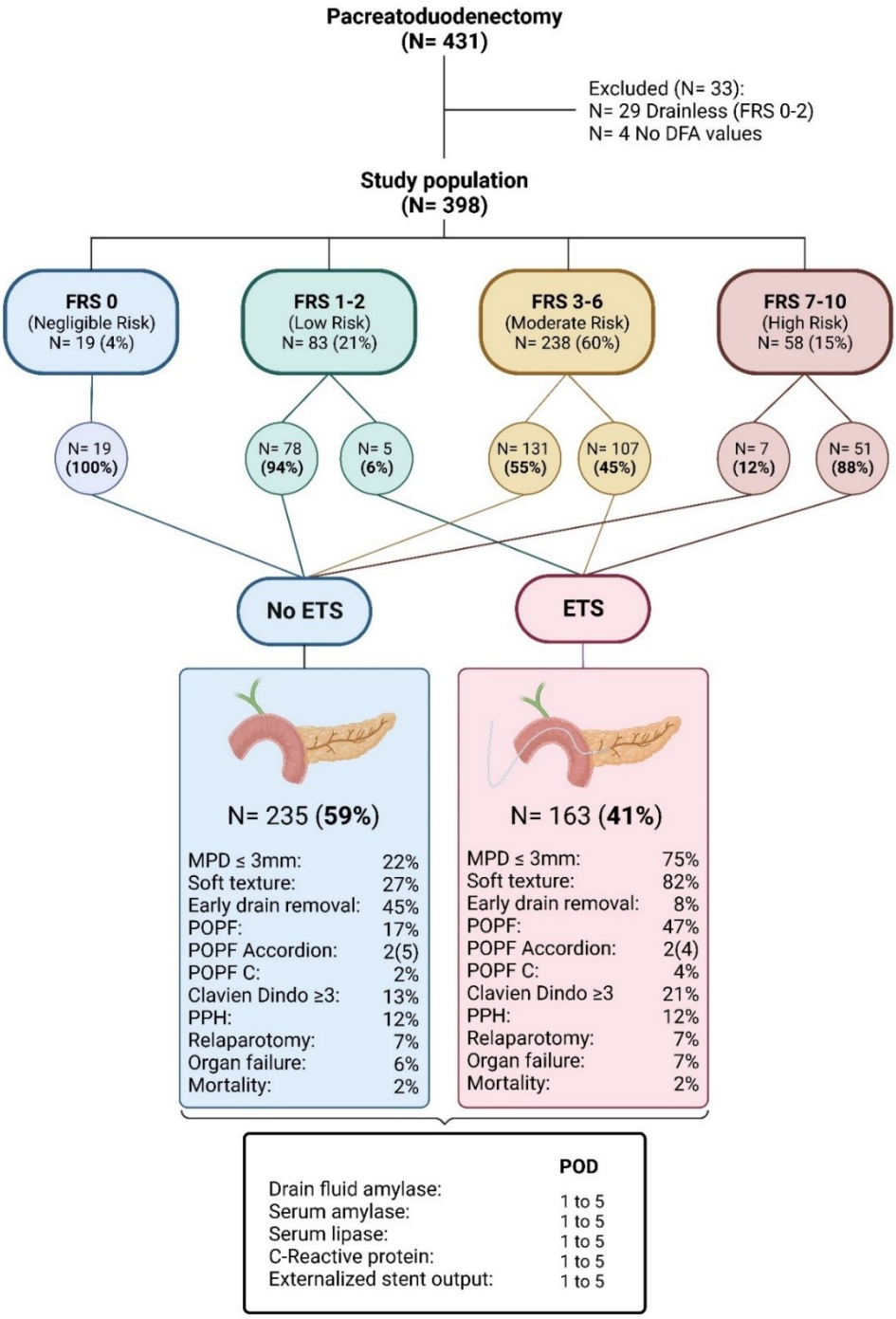


Figure 1. Study flow-chart. Note: POPF Accordion expressed as median (range).

Postoperative POPF predictors

Prospective collection of data was maintained during the entire duration of the study and included for all patient the daily assessment of DFA values from POD 1 to POD 5 and serum amylase (SA), serum lipase (SL), and C-RP assessed from POD 0 to POD 5. In patients where an ETS was positioned, the 24h output volume was recorded from POD 1 to POD 5. The study flowchart is displayed in **Figure 1**.

