RESEARCH ARTICLE

Postoperative pancreatic fistula affects recurrence-free survival of pancreatic cancer patients

Sameer A. Dhayat1*, Ahmad N. J. Tamim1, Marius Jacob1, Georg Ebeling1, Laura Kerschke2, Iyad Kabar3, Norbert Senninger1

1 Department of General and Visceral Surgery, University Hospital Muenster, Muenster, Germany, 2 Institute of Biostatistics and Clinical Research, University of Muenster, Muenster, Germany, 3 Department of Internal Medicine B, Gastroenterology and Hepatology, University Hospital Muenster, Muenster, Germany

* These authors contributed equally to this work.

* sameer.dhayat@ukmuenster.de

Abstract

Purpose
Postoperative pancreatic fistula (POPF) with reported incidence rates up to 45% contributes substantially to overall morbidity. In this study, we conducted a retrospective evaluation of POPF along with its potential perioperative clinical risk factors and its effect on tumor recurrence.

Methods
Clinical data on patients who had received pancreatoduodenectomy (PD), distal pancreatectomy (DP), or duodenum-preserving pancreatic head resection (DPPHR) were prospectively collected between 2007 and 2016. A Picrosirius red staining score was developed to enable morphologic classification of the resection margin of the pancreatic stump. The primary end point was the development of major complications. The secondary end points were overall and recurrence-free survival.

Results
340 patients underwent pancreatic resection including 222 (65.3%) PD, 87 (25.6%) DP, and 31 (9.1%) DPPHR. Postoperative major complications were observed in 74 patients (21.8%). In multivariable logistic regression analysis, POPF correlated with body mass index (BMI) (p = 0.025), prolonged stay in hospital (p<0.001), high Picrosirius red staining score (p = 0.049), and elevated postoperative levels of amylase or lipase in drain fluid (p≤0.001). Multivariable Cox regression analysis identified UICC stage (p<0.001), tumor differentiation (p<0.001), depth of invasion (p = 0.001), nodal invasion (p = 0.001), and the incidence of POPF grades B and C (p = 0.006) as independent prognostic markers of recurrence-free survival.
Conclusion

Besides the known clinicopathological risk factors BMI and amylase in the drain fluid, the incidence of POPF correlates with high Picrosirius red staining score in the resection margins of the pancreatic stumps of curatively resected pancreatic ductal adenocarcinoma (PDAC). Furthermore, clinically relevant POPF seems to be a prognostic factor for tumor recurrence in PDAC.

Introduction

Pancreatoduodenectomy (PD) is the preferred therapeutic method for treating benign and malignant diseases of the pancreatic head and its periampullary region. PD remains a complex and highly invasive visceral resection with mortality rates of about 5% and morbidity rates of up to 60% in experienced high-volume centres [1–3]. In line with the demographic changes resulting in an increasing number of multimorbid elderly patients in Europe and the USA, preoperative risk factor assessment and patient stratification have become increasingly popular. Postoperative pancreatic fistula (POPF) with reported incidence rates between 3% and 45% substantially contributes to overall morbidity with increased hospital stay, costs and re-intervention rates and in case of abscess formation, sepsis, and hemorrhage to mortality as well [4,5]. Moreover, clinically relevant POPF with prolonged hospital stay may delay adjuvant treatment and affect oncologic outcomes in malignant pancreatic diseases [6].

The crucial importance of standardized reporting of procedures and their complications with uniform definition and classification of POPF and widely supported recommendations for its diagnosis and treatment led to a first consensus statement of the International Study Group of Pancreatic Surgery (ISGSP) in 2005 [7]. In 2017, clinically relevant POPF was redefined as drainage fluid of any measurable volume with amylase level more than three times that of physiological serum amylase activity, associated with a clinically relevant condition related directly to the POPF, which can originate from pancreaticoenteric or pancreaticogastrointestinal anastomosis after head resection or drainage procedures and of pancreatic remnant after distal pancreatectomy or enucleation [8].

The efficacy of anastomotic techniques like pancreaticojejunostomy versus pancreaticogastrostomy, invagination versus duct-to-mucosa, internal versus external pancreatic duct stenting, fibrin glue versus other topical haemostatic occlusive agents to seal the pancreatic anastomosis, and the use of various somatostatin analogues to decrease pancreatic enzyme secretion were investigated in various trials [9–16]. So far, there is no consensus on the optimal management of POPF and no standardized intra- and perioperative treatment [17]. Therefore, the attention has been focused on the assessment of POPF risk factors. Many studies addressed the role of single factors or comprehensive risk scores on multiple risk factors, such as age, gender, body mass index (BMI), pathologic diagnosis, operative time, blood loss, diameter of the main pancreatic duct, texture of pancreatic parenchyma, American Society of Anaesthesiologists (ASA) score, heart rate, systolic blood pressure, haemoglobin, and albumin levels [18]. POPF risk stratification customized for individual patients may help in increasing the number of patients eligible for pancreatic surgery by preoperative nutritional support, optimizing cardiovascular medication, starting exercise therapy, and postoperative intensive monitoring or even to select high-risk patients who might be excluded from surgical resection. However, current guidelines for pancreatic surgery do not recommend any of the proposed clinical risk prediction scores for morbidity and mortality such as ASA and POSSUM.
(Physiological and Operative Severity Score for the enUmeration of Mortality and Morbidity) nor POPF risk scores such as PREPARE (Preoperative Pancreatic Resection) score by Uzuno-glu et al., FRS (Fistula Risk Score) by Callery et al., and a-FRS (Alternative Fistula Risk Score for Pancreatosduodenectomy) by Mungroop et al. [19–23].

In this study, we conducted a retrospective evaluation of POPF along with its potential peri-operative clinical and histopathological risk factors and its impact on tumor recurrence.

Patients and methods

Study design

This retrospective monocentric study included patients who underwent pancreatic resection between January 2007 and December 2016 at the Department of General and Visceral Surgery of the University Hospital Muenster and were followed up for at least 36 months. All operative procedures were performed by surgeons experienced in the field of pancreatic surgery who conducted at least 30 pancreatic resections per year. Perioperative clinical data, histopathological information and follow-up data on all patients were prospectively collected in an electronic database. Ethical approval for postoperative tissue collection, analysis, and retrospective analysis of patient-related clinical data were obtained by the Ethics committee of the University Muenster (Az: IXHai v. 19.9.2001 and Az: IXHai v.11.08.2011) and all patients provided informed written consent. All patients underwent PD, left pancreatic resection, or duodenum-preserving pancreatic head resection. Patients that received emergency pancreatic resection, primary total pancreatectomy, immunosuppression, neoadjuvant chemotherapy, neoadjuvant radiotherapy, and/or missing follow-up data were excluded from this study.

The primary end point of the study was the development of major complications, in particular pancreatic fistula. The secondary end points were overall survival and recurrence free survival.