Outcomes

The primary outcome was the incidence of POPF defined according to the International Study Group for Pancreatic Surgery (ISGPS).[47] Secondary outcomes were POPF severity (grade B or C according to ISGPS), ETS malfunction defined as occlusion (no fluid output) or displacement (bilio-enteric fluid output) during POD 1 to 5. All postoperative outcomes were registered prospectively, including: post-pancreatectomy hemorrhage (PPH)[48], delayed gastric emptying (DGE)[49], postoperative hyperamylasemia (POH) and post-pancreatectomy acute pancreatitis (PPAP)[38], 90-days in hospital mortality and readmission, major morbidity defined as Clavien-Dindo ≥3[50], length of hospital stay (LOS). The severity of POPF was recorded by assigning a severity grade ranging from 0 to 6 on the basis of the Modified Accordion Severity Grading System.[125]

Statistical analysis

Continuous variables were expressed as the median and inter-quartile range (IQR). Differences in variables between or among groups were tested using Student's t-test, Chi- squared test, or Fisher's exact test. ROC curves were used to find the optimal predictors cut-off values with the highest performance ruling out the occurrence of POPF and were compared using area under the curve (AUC). The Youden index was calculated and used to select the most appropriate cutoff combined with clinical relevance. For each cut-off, sensitivity (SENS), specificity (SPEC), positive predictive value (PPV), negative predictive value (NPV), and prevalence of positive cut-offs in the examined population (Prev +) were calculated. Multivariate analysis was performed using a logistic regression model expressed as an odds ratio. P-values less than 0.05 were considered statistically significant.

RESULTS

A total of 431 patients were initially enrolled (**Figure 1**). Of them, 29 in the negligible and low risk groups (FRS 0-2) underwent drainless PD and were therefore excluded. Four additional patients were excluded because of unavailable DFA values on POD 1-3. A total of 398 patients were included in the final analysis.

Clinical characteristics

A total of 163 patients (41%) underwent ETS positioning (**Supplementary Table 1**). Compared to patients without ETS (N= 235, 59%), patients with ETS had higher rates of presumed high-risk diagnosis (52% vs 32%), lower median MPD size (3 vs 5mm), and higher rates of soft pancreatic texture (82% vs 27%) (all p< 0.01). The ETS patients were almost exclusively classified in the moderate (66%) or high (31%) FRS zones.

Supplementary Table 1. Preoperative, intraoperative, and postoperative profile of included patients (N= 398)

Characteristics	Total, No. (%) (N= 398)	ETS, No. (%)		*P*
		No (N= 235, 59%)	Yes (N= 163, 41%)	
Preoperative				
Age, median (iqr), y	66 (15)	66 (14)	64 (15)	**0.039**
Female sex, No. (%)	187 (47)	120 (51)	67 (41)	0.050
BMI, median (iqr)	24 (5)	24 (5)	25 (5)	**0.005**
Smoker, No. (%)	114 (29)	71 (30)	43 (26)	0.406
Alcohol abuse, No. (%)	9 (2)	6 (3)	3 (2)	0.638
Diabetes, No. (%)	70 (18)	51 (22)	19 (12)	**0.010**
Weight loss, No. (%)	178 (45)	113 (48)	65 (40)	0.105
Jaundice, No. (%)	228 (57)	155 (66)	73 (45)	**<0.001**
Jaundice palliation, No. (%)				
Endoscopic stent	205 (52)	136 (58)	69 (42)	**0.002**
Percutaneous drain	13 (3)	9 (4)	4 (2)	0.454
Preoperative MDR colonization, No. (%)	27 (7)	17 (7)	10 (6)	0.680
Comorbidities, No. (%)	316 (79)	187 (80)	129 (79)	0.916
Ischemic cardiac disease, No. (%)	14 (4)	7 (3)	7 (4)	0.484
Hypertension, No. (%)	170 (43)	98 (42)	72 (44)	0.624
COPD, No. (%)	20 (5)	11 (5)	9 (6)	0.706
CKD, No. (%)	11 (3)	8 (3)	3 (2)	0.349
Previous laparotomy, No. (%)	88 (22)	48 (21)	40 (25)	0.467
ASA score, No. (%)				0.738
1-2	297 (75)	173 (74)	124 (76)	
3-4	101 (25)	62 (26)	39 (24)	
Neoadjuvant therapy, No. (%)	176 (44)	110 (47)	66 (41)	0.232
Presumed diagnosis				**<0.001**
PDAC/chronic pancreatitis	237 (60)	159 (68)	78 (48)	
Duodenal/ampullary/cystic/NET	161 (40)	76 (32)	85 (52)	
Intraoperative				
PD type, No. (%)				0.732
Pylorus-preserving	324 (81)	190 (81)	134 (82)	
Whipple	74 (19)	45 (19)	29 (18)	
Vascular resection, No. (%)	56 (14)	37 (16)	19 (12)	0.249
Operative time, median (iqr), min	429 (144)	437 (151)	412 (144)	0.08
Intraoperative transfusion, No. (%)	69 (17)	40 (17)	29 (18)	0.842