Selection of perioperative variables

Perioperative variables included patient characteristics, medical history, physiological parameters, laboratory tests, and variables related to the performed surgery. Only variables that could be assessed objectively were accepted for inclusion. The cut off values based on physiological relevance or on established scoring systems.

Preoperative variables included age, gender, BMI, ASA status, heart rate, systolic blood pressure, biliary drainage, New York Heart Association (NYHA) Functional Classification, cardiac or pulmonary comorbidity, diabetes mellitus, smoking, alcohol abuse, weight loss over 10% in the last 6 months preceding the operation, acute or chronic pancreatitis, septic cholangitis, ascites, diagnosis, preoperative stay and other relevant comorbidities assessed by the Charlson comorbidity index [24]. Preoperative variables included standardized analyses of different blood parameters such as hemoglobin, leukocytes, electrolytes, creatinine, lipase, amylase, bilirubin, gamma-glutamyltransferase, transaminases, alkaline phosphatase, C-reactive protein (CRP), Cancer Antigen 19–9 (CA-19–9), and Carcinoembryonic antigen (CEA).

Intraoperative variables included operation and anesthesia time, the set-up of an epidural catheter for continuous analgesia, heart rate, blood pressure, hemoglobin, blood transfusion, the surgical procedure, usage of internal or external drainages, kind of anastomosis, consistency of the pancreatic tissue, and the diameter size of the main duct at the pancreatic resection margin. Corresponding morphological features regarding the degree of fibrosis, inflammatory and fatty infiltration of the pancreatic resection margin of haematoxylin and eosin (H&E) stained specimens were also considered. Postoperative blood parameters included CRP, lipase, and amylase. In addition, lipase and amylase activities were assessed in the drainage fluid from
postoperative day (POD) 3. Postoperative incidence and treatment of complications, such as pneumonia, heart attack, pulmonary embolism, thrombosis, urinary tract infection, wound infection, delayed gastric emptying, POPF, bile and chyle leakage, abdominal haemorrhage, and abscess were documented. Further data were obtained concerning the length of postoperative hospital stay particularly in the intensive care unit, reoperation, hospital mortality, the histopathological diagnosis, adjuvant chemotherapy regimens, tumor relapse, and tumor related deaths. In total, 72 variables were evaluated in this study and are presented in Table 1.

**Definitions of postoperative complications**

Major complications were defined according to the Clavien-Dindo classification as grades IIIb to V, validated for pancreatic resections [25,26]. Grade III includes complications requiring surgical, endoscopic or radiological intervention, followed by grade IV life-threatening complications requiring intensive care unit (ICU) management, and grade V as the complication-related death of the patient. POPF was defined according to the reclassification of the ISGPS in 2017 [8]. Depending on the clinical course three grades of severity were defined: 1. Biochemical leak (BL), former POPF grade A, with increased amylase activity in the fluid of a drain from POD 3 but without clinical implications. 2. POPF grade B that requires a change in the expected postoperative management with prolonged drainage, therapeutic agents and less-invasive treatment including percutaneous, endoscopic, or angiographic interventional procedures. 3. A shift to POPF grade C is characterized by organ failure, clinical instability, and high mortality, requiring reoperation and stay in an ICU.

Postoperative bile leakage was defined according to the definitions of the International Study Group of Liver Surgery (ISGLS) as discharge of fluid of an intra-abdominal drain with a bilirubin concentration at last three times greater than the serum bilirubin persisting on POD 3 [27]. Chyle leak after pancreatic resection was defined as the output of milky-colored fluid of a drain from POD 3 with a triglyceride content ≥110mg/dl [28]. In accordance with the definitions of the ISGPS, post-pancreatectomy hemorrhage was defined according to the time of onset, location, and clinical severity [29]. Delayed gastric emptying was diagnosed if the patient was unable to tolerate a solid diet on POD 7 or if reinsertion of the nasogastric tube was required between POD 4 and 7 according to the definitions of the ISGPS [30]. Mortality was defined as death occurring during the hospital stay.

**Histochemical staining by picrosirius red**

The resection margins of pancreatic stumps of all cases treated by pancreatic head resection were submitted for pathological diagnosis by classic H&E staining. In addition, 6μm thick formalin-fixed paraffin embedded (FFPE) tissue sections of the resection margins were deparaffinized through xylene and a graded alcohol series. Tissue samples were stained for 30 min in Picrosirius red solution consisting of 0.1% solution of Sirius Red F3B (Sigma-Aldrich, St Louis, MO, USA) in saturated aqueous picric acid [31]. The stained sections were then washed for 2 min in 0.01 N HCl, dehydrated, cleared and mounted in synthetic resin. Negative controls without Sirius Red and positive controls (liver fibrosis and cirrhosis) were included in all experiments using the same experimental conditions. Picrosirius red planar staining was evaluated separately by two independent investigators in a blinded manner using light microscopy (Eclipse E1000M and NIS-Elements D3.1 imaging software, Nikon, Tokyo, Japan) with a modified score range from 1 to 3 according to Ridolfi et al. and Gaujoux et al. [32,33] Three grades of fibrosis were defined: 1. Normal pancreatic parenchyma, consisting in lobes separated by connective tissue organized in fine septa. 2. focal perilobular or periacinar fibrosis (Fig 1A), and 3. complete replacement of the parenchyma by fibrosis with rare residual areas of acinar...
Table 1. Clinicopathological characteristics for postoperative complications.

|                                | Total  | No Complication       | Complication | p-value |
|--------------------------------|--------|-----------------------|--------------|---------|
|                                | n = 340| n = 339 (99.7%)       | n = 15 (4.4%)|         |
|                                |        | non severe (<IIIb) n = 149 (45.8%) | severe (≥IIIb) n = 74 (21.8%) |         |
| Age (years)                    |        |                       |              |         |
| Median [range]                 | 64 [15–88] | 59 [15–86]           | 65 [15–88]  | 69.5 [20–82] | 0.002 |
| < 60                           | 144    | 64                    | 57           | 23      |
| ≥ 60                           | 196    | 92                    | 51           |         |
| Gender                         |        |                       |              |         |
| female                        | 157    | 73                    | 32           |         |
| male                          | 183    | 76                    | 42           |         |
| Body mass index (kg/m²)        |        |                       |              |         |
| Median [range]                 | 24.6 [13.1–51.6] | 24.4 [16.4–35.7] | 24 [16.6–51.6] | 25.9 [13.1–39.8] | 0.015 |
| 18–24                          | 165    | 60                    | 80           | 25      |
| > 25                           | 175    | 57                    | 69           | 49      |
| Smoking                        |        |                       |              |         |
| No                             | 268    | 118                   | 62           |         |
| Yes                            | 72     | 31                    | 12           |         |
| Alcohol                        |        |                       |              |         |
| No                             | 310    | 140                   | 70           |         |
| Yes                            | 30     | 9                     | 4            |         |
| Weight loss                    |        |                       |              |         |
| No                             | 311    | 138                   | 70           |         |
| Yes                            | 29     | 11                    | 4            |         |
| ASA                            |        |                       |              |         |
| 2-Jan                          | 247    | 113                   | 47           |         |
| 4-Mar                          | 93     | 36                    | 27           |         |
| NYHA                           |        |                       |              |         |
| 2-Jan                          | 317    | 140                   | 66           |         |
| 4-Mar                          | 23     | 9                     | 8            |         |
| Cardiac comorbidity            |        |                       |              |         |
| No                             | 161    | 67                    | 25           |         |
| Yes                            | 179    | 82                    | 49           |         |
| Pulmonary comorbidity          |        |                       |              |         |
| No                             | 305    | 133                   | 60           |         |
| Yes                            | 35     | 16                    | 14           |         |
| Pre-op diabetes mellitus       |        |                       |              |         |
| No                             | 252    | 112                   | 52           |         |
| Yes                            | 88     | 37                    | 22           |         |
| Pre-op acute pancreatitis      |        |                       |              |         |
| No                             | 325    | 146                   | 69           |         |
| Yes                            | 15     | 3                     | 5            |         |
| Pre-op chronic pancreatitis    |        |                       |              |         |
| No                             | 247    | 106                   | 65           |         |
| Yes                            | 93     | 43                    | 9            |         |
| Pre-op septic cholangitis      |        |                       |              |         |
| No                             | 326    | 140                   | 71           |         |
| Yes                            | 14     | 9                     | 3            |         |
| Pre-op ascites                 |        |                       |              |         |

(Continued)
Table 1. (Continued)

|                  | Total n = 340 | No Complication | Complication |
|------------------|--------------|-----------------|--------------|
|                  | n = 117 (34.4%) | non severe (< IIIb) n = 149 (43.8%) | severe (≥ IIIb) n = 74 (21.8%) | p-value |
| No               | 337          | 117             | 148          | 72      | 0.161 |
| Yes              | 3            | 0               | 1            | 2       | 0.016* |

Charlson comorbidity index

|       | 0 | 1 | 2 | 3 | 4 | 5 | ≥6 |
|-------|---|---|---|---|---|---|----|
| n     | 27 | 21 | 146 | 82 | 35 | 12 | 17 |
| p-value | 2 | 4 | 22 | 23 | 12 | 6 | 5 |

Pre-op S-CA19-9 (U/ml)

|       | Median [range] |
|-------|----------------|
| No    | 23 [0–15250]   |
| Yes   | 27.5 [0.6–91630.3] |
| p-value | 0.92 |

Pre-op S-CEA (ng/ml)

|       | Median [range] |
|-------|----------------|
| No    | 2.2 [1.4–83.6] |
| Yes   | 1.7 [0.2–284.4] |
| p-value | 0.45 |

Pre-op S-Creatinine (mg/dl)

|       | Median [range] |
|-------|----------------|
| No    | 0.8 [0.1–9]    |
| Yes   | 0.9 [0.2–2]    |
| p-value | 0.764 |

Pre-op S-Bilirubin (mg/dl)

|       | Median [range] |
|-------|----------------|
| No    | 0.6 [0.2–7.8]  |
| Yes   | 0.8 [0.1–24.3] |
| p-value | 0.116 |

Pre-op S-Gamma-GT (U/l)

|       | Median [range] |
|-------|----------------|
| No    | 88 [9–1561]    |
| Yes   | 74 [7–3550]    |
| p-value | 0.4 |

Pre-op S-Alkaline phosphatase (U/I)

|       | Median [range] |
|-------|----------------|
| No    | 109 [33–1219]  |
| Yes   | 101 [34–1731]  |
| p-value | 0.243 |

Pre-op S-Amylase (U/I)

|       | Median [range] |
|-------|----------------|
| No    | 53.5 [13–267]  |
| Yes   | 60 [8–615]     |
| p-value | 0.627 |

Pre-op S-Lipase (U/I)

|       | Median [range] |
|-------|----------------|
| No    | 50.5 [3–555]   |
| Yes   | 43 [10–7000]   |
| p-value | 0.388 |

Pre-op S-CRP (mg/dl)

|       | Median [range] |
|-------|----------------|
| No    | 0.5 [0.5–30.8] |
| Yes   | 0.5 [0.1–32]   |
| p-value | 0.037 |

(Continued)
Table 1. (Continued)

|                                      | Total  | No Complication | Complication | p-value |
|--------------------------------------|--------|-----------------|--------------|---------|
|                                      | n = 340| n = 117 (34.4%) | n = 149      |         |
|                                      |        | non severe (<IIIb) | severe (≥IIIb) |         |
|                                      |        | (43.8%)          | n = 74      | (21.8%) |
| Pre-op P-Leukocytes (1/μl)           |        |                  |              |         |
| Median [range]                       |        | 6600 [1000–12500] | 7000 [890–24060] | 6980 [3250–23610] | 0.612 |
| <10,000                              | 300    | 106             | 130          | 64      |
| ≥10,000                              | 40     | 11              | 19           | 10      |
| Pre-op P-Hemoglobin (mg/dl)          |        |                  |              |         |
| Median [range]                       |        | 11.9 [7.5–16.7]  | 11.4 [5.7–16.1] | 10.9 [6.7–15.3]  | 0.004 |
| <11.5                                | 170    | 47              | 75           | 48      |
| 11.5–17                              | 170    | 70              | 74           | 26      |
| Pre-op heart rate (1/min)            |        |                  |              |         |
| Median [range]                       |        | 75 [40–100]     | 75 [50–110]  | 75 [55–120] | 0.025 |
| 60–100                               | 304    | 97              | 138          | 69      |
| <60 or >100                          | 36     | 20              | 11           | 5       |
| Pre-op systolic blood pressure (mmHg)|        |                  |              |         |
| Median [range]                       |        | 110–140         | 110–140      | 110–140 |
| 110–140                              | 234    | 79              | 106          | 49      |
| <110 or >140                         | 106    | 38              | 43           | 25      |
| Pre-op hospital stay (d)             |        |                  |              |         |
| Median [range]                       |        | 1 [1–43]        | 1 [1–25]     | 1 [1–34] | 0.145 |
| <5                                   | 290    | 105             | 126          | 59      |
| ≥5                                   | 15     | 12              | 23           | 15      |
| Anaesthesia time (h)                 |        |                  |              |         |
| Median [range]                       |        | 7.75 [2.75–13]  | 8 [3.25–12.5] | 7.3 [2.75–13] | 8.125 [3.5–13] | 0.006 |
| <7                                   | 112    | 34              | 62           | 16      |
| ≥7                                   | 228    | 83              | 87           | 58      |
| Operation time (h)                   |        |                  |              |         |
| Median [range]                       |        | 6 [1.5–12]      | 6 [1.5–10.3] | 5.75 [2–12] | 6.275 [2.5–11.5] | 0.104 |
| <3.5                                 | 105    | 36              | 53           | 16      |
| ≥3.5                                 | 235    | 81              | 96           | 58      |
| Epidural anaesthesia                 |        |                  |              |         |
| No                                   | 63     | 20              | 26           | 17      |
| Yes                                  | 277    | 97              | 123          | 57      |
| Intra-op blood transfusion           |        |                  |              |         |
| No                                   | 240    | 93              | 107          | 40      |
| Yes                                  | 100    | 24              | 42           | 34      |
| Surgical procedure                   |        |                  |              |         |
| Traverso-Longmire/Whipple            | 222    | 72              | 91           | 59      |
| Distal pancreatectomy                | 87     | 24              | 50           | 13      |
| Drainage operation (Beger, Frey, Partington-Rochelle) | 31 | 21 | 8 | 2 |
| Pancreateojunostomy                  |        |                  |              |         |
| No                                   | 87     | 28              | 47           | 12      |
| Yes                                  | 253    | 89              | 102          | 62      |
| Internal/external drainage           |        |                  |              |         |
| No                                   | 256    | 98              | 109          | 49      |
| Yes                                  | 84     | 19              | 40           | 25      |
| Spleen resection                     |        |                  |              |         |