Blood loss, median (iqr), ml	600 (600)	600 (650)	600 (514)	0.888
MPD size, median (iqr), mm	4 (2)	5 (1)	3 (2)	**<0.001**
MPD size ≤ 3mm, No. (%)	174 (44)	52 (22)	122 (75)	**<0.001**
Pancreatic texture, No. (%)				**<0.001**
Hard	201 (50)	171 (73)	30 (18)	
Soft	197 (50)	64 (27)	133 (82)	
FRS zone, No. (%)				**<0.001**
Negligible (0)	19 (4)	19 (8)		
Low (1-2)	83 (21)	78 (33)	5 (3)	
Moderate (3-6)	238 (60)	131 (56)	107 (66)	
High (7-10)	58 (15)	7 (3)	51 (31)	
Pancreatic anastomosis, No. (%)				0.135
PJ	381 (96)	222 (94)	159 (98)	
PG	17 (4)	13 (6)	4 (2)	
ETS diameter, median (iqr), French			7.5 (1.5)	
Postoperative				
POPF, No. (%)	118 (30)	41 (17)	77 (47)	**<0.001**
POPF grade, No. (%)				0.617
BL	14 (4)	6 (13)	8 (9)	
B	107 (27)	36 (76)	71 (84)	
C	11 (3)	5 (11)	6 (7)	
POH, No. (%)	103 (26)	41 (17)	62 (38)	**<0.001**
PPAP, No. (%)	24 (6)	16 (7)	8 (5)	0.613
Fluid collection, No. (%)	146 (37)	63 (27)	83 (51)	**<0.001**
Abscess, No. (%)	22 (6)	10 (4)	12 (7)	0.182
Biliary fistula, No. (%)	38 (10)	23 (10)	15 (9)	0.845
DJ/GJ fistula, No. (%)	6 (2)	5 (2)	1 (1)	0.223
Chyle leak, No. (%)	22 (6)	16 (7)	6 (4)	0.179
PPH, No. (%)	49 (12)	28 (12)	20 (12)	0.915
PPH grade, No. (%)				0.819
A	6 (2)	3 (10)	3 (15)	
B	25 (6)	15 (54)	9 (45)	
C	18 (5)	10 (36)	8 (40)	
DGE, No. (%)	64 (16)	22 (9)	42 (26)	**<0.001**
DGE grade, No. (%)				0.769
A	15 (4)	4 (18)	11 (26)	
B	35 (9)	13 (59)	22 (52)	
C	14 (4)	5 (23)	9 (21)	
Septicemia, No. (%)	133 (33)	65 (28)	68 (42)	**0.003**
Pleural effusion, No. (%)	61 (15)	32 (14)	29 (18)	0.256
Postoperative pneumonia, No. (%)	35 (9)	14 (6)	21 (13)	**0.016**
Sars-Cov-2 infection, No. (%)	11 (3)	4 (2)	7 (4)	0.121
AF w/ RVR, No. (%)	33 (8)	23 (10)	10 (6)	0.194
Inotropes, No. (%)	29 (7)	15 (6)	14 (9)	0.405
UTI, No. (%)	15 (4)	7 (3)	8 (6)	0.204
Acute Kidney Injury, No. (%)	34 (9)	20 (9)	15 (9)	0.811
SSI, No. (%)	40 (10)	23 (10)	17 (10)	0.834
Antibiotic therapy, No. (%)	196 (49)	95 (40)	101 (62)	**<0.001**
Enteral nutrition, No. (%)	104 (26)	37 (16)	67 (41)	**<0.001**
TPN, No. (%)	156 (39)	69 (29)	87 (53)	**<0.001**
Therapeutic octreotide, No. (%)	42 (11)	13 (6)	29 (18)	**<0.001**
Percutaneous drainage, No. (%)	21 (5)	9 (4)	12 (7)	0.121
ETS displacement, No. (%)			5 (3)	
ETS occlusion, No. (%)			17 (11)	
ETS malfunction, No. (%)			22 (14)	
POD of ETS malfunction, median (range), days			1 (0-4)	
Transfusions, No. (%)	113 (28)	60 (26)	53 (33)	0.119
Re-laparotomy, No. (%)	27 (7)	16 (7)	11 (7)	0.981
Unplanned ICU admission, No. (%)	74 (19)	43 (18)	31 (19)	0.856
Single/multiple organ failure, No. (%)	26 (7)	14 (6)	12 (7)	0.577
POD of drain removal, median (iqr), days	5 (12)	4 (4)	8 (21)	**<0.001**
Early drain removal, No. (%)	119 (30)	106 (45)	13 (8)	**<0.001**

Discharged with drains, No. (%)	32 (8)	12 (5)	20 (12)	**0.010**
LOS, median (iqr), days	14 (22)	10 (12)	21 (18)	**<0.001**
Readmission (30-days), No. (%)	38 (10)	15 (6)	23 (14)	**0.010**
POPF Accordion, No. (%)				0.209
1	9 (7)	4 (10)	5 (6)	
2	88 (75)	30 (73)	58 (75)	
3	11 (9)	2 (5)	6 (12)	
4	6 (5)	3 (7)	3 (4)	
5	2 (2)	_	2 (3)	
6	2 (2)	2 (5)	_	
Clavien-Dindo, No (%)				**<0.001**
0	139 (35)	105 (45)	34 (21)	
1	21 (5)	15 (6)	6 (4)	
2	173 (43)	84 (36)	89 (55)	
3a	24 (6)	9 (4)	15 (9)	
3b	15 (4)	8 (3)	7 (4)	
4a	11 (3)	7 (3)	4 (2)	
4b	7 (2)	2 (1)	5 (3)	
5	8 (2)	5 (2)	3 (2)	
Major morbidity, No (%)	65 (16)	31 (13)	34 (21)	**0.042**
Mortality, No. (%)	8 (2)	5 (2)	3 (2)	0.841