(Continued)
Table 1. (Continued)

|                  | Total n = 340 | No Complication n = 117 (34.4%) | Complication non severe (<IIIb) n = 149 (43.8%) | Complication severe (≥IIIb) n = 74 (21.8%) | p-value |
|------------------|---------------|---------------------------------|-----------------------------------------------|---------------------------------------------|---------|
| No               | 254           | 90                              | 109                                           | 55                                          | 0.794   |
| Yes              | 86            | 27                              | 40                                            | 19                                          |         |
| Pancreatic tissue|               |                                 |                                               |                                             |         |
| Soft             | 181           | 55                              | 77                                            | 49                                          | 0.03    |
| Hard             | 159           | 62                              | 72                                            | 25                                          |         |
| Main pancreatic duct (mm) | |                                 |                                               |                                             |         |
| ≤2               | 178           | 60                              | 74                                            | 44                                          | 0.189   |
| >2               | 162           | 57                              | 75                                            | 30                                          |         |
| Histological diagnosis | |                                 |                                               |                                             |         |
| malignant        |               |                                 |                                               |                                             |         |
| Pancreatic Adenocarcinoma | 126          | 46                              | 60                                            | 20                                          | 0.209*  |
| Papillary carcinoma | 36            | 11                              | 10                                            | 15                                          |         |
| NET              | 36            | 10                              | 18                                            | 8                                           |         |
| Distal bile duct carcinoma | 19           | 2                               | 8                                             | 9                                           |         |
| Duodenal carcinoma | 3            | 0                               | 1                                             | 2                                           |         |
| Othertumors/metastasis | 5            | 2                               | 3                                             | 0                                           |         |
| benign           |               |                                 |                                               |                                             |         |
| Chronic pancreatitis | 68           | 33                              | 29                                            | 6                                           |         |
| IPMN             | 12            | 1                               | 7                                             | 4                                           |         |
| Pancreatic cystadenoma | 28           | 10                              | 10                                            | 8                                           |         |
| Adenoma of the papilla Vateri | 7        | 2                               | 3                                             | 2                                           |         |
| Tumor size (cm)  |               |                                 |                                               |                                             |         |
| ≤3               | 163           | 53                              | 69                                            | 41                                          | 0.619   |
| >3               | 62            | 18                              | 31                                            | 13                                          |         |
| UICC             |               |                                 |                                               |                                             |         |
| I-II             | 95            | 31                              | 41                                            | 23                                          | 0.904   |
| III-IV           | 113           | 36                              | 52                                            | 25                                          |         |
| T                |               |                                 |                                               |                                             |         |
| T1-T2            | 65            | 20                              | 25                                            | 20                                          | 0.203   |
| T3-T4            | 143           | 47                              | 68                                            | 28                                          |         |
| N                |               |                                 |                                               |                                             |         |
| N0               | 100           | 33                              | 43                                            | 24                                          |         |
| N1-2             | 108           | 34                              | 50                                            | 24                                          |         |
| M                |               |                                 |                                               |                                             |         |
| M0               | 200           | 65                              | 89                                            | 46                                          | 1       |
| M1               | 8             | 2                               | 4                                             | 2                                           |         |
| L                |               |                                 |                                               |                                             |         |
| L0               | 133           | 41                              | 60                                            | 32                                          | 0.835   |
| L1               | 75            | 26                              | 33                                            | 16                                          |         |
| V                |               |                                 |                                               |                                             |         |
| V0               | 174           | 59                              | 73                                            | 42                                          | 0.281   |
| V1               | 34            | 8                               | 20                                            | 6                                           |         |
| G                |               |                                 |                                               |                                             |         |
| G1               | 20            | 8                               | 6                                             | 6                                           | 0.558   |
| G2               | 116           | 35                              | 57                                            | 24                                          |         |
| G3               | 72            | 24                              | 30                                            | 18                                          |         |

(Continued)
Table 1. (Continued)

| Total | No Complication | Complication | p-value |
|-------|-----------------|--------------|---------|
| n = 340 | n = 117 (34.4%) | n = 149 (43.8%) | severe (≥IIb) n = 74 (21.8%) |

**R**
- R0: 175 (62) 75 (39) 0.074
- R1: 33 (5) 18 (9)

**Sirius red global score**
- A: 85 (40) 35 (10) <0.001*
- B: 81 (18) 40 (23)
- C: 12 (1) 3 (8)

**Stay in ICU/IMC (d)**
- Median [range]: 6 [0–150] 5 [0–14] 6 [1–48] 14.5 [1–150] <0.001
- <6: 163 (77) 74 (12)
- ≥6: 177 (40) 75 (62)

**Total stay in hospital (d)**
- Median [range]: 20 [2–377] 14 [8–43] 21 [9–67] 34 [2–377] <0.001
- <21: 186 (96) 72 (18)
- ≥21: 154 (21) 77 (56)

**Post-op S-Amylase (U/l)**
- Median [range]: 62 [10–903] 59 [3–825] 78 [7–1035] 0.025
- <40: 196 (77) 85 (34)
- ≥40: 144 (40) 64 (40)

**Amylase in drain fluid (U/l)**
- Median [range]: 30 [6–815] 248 [2–7693] 827 [7–52277] <0.001
- <120: 220 (117) 80 (24)
- ≥120: 120 (0) 69 (50)

**Post-op S-Lipase (U/l)**
- Median [range]: 39 [0–1088] 44.5 [1–1075] 68 [1–2648] 0.017
- <60: 204 (72) 98 (34)
- ≥60: 136 (45) 51 (40)

**Lipase in drain fluid (U/l)**
- Median [range]: 17.5 [4–1059] 236.5 [2–18940] 1935 [1–80561] <0.001
- <180: 220 (117) 78 (25)
- ≥180: 120 (0) 71 (49)

**Post-op S-CRP (mg/dl)**
- Median [range]: 8.1 [0.5–45] 7.6 [0.5–32.9] 8 [0.5–45] 11 [0.5–39.8] 0.139
- <8: 166 (63) 74 (29)
- ≥8: 174 (54) 75 (45)

**Post-op chemotherapy**
- No: 112 (26) 46 (40) <0.001
- Yes: 113 (45) 54 (14)

**Tumor recurrence**
- No: 111 (34) 48 (29) 0.782
- Yes: 114 (37) 52 (25)

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glandular tissue. Similarly, the grade of inflammation by lymphocytes and of fatty infiltration by adipocytes was classified in three grades as absent, focal, and generalized (Fig 1B). Based on this, a global scoring system for morphological classification of the resection margin of the pancreatic stump was developed. Resulting from the sum of the values for each of the three morphological features of fibrosis, inflammatory and fatty infiltration, three groups A-C were defined: A: resection margin of the pancreatic stump with no or marginal alterations of inflammation and steatosis but generalized fibrosis (3–4), B: resection margin of the pancreatic stump with moderate alterations (5–6), and C: resection margin of the pancreatic stump with severe alterations of inflammation and steatosis but absent or marginal fibrosis (7–9).

**Statistical analysis**

Statistical analyses were performed by SPSS Statistics Version 22 (IBM Corp. Armonk, NY) for Windows. Categorical parameters are reported as absolute and relative frequencies and

| Total | No Complication | Complication | p-value |
|-------|----------------|--------------|---------|
|       | n = 340        | n = 117 (34.4%) | non severe (<IIIb) n = 149 (43.8%) | severe (≥IIIb) n = 74 (21.8%) |
| No    | 117            | 37           | 50      | 30      | 0.795 |
| Yes   | 108            | 34           | 50      | 24      |

Bold values indicate significance (p ≤ 0.05, Fisher’s exact test Chi-Quadrat-test).

ASA, American Society of Anesthesiologists; CA-19-9, Cancer Antigen 19–9; CEA, Carcinoembryonic antigen; CRP, C-reactive protein; d, day; Gamma-GT, Gamma-glutamyl transferase; ICU, intensive care unit; IMC, intermediate care unit; IPMN, intraductal papillary mucinous neoplasm; n, number of patients; NET, Neuroendocrine tumor; NYHA, New York Heart Association; op, operative; P, Plasma; S, Serum; UICC, Union for international cancer control.

[Table 1. (Continued)]

![Fig 1. Histochemical staining by picrosirius red F3B. A: Resection margin of the pancreatic stump with focal perilobular and periacinar fibrosis, marginal steatosis, and absent inflammation. B: Resection margin of the pancreatic stump with generalized steatosis and marginal fibrosis.](https://doi.org/10.1371/journal.pone.0252727.g001)
continuous parameters as median [minimum–maximum]. All continuous variables were considered in a dichotomized form in further analyses. The association between clinicopathological parameters and the incidence of (no, non-severe, major) complications, and (no, BL, B, C) POPF was assessed by Fisher’s exact test. Uni- and multivariable logistic regression analyses were performed to identify independent risk factors for grade B/C POPF and major postoperative complications. All parameters with p ≤ 0.05 were included in the multivariable analyses. Results are reported as odds ratios (OR), corresponding 95% confidence intervals (CI) and p-values.

Overall survival and recurrence free survival were measured from the date of surgery to the time of the last follow-up or cancer-related death or tumor relapse, respectively, considering patients who were still alive or without evidence of tumor relapse at the end of the study as censored. The Kaplan-Meier method was used to generate overall and recurrence-free survival curves of curatively treated PDAC patients with and without major postoperative complications and grade B/C POPF. Survival curves were compared by the log-rank test. A Cox proportional-hazards regression model was used to assess the impact of potential risk factors on overall and recurrence-free survival. All parameters with p ≤ 0.05 were included in the multivariable models. A forward stepwise variable selection procedure based on the likelihood ratio test was applied. Results are reported as hazard ratios (HR), corresponding 95% CIs and p-values. All inferential statistics were intended to be exploratory. P ≤ 0.05 was considered statistically noticeable.

Results

Clinicopathologic characteristics

A total of 340 patients underwent pancreatic resection during the study period including 222 (65.3%) PD according to either Traverso-Longmire (211; 95%) or Whipple-Kausch (11; 5%), 87 (25.6%) DP, and 31 (9.1%) DPPHR. The median age of the patients was 64 (15–88) years, their median BMI 24.6 (13.1–51.6) kg/m², and 53.8% of patients were male. The distribution of various patient characteristics and physiological parameters by postoperative complication and POPF grade is shown in Tables 1 and 2. Postoperative complications were associated with age ≥ 60 years (p = 0.002), high BMI (p = 0.015), alcohol abuse (p = 0.035), cardiac and pulmonary comorbidity (p = 0.002; p = 0.005), absence of preoperative chronic pancreatitis (p = 0.001), high Charlson comorbidity index (p = 0.016), preoperative elevated serum level of CRP (p = 0.037), anemia (p = 0.004), preoperative heart rate up to 100/min (p = 0.025), prolonged anaesthesia time (p = 0.006), intraoperative blood transfusion (p = 0.001), pancreatojunostomy procedure (p = 0.004), use of internal or external pancreatic duct drainage (p = 0.015), soft pancreatic tissue (p = 0.03), high Picrosirius red histochemical staining score (p < 0.001), prolonged stay in ICU and hospital (each p < 0.001), elevated postoperative serum levels of amylase (p = 0.025) and lipase (p = 0.017), as well as high concentrations of amylase and lipase (each p < 0.001) in the drain fluid. POPF was associated with high BMI and ASA score (each p = 0.001), cardiac comorbidity (p = 0.011), absence of preoperative chronic pancreatitis (p < 0.001), high preoperative bilirubin serum level (p = 0.023), preoperative heart rate up to 100/min (p = 0.01), pancreatojunostomy procedure (p = 0.004), use of internal or external pancreatic duct drainage (p < 0.001), soft pancreatic tissue (p < 0.001), high Picrosirius red histochemical staining score (p < 0.001), prolonged stay in ICU and hospital (each p < 0.001), elevated postoperative serum levels of lipase (p = 0.017), as well as high concentrations of amylase and lipase (each p < 0.001) in the drain fluid.