Abbreviations: ETS, externalized trans-anastomotic stent; BMI, body mass index; MDR, multi-drug resistant bacteria; COPD, chronic obstructive pulmonary disease; CKD, chronic kidney disease; ASA, American Society of Anesthesiologists Classification; PDAC, pancreatic ductal adenocarcinoma; NET, neuroendocrine tumor; PD, pancreatoduodenectomy; MPD, main pancreatic duct; FRS, fistula risk score; PJ, pancreatico-jejunostomy; PG, pancreatico-gastrostomy; POPF, clinically relevant postoperative pancreatic fistula; BL, biochemical leak; POH; postoperative hyperamylasemia; POAP, postoperative acute pancreatitis; DJ, duodeno-jejunal anastomosis; GJ, gastro-jejunal anastomosis; PPH, post pancreatectomy hemorrhage; DGE, delayed gastric emptying; AF w/RVR, atrial fibrillation with rapid ventricular rate; UTI, urinary tract infection; SSI, surgical site infection; TPN, total parenteral nutrition; POD, postoperative day; ICU, intensive care unit; LOS, length of hospital stay.

The POPF rate in the ETS group was significantly higher (47% vs 17%; p< 0.01), as well as that of POH (38% vs 17%; p< 0.01), fluid collections (51% vs 27%; p< 0.01), septicemia (42% vs 28%; p< 0.01), LOS (21 vs 10 days; p< 0.01), and major morbidity (Clavien-Dindo ≥3: 21% vs 13%; p= 0.04). Patients in the ETS group were more frequently treated with antibiotic therapy, enteral or total parenteral nutrition, and therapeutic octreotide. However, POPF Accordion (p= 0.20) and POPF grade distribution (C grade: 7% vs 11% of patients with BL/POPF; p= 0.61) were not different between ETS and non-ETS patients, as well as rates of percutaneous drainage (7% vs 4%; p= 0.12), PPH (both 12%; p= 0.91), re-laparotomy (both 7%; p= 0.98), single/multiple organ failure (7% vs 6%; p= 0.57), and mortality (both 2%; p= 0.84), respectively. Early drain removal was performed in 45% of non-ETS patients, compared to only 8% of ETS patients (p< 0.01).

Postoperative POPF predictors – dynamic analysis

The median value of each postoperative POPF predictor from POD 1 to 5 and according to ETS presence is showed in **Supplementary Table 2**.

Supplementary Table 2. Median values for examined postoperative POPF predictors, in patients with and without ETS

Predictors	Total, median (iqr) (N= 398)	ETS, median (iqr)		*P*
		No (N= 235, 59%)	Yes (N= 163, 41%)	
DFA (UI/L)				
POD 1	319 (1709)	67 (478)	1284 (2311)	**<0.001**
POD 2	211 (1038)	47 (332)	763 (1906)	**<0.001**
POD 3	98 (456)	34 (165)	296 (811)	**<0.001**
POD 4	80 (274)	40 (145)	132 (388)	**<0.001**
POD 5	65 (396)	33 (137)	84 (562)	**<0.001**
SA (UI/L)				
POD 1	28 (93)	8 (46)	73 (115)	**<0.001**
POD 2	16 (64)	5 (26)	40 (86)	**<0.001**
POD 3	8 (26)	4 (12)	22 (37)	**<0.001**
POD 4	6 (14)	3 (6)	15 (24)	**<0.001**
POD 5	6 (12)	3 (5)	13 (20)	**<0.001**
SL (UI/L)				
POD 1	35 (120)	10 (48)	96 (141)	**<0.001**
POD 2	17 (58)	7 (24)	41 (97)	**<0.001**
POD 3	11 (22)	6 (10)	23 (42)	**<0.001**
POD 4	9 (15)	6 (6)	17 (20)	**<0.001**
POD 5	9 (12)	6 (6)	15 (18)	**<0.001**
C-RP				
POD 1	89 (49)	92 (49)	85 (47)	0.09
POD 2	181 (110)	177 (102)	191 (115)	0.06
POD 3	187 (125)	169 (126)	209 (113)	**<0.001**
POD 4	142 (121)	119 (115)	167 (106)	**<0.001**
POD 5	104 (112)	81 (114)	130 (104)	**<0.001**
ETS output (ml)				
POD 1			10 (50)	
POD 2			100 (125)	
POD 3			100 (120)	
POD 4			200 (200)	
POD 5			200 (200)	

Abbreviations: ETS, externalized trans-anastomotic stent; POD, postoperative day; DFA, drain fluid amylase; SA, serum amylase; SL, serum lipase; C-RP, c-reactive protein.