Pancreatic anastomosis after PD was performed by duct-to-mucosa and end-to-side pancreaticojejunostomy in all patients. A surgical drain was placed in all patients. PD with
Table 2. Clinicopathological characteristics for postoperative pancreatic fistula.

|                        | Total n = 340 | w/o POPF n = 220 (64.7%) | Biochemical leak n = 59 (17.4%) | POPF B n = 30 (8.8%) | POPF C n = 31 (9.1%) | p-value |
|------------------------|---------------|--------------------------|---------------------------------|----------------------|----------------------|---------|
| Age (years)            |               |                          |                                 |                      |                      |         |
| Median [range]         | 64 (15–88)    | 64 (15–86)               | 63 (15–88)                      | 62.5 (21–81)         | 70 (46–82)           | 0.395   |
| ≤60                    | 144           | 97                       | 24                              | 13                   | 10                   |         |
| ≥60                    | 196           | 123                      | 35                              | 17                   | 21                   |         |
| Gender                 |               |                          |                                 |                      |                      |         |
| Female                 | 157           | 96                       | 32                              | 16                   | 13                   | 0.778   |
| Male                   | 183           | 124                      | 27                              | 14                   | 18                   |         |
| Body mass index (kg/m²)|               |                          |                                 |                      |                      |         |
| Median [range]         | 24.6 (13.1–51.6) | 24.3 (16.4–41)             | 24.1 (13.1–51.6) | 27.5 (19.2–35.6) | 25.9 (18.6–37.6) | 0.001   |
| ≤18                    | 165           | 117                      | 30                              | 7                    | 11                   |         |
| >18                    | 175           | 103                      | 29                              | 23                   | 20                   |         |
| Smoking                |               |                          |                                 |                      |                      |         |
| No                     | 268           | 171                      | 47                              | 25                   | 25                   | 0.864   |
| Yes                    | 72            | 49                       | 12                              | 5                    | 6                    |         |
| Alcohol                |               |                          |                                 |                      |                      |         |
| No                     | 310           | 195                      | 57                              | 28                   | 30                   | 0.322   |
| Yes                    | 30            | 25                       | 2                               | 2                    | 1                    |         |
| Weight loss            |               |                          |                                 |                      |                      |         |
| No                     | 312           | 197                      | 57                              | 29                   | 29                   | 0.442   |
| Yes                    | 28            | 23                       | 2                               | 1                    | 2                    |         |
| ASA                    |               |                          |                                 |                      |                      |         |
| 2-Jan                  | 247           | 155                      | 54                              | 20                   | 18                   | 0.001   |
| 4-Mar                  | 93            | 65                       | 5                               | 10                   | 13                   |         |
| NYHA                   |               |                          |                                 |                      |                      |         |
| 2-Jan                  | 315           | 206                      | 55                              | 27                   | 27                   | 0.561   |
| 4-Mar                  | 25            | 14                       | 4                               | 3                    | 4                    |         |
| Cardiac comorbidity    |               |                          |                                 |                      |                      |         |
| No                     | 161           | 108                      | 33                              | 9                    | 11                   | 0.011   |
| Yes                    | 179           | 112                      | 26                              | 21                   | 20                   |         |
| Pulmonary comorbidity  |               |                          |                                 |                      |                      |         |
| No                     | 305           | 199                      | 55                              | 23                   | 28                   | 0.106   |
| Yes                    | 35            | 21                       | 4                               | 7                    | 3                    |         |
| Pre-op diabetes mellitus|             |                          |                                 |                      |                      |         |
| No                     | 252           | 162                      | 47                              | 21                   | 22                   | 0.340   |
| Yes                    | 88            | 58                       | 12                              | 9                    | 9                    |         |
| Pre-op acute pancreatitis|            |                          |                                 |                      |                      |         |
| No                     | 325           | 208                      | 57                              | 29                   | 31                   | 0.323   |
| Yes                    | 15            | 12                       | 2                               | 1                    | 0                    |         |
| Pre-op chronic pancreatitis|         |                          |                                 |                      |                      | <0.001  |
| No                     | 247           | 147                      | 45                              | 27                   | 28                   |         |
| Yes                    | 93            | 73                       | 14                              | 3                    | 3                    |         |
| Pre-op septic cholangitis|            |                          |                                 |                      |                      |         |
| No                     | 327           | 209                      | 59                              | 29                   | 30                   | 1.000   |
| Yes                    | 13            | 11                       | 0                               | 1                    | 1                    |         |
| Pre-op ascites         |               |                          |                                 |                      |                      |         |
| No                     | 337           | 220                      | 57                              | 30                   | 30                   | 0.454   |
| Yes                    | 3             | 0                        | 2                               | 0                    | 1                    |         |

(Continued)
Table 2. (Continued)

|                        | Total n = 340 | w/o POPF n = 220 (64.7%) | Biochemical leak n = 59 (17.4%) | POPF B n = 30 (8.8%) | POPF C n = 31 (9.1%) | p-value |
|------------------------|--------------|--------------------------|-------------------------------|-------------------|-------------------|---------|
| **Charlson comorbidity index** |              |                          |                               |                   |                   |         |
| 0                      | 27           | 18                       | 8                             | 1                 | 0                 | 0.051†  |
| 1                      | 21           | 15                       | 2                             | 2                 | 2                 |         |
| 2                      | 146          | 96                       | 30                            | 12                | 8                 |         |
| 3                      | 82           | 54                       | 9                             | 7                 | 12                |         |
| 4                      | 35           | 19                       | 6                             | 6                 | 4                 |         |
| 5                      | 12           | 7                        | 0                             | 0                 | 5                 |         |
| ≥                      | 17           | 11                       | 4                             | 2                 | 0                 |         |

**Pre-op S-CA.19-9 (U/ml)**

|                        | Total n = 340 | w/o POPF n = 220 (64.7%) | Biochemical leak n = 59 (17.4%) | POPF B n = 30 (8.8%) | POPF C n = 31 (9.1%) | p-value |
|------------------------|--------------|--------------------------|-------------------------------|-------------------|-------------------|---------|
| Median [range]         |              |                          |                               |                   |                   |         |
| ≤30                    | 187          | 113                      | 41                            | 13                | 20                | 0.888   |
| >30                    | 153          | 107                      | 18                            | 17                | 11                |         |

**Pre-op S-CEA (ng/ml)**

|                        | Total n = 340 | w/o POPF n = 220 (64.7%) | Biochemical leak n = 59 (17.4%) | POPF B n = 30 (8.8%) | POPF C n = 31 (9.1%) | p-value |
|------------------------|--------------|--------------------------|-------------------------------|-------------------|-------------------|---------|
| Median [range]         |              |                          |                               |                   |                   |         |
| ≤5                     | 309          | 196                      | 57                            | 27                | 29                | 1.000   |
| >5                     | 31           | 24                       | 2                             | 3                 | 2                 |         |