Median DFA, SA and SL values were higher in the ETS group at each POD. However, in both groups the median DFA, SA, and SL values decreased during time. Similarly, the median C-RP was higher in the ETS group (in POD 3 to 5), but median values followed the same pattern than those in the non-ETS group, peaking in POD 3. The median ETS output gradually increased from POD 1 to 5, from 10ml to 200ml.

The ROC and AUC of each clinical indicator, from POD 1 to 5 and according to ETS presence, is displayed in **Figure 2**.

Figure 2. ROC curves and AUC of each indicator for POPF classification, stratified by POD and ETS presence.

In the non-ETS group, DFA had a high AUC in POD 1 to 3 (0.87-0.87-0.84), with a reduction in POD 4 and 5 (0.73-0.72). In the ETS group, DFA had a lower AUC in POD 1 to 3 (0.68-0.71-0.69) compared to non-ETS (all p=0.01), reaching similar AUC in POD 4 and 5 (0.73-0.78). In the non-ETS group, SA maintained a high AUC

from POD 1 to 5 (0.86-0.87-0.81-0.77-0.75), differently to the ETS group (all p< 0.01) where its AUC was reduced regardless of the POD. The SL ROC patterns were like the SA ones, for both groups and for each POD, with a slightly reduced AUC. The C-RP ROC patterns were similar for both non-ETS and ETS groups, with higher AUC during POD 3, 4 and 5. The ETS output had a reduced AUC regardless of the POD.

Postoperative POPF predictors – cut offs

The diagnostic performances of different DFA cut-offs in different PODs and according to the presence of ETS are showed in **Table 1**.

Table 1. Diagnostic performances (for POPF) of different DFA cutoffs according to POD and ETS presence

		No ETS (N= 230, 98%)						ETS (N= 159, 98%)					
POD	**DFA (IU/L)**	**SENS**	**SPEC**	**PPV**	**NPV**	**Prev +**	**Youden**	**SENS**	**SPEC**	**PPV**	**NPV**	**Prev +**	**Youden**
	5000	30%	99%	92%	87%	6%	0.295	19%	91%	64%	56%	14%	0.095
	2500	43%	95%	65%	89%	11%	0.378	36%	76%	57%	58%	30%	0.130
	1000	60%	89%	55%	91%	19%	0.495	69%	54%	57%	67%	57%	0.230
1	600	67%	87%	52%	93%	23%	0.543	86%	46%	58%	80%	69%	0.324
	400	78%	83%	48%	95%	28%	0.601	93%	41%	58%	88%	75%	0.344
	300	83%	79%	45%	96%	32%	0.614	95%	36%	56%	89%	78%	0.311
	150	93%	72%	41%	98%	39%	0.646	96%	25%	53%	88%	85%	0.207
		No ETS (N= 227, 97%)						**ETS (N= 156, 96%)**					
POD	**DFA (IU/L)**	**SENS**	**SPEC**	**PPV**	**NPV**	**Prev +**	**Youden**	**SENS**	**SPEC**	**PPV**	**NPV**	**Prev +**	**Youden**
	5000	22%	98%	73%	87%	5%	0.200	15%	92%	61%	57%	12%	0.073
	2500	43%	96%	70%	90%	10%	0.395	31%	84%	61%	59%	23%	0.145
	1000	59%	94%	67%	92%	15%	0.536	59%	69%	62%	67%	44%	0.286
2	600	68%	92%	63%	94%	18%	0.586	76%	59%	61%	74%	57%	0.349
	400	78%	88%	56%	95%	23%	0.662	83%	47%	57%	77%	67%	0.302
	300	81%	82%	47%	96%	28%	0.631	89%	41%	56%	81%	72%	0.299
	150	86%	71%	36%	96%	38%	0.570	94%	36%	55%	89%	78%	0.308
		No ETS (N= 230, 98%)						**ETS (N= 157, 96%)**					
POD	**DFA (IU/L)**	**SENS**	**SPEC**	**PPV**	**NPV**	**Prev +**	**Youden**	**SENS**	**SPEC**	**PPV**	**NPV**	**Prev +**	**Youden**
	600	56%	94%	65%	91%	15%	0.501	43%	78%	61%	63%	31%	0.208
	500	56%	93%	61%	91%	16%	0.490	49%	76%	62%	64%	35%	0.242
3	400	64%	92%	63%	93%	18%	0.562	57%	69%	60%	66%	43%	0.257
	300	68%	92%	62%	93%	18%	0.582	64%	63%	58%	68%	49%	0.271
	150	79%	84%	50%	95%	27%	0.631	79%	45%	54%	71%	65%	0.239
		No ETS (N= 120, 52%)						**ETS (N= 143, 88%)**					
POD	**DFA (IU/L)**	**SENS**	**SPEC**	**PPV**	**NPV**	**Prev +**	**Youden**	**SENS**	**SPEC**	**PPV**	**NPV**	**Prev +**	**Youden**
4	600	28%	93%	63%	74%	14%	0.204	33%	89%	73%	59%	22%	0.217