**Pre-op S-Creatinine (mg/dl)**

|                        | Total n = 340 | w/o POPF n = 220 (64.7%) | Biochemical leak n = 59 (17.4%) | POPF B n = 30 (8.8%) | POPF C n = 31 (9.1%) | p-value |
|------------------------|--------------|--------------------------|-------------------------------|-------------------|-------------------|---------|
| Median [range]         |              |                          |                               |                   |                   |         |
| ≤1.1                   | 253          | 168                      | 47                            | 20                | 18                | 0.826   |
| >1.1                   | 87           | 52                       | 12                            | 10                | 13                |         |

**Pre-op S-Bilirubin (mg/dl)**

|                        | Total n = 340 | w/o POPF n = 220 (64.7%) | Biochemical leak n = 59 (17.4%) | POPF B n = 30 (8.8%) | POPF C n = 31 (9.1%) | p-value |
|------------------------|--------------|--------------------------|-------------------------------|-------------------|-------------------|---------|
| Median [range]         |              |                          |                               |                   |                   |         |
| ≤1.1                   | 89           | 62                       | 17                            | 5                 | 5                 | 0.023   |
| >1.1                   | 251          | 158                      | 42                            | 25                | 26                |         |

**Pre-op S-Gamma-GT (U/I)**

|                        | Total n = 340 | w/o POPF n = 220 (64.7%) | Biochemical leak n = 59 (17.4%) | POPF B n = 30 (8.8%) | POPF C n = 31 (9.1%) | p-value |
|------------------------|--------------|--------------------------|-------------------------------|-------------------|-------------------|---------|
| Median [range]         |              |                          |                               |                   |                   |         |
| ≤28                    | 178          | 113                      | 33                            | 17                | 15                | 0.889   |
| ≥28                    | 162          | 107                      | 26                            | 13                | 16                |         |

**Pre-op S-Alkaline phosphatase (U/I)**

|                        | Total n = 340 | w/o POPF n = 220 (64.7%) | Biochemical leak n = 59 (17.4%) | POPF B n = 30 (8.8%) | POPF C n = 31 (9.1%) | p-value |
|------------------------|--------------|--------------------------|-------------------------------|-------------------|-------------------|---------|
| Median [range]         |              |                          |                               |                   |                   |         |
| ≤104                   | 216          | 144                      | 39                            | 15                | 18                | 0.110   |
| ≥104                   | 124          | 76                       | 20                            | 15                | 13                |         |

**Pre-op S-AMylase (U/I)**

|                        | Total n = 340 | w/o POPF n = 220 (64.7%) | Biochemical leak n = 59 (17.4%) | POPF B n = 30 (8.8%) | POPF C n = 31 (9.1%) | p-value |
|------------------------|--------------|--------------------------|-------------------------------|-------------------|-------------------|---------|
| Median [range]         |              |                          |                               |                   |                   |         |
| ≤40                    | 223          | 144                      | 41                            | 19                | 19                | 0.658   |
| ≥40                    | 117          | 76                       | 18                            | 11                | 12                |         |

**Pre-op S-CRP (mg/dl)**

|                        | Total n = 340 | w/o POPF n = 220 (64.7%) | Biochemical leak n = 59 (17.4%) | POPF B n = 30 (8.8%) | POPF C n = 31 (9.1%) | p-value |
|------------------------|--------------|--------------------------|-------------------------------|-------------------|-------------------|---------|
| Median [range]         |              |                          |                               |                   |                   |         |
| ≤8                     | 314          | 199                      | 58                            | 28                | 29                | 1.000   |
| ≥8                     | 26           | 21                       | 1                             | 2                 | 2                 |         |

**Pre-op P-Leukocytes (1/μl)**

|                        | Total n = 340 | w/o POPF n = 220 (64.7%) | Biochemical leak n = 59 (17.4%) | POPF B n = 30 (8.8%) | POPF C n = 31 (9.1%) | p-value |
|------------------------|--------------|--------------------------|-------------------------------|-------------------|-------------------|---------|
| Median [range]         |              |                          |                               |                   |                   |         |
| ≤10,000                | 300          | 191                      | 52                            | 26                | 31                | 0.193   |
| ≥10,000                | 40           | 29                       | 7                             | 4                 | 0                 |         |
| Table 2. (Continued) | Total n = 340 | w/o POPF n = 220 (64.7%) | Biochemical leak n = 59 (17.4%) | POPF B n = 30 (8.8%) | POPF C n = 31 (9.1%) | p-value |
|---------------------|--------------|--------------------------|-------------------------------|-------------------|-------------------|---------|
| **Pre-op P-Hemoglobin (mg/dl)** | | | | | | 0.483 |
| Median [range] | 11.5 (5.7–16.7) | 11.5 (7.1–15.7) | 11.6 (7.2–16.1) | 11 (6.7–14.9) | | |
| <11.5 | 170 (49.4%) | 108 (48.6%) | 28 (47.4%) | 13 (43.3%) | 21 (67.7%) | |
| 11.5–17 | 170 (49.4%) | 112 (50.6%) | 31 (52.5%) | 17 (56.7%) | 10 (32.3%) | |
| **Pre-op heart rate (1/min)** | | | | | | 0.010 |
| Median [range] | 75 (40–120) | 80 (55–115) | 70 (60–105) | 70 (60–100) | | |
| 60–100 | 304 (89.4%) | 192 (87%) | 52 (88.1%) | 29 (96.7%) | 31 (100%) | |
| <60 or >100 | 36 (10.6%) | 28 (13%) | 7 (11.9%) | 1 (3.3%) | 0 (0%) | |
| **Pre-surgical systolic blood pressure (mmHg)** | | | | | | 1.000 |
| Median [range] | 110–140 | 110–140 | 110–140 | 110–140 | | |
| <110–140 | 234 (68.8%) | 149 (66.8%) | 43 (72.9%) | 22 (73.3%) | 20 (64.5%) | |
| ≥110 or >140 | 106 (31.2%) | 71 (33.2%) | 16 (27.1%) | 8 (26.7%) | 11 (35.5%) | |
| **Pre-op hospital stay (d)** | | | | | | 0.843 |
| Median [range] | 1 (1–43) | 1 (1–23) | 1 (1–34) | 1 (1–17) | | |
| <5 | 290 (85.3%) | 187 (83.6%) | 50 (83.3%) | 27 (89.4%) | 26 (83.9%) | |
| ≥5 | 50 (14.7%) | 33 (16.4%) | 9 (16.7%) | 3 (10.6%) | 5 (16.1%) | |
| **Anaesthesia time (h)** | | | | | | 0.766 |
| Median [range] | 7.75 (2.75–13) | 8 (2.75–12.5) | 6.5 (4–13) | 7.75 (4–11.75) | 8.25 (3.5–11.5) | |
| <7 | 112 (32.9%) | 61 (27.7%) | 33 (55.9%) | 12 (40%) | 6 (20%) | |
| ≥7 | 228 (67.1%) | 159 (72.3%) | 26 (44.1%) | 18 (60%) | 25 (80%) | |
| **Operation time (h)** | | | | | | 0.366 |
| Median [range] | 6 (1.5–12) | 6 (1.5–12) | 5 (2.25–11.5) | 5.8 (2.5–9) | 6.5 (3–8.75) | |
| <3.5 | 105 (30.3%) | 62 (28%) | 28 (47.4%) | 11 (36.7%) | 4 (12.9%) | |
| ≥3.5 | 235 (69.7%) | 158 (72%) | 31 (52.6%) | 19 (63.3%) | 27 (87.1%) | |
| **Epidural anaesthesia** | | | | | | 0.209 |
| No | 63 (18.5%) | 41 (18.6%) | 7 (12%) | 7 (23.3%) | 8 (26.6%) | |
| Yes | 277 (81.5%) | 179 (81.4%) | 52 (88%) | 23 (76.7%) | 23 (73.4%) | |
| **Intra-op blood transfusion** | | | | | | 0.878 |
| No | 240 (70.6%) | 149 (67%) | 49 (83.1%) | 24 (80%) | 18 (58.1%) | |
| Yes | 100 (29.4%) | 71 (33%) | 10 (16.9%) | 6 (20%) | 13 (41.9%) | |
| **Surgical procedure** | | | | | | 0.184 |
| Traverso-Longmire/Whipple | 222 (65.3%) | 147 (66.1%) | 29 (49.1%) | 18 (60%) | 28 (87.1%) | |
| Distal pancreatectomy | 87 (25.6%) | 48 (21.8%) | 27 (45.8%) | 9 (30%) | 3 (9.7%) | |
| Drainage operation (Beger, Frey, Partington-Rochelle) | 31 (9.1%) | 25 (11.1%) | 3 (5%) | 3 (10%) | 0 (0%) | |
| Pancreateojunostomy | | | | | | 0.004 |
| No | 87 (25.6%) | 54 (24.1%) | 24 (41%) | 7 (23.3%) | 2 (6.5%) | |
| Yes | 253 (74.4%) | 166 (75.9%) | 35 (59%) | 23 (76.7%) | 29 (93.5%) | |
| **Internal/external drainage** | | | | | | <0.001 |
| No | 256 (74.7%) | 183 (82.7%) | 40 (68%) | 16 (53.3%) | 17 (54.8%) | |
| Yes | 84 (25.3%) | 37 (17.3%) | 19 (32%) | 14 (46.7%) | 14 (45.2%) | |
| **Spleen resection** | | | | | | 0.262 |
| No | 254 (74.4%) | 161 (72.7%) | 44 (74.1%) | 24 (80%) | 25 (80.6%) | |
| Yes | 86 (25.6%) | 59 (27.3%) | 15 (25.9%) | 6 (20%) | 6 (20%) | |
| **Pancreatic tissue** | | | | | | <0.001 |
| Soft | 181 (53.2%) | 96 (43.6%) | 40 (68%) | 21 (69.4%) | 24 (77.4%) | |
| Hard | 159 (46.8%) | 124 (56.4%) | 19 (32%) | 9 (30.6%) | 7 (22.6%) | (Continued) |
Table 2. (Continued)