	300	36%	88%	57%	76%	20%	0.238	46%	86%	76%	63%	30%	0.324
	150	47%	81%	53%	78%	27%	0.287	61%	69%	65%	66%	45%	0.306
		No ETS (N= 97, 42%)						**ETS (N= 142, 88%)**					
POD	**DFA (IU/L)**	**SENS**	**SPEC**	**PPV**	**NPV**	**Prev +**	**Youden**	**SENS**	**SPEC**	**PPV**	**NPV**	**Prev +**	**Youden**
5	600	27%	94%	69%	71%	14%	0.209	38%	90%	79%	59%	24%	0.280
	300	39%	90%	68%	74%	20%	0.299	55%	86%	80%	65%	35%	0.406
	150	42%	86%	61%	74%	24%	0.281	66%	83%	80%	71%	42%	0.491

Abbreviations: ETS, externalized trans-anastomotic stent; POD, postoperative day; DFA, drain fluid amylase; SENS, sensitivity; SPEC, specificity; PPV, positive predictive value; NPV, negative predictive value; Prev +, prevalence of positive cut-offs in the examined population; Youden, Youden's index.

In the non ETS group, a DFA= 300 UI/L was chosen as the best cut-off on POD 1 (SENS 83%, SPEC 79%, PPV 45%, NPV 96%, Prev+ 32%, Youden 0.614) and POD 2 (SENS 81%, SPEC 82%, PPV 47%, NPV 96%, Prev+ 28%, Youden 0.631), while a DFA= 150 UI/L was chosen as the POD 3 cut-off (SENS 79%, SPEC 84%, PPV 50%, NPV 95%, Prev+ 27%, Youden 0.631). No DFA cut-off was identified from POD 1 to 3 for the ETS group, while a DFA= 150 UI/ml was chosen as the POD 5 cut-off (SENS 66%, SPEC 83%, PPV 80%, NPV 71%, Prev+ 42%, Youden 0.491). The presence of POH (sustained SA >52 UI/L in both POD 1 &2) was chosen as the best cut-off for SA in the non-ETS group (SENS 51%, SPEC 90%, PPV 51%, NPV 90%, Prev+ 17%, Youden 0.409) (**Table 2**). No cut-offs for SA were identified in the ETS group.

Table 2. Diagnostic performances (for POPF) of POH (SA >52 UI/L POD 1 & 2) according to ETS presence

	No ETS (N= 235, 100%)						**ETS (N= 163, 100%)**					
POH	**SENS**	**SPEC**	**PPV**	**NPV**	**Prev +**	**Youden**	**SENS**	**SPEC**	**PPV**	**NPV**	**Prev +**	**Youden**
SA >52 UI/L POD 1 & 2	51%	90%	51%	90%	17%	0.409	38%	62%	47%	52%	47%	0.000

Abbreviations: ETS, externalized trans-anastomotic stent; POH, postoperative hyperamylasemia; SA, serum amylase; SENS, sensitivity; SPEC, specificity; PPV, positive predictive value; NPV, negative predictive value; Prev +, prevalence of positive cut-offs in the examined population; Youden, Youden's index.

The cut-offs for C-RP in all patients (both ETS and non-ETS group) are showed in **Table 3**. A C-RP= 150 mg/L was identified as the cut-off for POD 5 (SENS 66%, SPEC 82%, PPV 60%, NPV 85%, Prev+ 33%, Youden 0.473).

Table 3. Diagnostic performances (for POPF) of C-RP best cutoffs according to POD

		All (N= 382, 96%)					
POD	**C-RP (mg/L)**	**SENS**	**SPEC**	**PPV**	**NPV**	**Prev +**	**Youden**
1	100	41%	63%	32%	71%	38%	0.039
		All (N= 394, 99%)					
2	200	69%	71%	50%	84%	41%	0.400
		All (N= 392, 98%)					
3	250	55%	88%	66%	82%	25%	0.432
		All (N= 395, 99%)					
4	150	79%	68%	51%	89%	46%	0.478
		All (N= 393, 98%)					
5	150	66%	82%	60%	85%	33%	0.473

Abbreviations: POD, postoperative day; C-RP, c-reactive protein; SENS, sensitivity; SPEC, specificity; PPV, positive predictive value; NPV, negative predictive value; Prev +, prevalence of positive cut-offs in the examined population; Youden, Youden's index.

Criteria for early vs. late drain removal

Criteria for early and late drain removal were selected (**Table 4**) based on the previous analysis. Criteria for early removal were DFA <300 UI/L in POD 1&2, DFA <150 UI/L in POD 3, and absence of POH. Criteria for late removal were DFA <150 UI/L and C-RP <150 mg/ml in POD 5. Criteria for early removal accurately predicted the absence of POPF in the non-ETS group (SENS 73%, SPEC 88%, PPV 97%, NPV 40%, Prev+ 62%, POPF 3%, Youden 0.605), while criteria for late removal accurately predicted the absence of POPF in the ETS group (SENS 67%, SPEC 93%, PPV 90%, NPV 75%, Prev+ 36%, POPF 10%, Youden 0.605).

Table 4. Diagnostic performances (for POPF absence) of early and late drain removal criteria according to ETS presence

		No ETS (N= 235, 100%)							ETS (N= 163, 100%)						
Policy	**Criteria**	**SENS**	**SPEC**	**PPV**	**NPV**	**Prev +**	**POPF**	**Youden**	**SENS**	**SPEC**	**PPV**	**NPV**	**Prev +**	**POPF**	**Youden**
Early Drain Removal	DFA POD 1&2 <300 UI/L, DFA POD 3 <150 U/L, No POH	73%	88%	97%	40%	62%	3%	0.605	31%	95%	87%	55%	19%	13%	0.262
		No ETS (N= 128, 54%)							**ETS (N= 145, 89%)**						
Policy	**Criteria**	**SENS**	**SPEC**	**PPV**	**NPV**	**Prev +**	**POPF**	**Youden**	**SENS**	**SPEC**	**PPV**	**NPV**	**Prev +**	**POPF**	**Youden**
Late Drain Removal	DFA POD 5 <150 UI/L, C-RP POD 5 <150 mg/l	72%	72%	85%	53%	59%	15%	0.437	67%	93%	90%	75%	36%	10%	0.605

Abbreviations: ETS, externalized trans-anastomotic stent; POD, postoperative day; DFA, drain fluid amylase; POH, postoperative hyperamylasemia; C-RP, c-reactive protein; SENS, sensitivity; SPEC, specificity; PPV, positive predictive value; NPV, negative predictive value; Prev +, prevalence of positive cut-offs in the examined population; POPF, prevalence of POPF in the examined population; Youden, Youden's index.

Based on the previous findings, a tailored drain management protocol was proposed for patients with and without ETS, as showed in **Figure 3**.

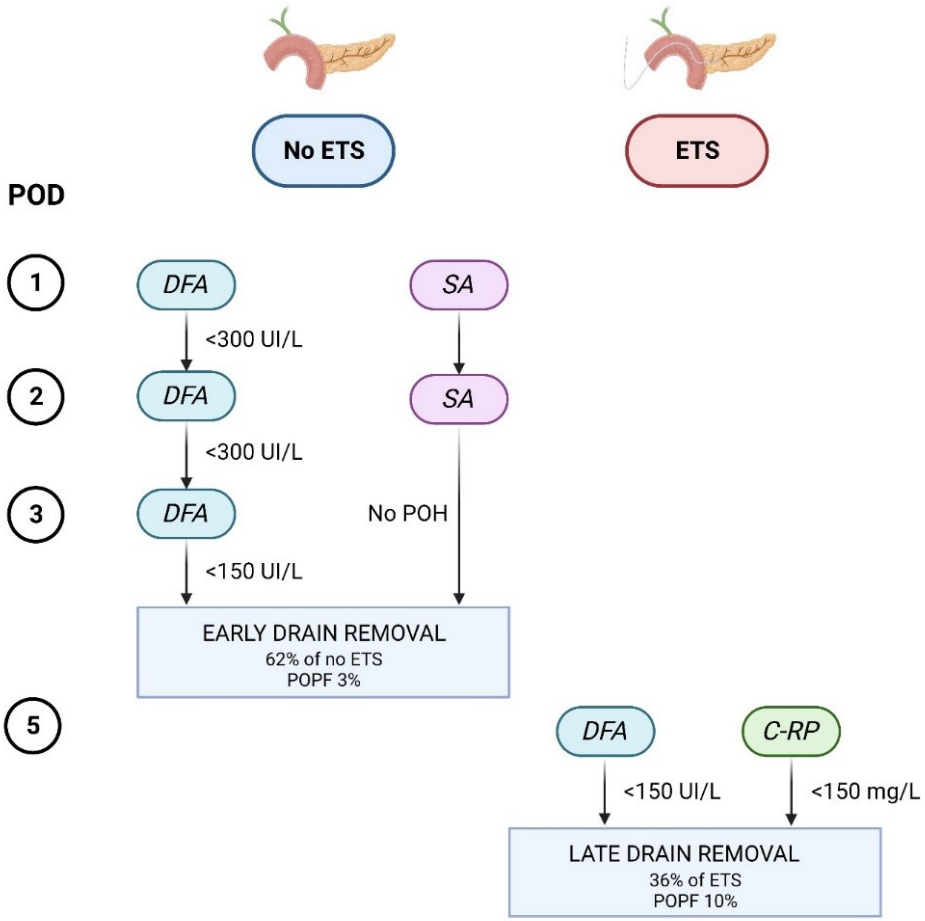


Figure 3. Proposal of tailored drain management protocol for patients with or without ETS.

DISCUSSION

The present study aimed to establish a novel, risk-based protocol for the postoperative drain management of patients undergoing PD with or without ETS, thorough the dynamic evaluation of the diagnostic performance of different postoperative POPF predictors. In the present series, the ETS positioning successfully mitigated POPF severity in the higher risk patients, achieving results comparable to that of lower risk patients, despite the higher POPF incidence. In patients with ETS, DFA was not a reliable predictor of POPF in the early (POD 1-3) postoperative period, neither were SA or C-RP. Therefore, while in patients without ETS an early drain removal policy is recommended based on DFA and SA, in patients with ETS drain removal should be probably postponed until POD 5, when DFA and C-RP reach acceptable diagnostic accuracy. The positioning of a trans-anastomotic stents is considered among optimal POPF mitigation

strategies for high-risk pancreatic anastomoses[117]. Compared to internal stents, ETS have the possibility for real time monitoring of their correct functioning, measuring the quality and the amount of externalized pancreatic juice. Several trials and prospective studies demonstrated the ability of ETS to reduce not only POPF incidence, but also the general and POPF-related morbidity burden, especially in the high-risk setting [126] [127] [118] [120]. In the present observational series, patients with ETS positioning, who were selected intraoperatively as high-risk, experienced an almost three-fold rate of POPF compared to the lower risk patients in which an ETS was not deemed necessary. However, despite longer hospital stays and higher degree of medicalization (i.e., use of postoperative enteral/parenteral nutrition, octreotide, and antibiotics) ETS patients did not experience higher median POPF Accordion, or a higher proportion of C grade POPF. Moreover, in ETS patients the rates of percutaneous drainage, PPH, relaparotomy, ICU admission, single/multiple organ failure, or mortality were not increased compared to patients without ETS. Interestingly, probably due to further progress in the learning curve, the rate of ETS malfunction (occlusion or displacement) was lower (14%) compared to what reported in previous experiences (22%-28%[120] [121]). It is already well recognized that a risk-based approach to postoperative drain protocols is necessary after PD [109] [110] [112] [113]. In previous publications, tailored protocols based on intraoperative risk stratification (such as the FRS[122]) mostly relied on different DFA cut-offs adjusted for patient's risk, usually proposing higher thresholds for higher risk glands. However, the presence of ETS implies an external deviation of most of the pancreatic juice. The systematic positioning of an ETS in the high-risk patients group calls for a reappraisal of the current postoperative drain management and its predictors. As previously hypotized[114] in case of ETS positioning DFA values are not a reliable predictor of future POPF occurrence during POD 1-3. Interestingly, their overall median levels are higher than non ETS patients, irrespective of the eventual development of POPF and despite the external deviation of pancreatic juice. Their diagnostic performance increase again only in POD 4-5, when their levels start to drop in most patients with no POPF. Unfortunately, no other examined predictor was able to exclude POPF early enough in ETS patients to safely apply early drain removal policies. Even SA levels are not useful POPF predictors in this subset of patients, as the prevalence of elevated POD 1-2 SA and/or POH is very high, nor is the ETS fluid volume. Finally, for both ETS and non-ETS patients, C-RP

confirmed to be a valuable indicator of POPF clinical relevance, but mostly from POD 3 onward and as a compendium of other more specific predictors[115]. Consequently, it appears that avoiding "blind" early drain removal policies in high-risk patients with ETS may be the safest option, postponing the decision to POD 4-5 according to DFA and C-RP levels. In case of no ETS, conversely, POD 1-3 DFA levels are reliable parameters which can safely drive early drain removal in most patients, and can be implemented with POD1-2 SA to exclude the presence of POH[38] and improve the final accuracy of the model. This study has some limitations. The foremost is to represent an observational series where postoperative decisions for patients with ETS were not based on a pre-defined dedicated protocol, given the absence of prior evidence. It is important to notice that, despite an established institutional practice of DFA-based early drain removal, based on surgeon's decision such policy was almost never applied in the ETS group, while it was maintained in half of the non-ETS patients. However, not performing early removal in the ETS group allowed the comprehensive collection of drain/serum POPF predictors thorough POD 1 to 5 for the majority of this patients. A randomized controlled trial comparing early vs late removal policies in ETS patients would represent a higher level of evidence, but the present analysis suggests that in this high-risk category there may not be elements to support informed early drain removal. Finally, trans-anastomotic stents include also internal stents, widely used for example during minimally invasive surgery[128]. It is not clear if the results of the present analysis should be applied also to internal stents, given the macroscopic differences in their design and functioning. In conclusion, the postoperative management of patients undergoing pancreatic resection is challenging. New developments such as the application of data-driven algorithms[70] , based on daily assessment of clinical and serological parameters and a pro-active approach toward complications, have promising results preventing the most severe sequelae of pancreatic fistula, including mortality. However, tailored postoperative care should consider both the pancreas-specific risk of complications, and the use of available mitigations strategies such as ETS. In patients undergoing ETS positioning, the most commonly used postoperative predictors seem not able to accurately exclude the development of POPF during the first 72 hours after surgery. Therefore, in the absence of better predictors, early drain removal policies should be applied with cautions to this high-risk population.

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