|                           | Total n = 340 | w/o POPF n = 220 (64.7%) | Biochemical leak n = 59 (17.4%) | POPF B n = 30 (8.8%) | POPF C n = 31 (9.1%) | p-value |
|---------------------------|--------------|--------------------------|---------------------------------|----------------------|----------------------|---------|
| **Main pancreatic duct (mm)** |              |                          |                                 |                      |                      |         |
| ≤2                        | 178          | 103                      | 37                              | 19                   | 19                   | 0.091   |
| >2                        | 162          | 117                      | 22                              | 11                   | 12                   |         |
| **Histological diagnosis** |              |                          |                                 |                      |                      |         |
| malignant                 |              |                          |                                 |                      |                      |         |
| Pancreatic Adenocarcinoma | 126          | 100                      | 14                              | 6                    | 6                    | 0.018   |
| Papillary carcinoma       | 36           | 15                       | 11                              | 4                    | 6                    |         |
| NET                       | 36           | 16                       | 11                              | 6                    | 3                    |         |
| Distal bile duct carcinoma | 19         | 10                       | 2                               | 2                    | 5                    |         |
| Duodenal carcinoma        | 3            | 1                        | 0                               | 1                    | 1                    |         |
| Other tumors/metastasis   | 5            | 2                        | 2                               | 1                    | 0                    |         |
| benign                    |              |                          |                                 |                      |                      |         |
| Chronic pancreatitis      | 68           | 55                       | 9                               | 2                    | 2                    |         |
| IPMN                      | 12           | 4                        | 3                               | 4                    | 1                    |         |
| Pancreatic cystadenom      | 28           | 14                       | 7                               | 2                    | 5                    |         |
| Adenoma of the papilla Vateri | 7         | 3                        | 0                               | 2                    | 2                    |         |
| **Tumor size (cm)**       |              |                          |                                 |                      |                      |         |
| ≤3                        | 163          | 100                      | 26                              | 18                   | 19                   | 0.012   |
| >3                        | 62           | 44                       | 13                              | 2                    | 3                    |         |
| **UICC**                  |              |                          |                                 |                      |                      |         |
| I-I                       | 95           | 57                       | 21                              | 9                    | 8                    | 0.714   |
| III-IV                    | 113          | 82                       | 13                              | 7                    | 11                   |         |
| **T**                     |              |                          |                                 |                      |                      |         |
| T1-T2                     | 65           | 35                       | 15                              | 6                    | 9                    | 0.113   |
| T3-T4                     | 143          | 104                      | 19                              | 10                   | 10                   |         |
| **N**                     |              |                          |                                 |                      |                      |         |
| N0                        | 100          | 60                       | 22                              | 10                   | 8                    | 0.712   |
| N1-2                      | 108          | 79                       | 12                              | 6                    | 11                   |         |
| **M**                     |              |                          |                                 |                      |                      |         |
| M0                        | 200          | 134                      | 32                              | 15                   | 19                   | 1       |
| M1                        | 8            | 5                        | 2                               | 1                    | 0                    |         |
| **L**                     |              |                          |                                 |                      |                      |         |
| L0                        | 133          | 87                       | 25                              | 10                   | 11                   | 0.699   |
| L1                        | 75           | 52                       | 9                               | 6                    | 8                    |         |
| **V**                     |              |                          |                                 |                      |                      |         |
| V0                        | 174          | 114                      | 29                              | 13                   | 18                   | 0.462   |
| V1                        | 34           | 25                       | 5                               | 3                    | 1                    |         |
| **G**                     |              |                          |                                 |                      |                      |         |
| G1                        | 20           | 10                       | 6                               | 1                    | 3                    | 0.922   |
| G2                        | 116          | 77                       | 20                              | 12                   | 7                    |         |
| G3                        | 72           | 52                       | 8                               | 3                    | 9                    |         |
| **R**                     |              |                          |                                 |                      |                      |         |
| R0                        | 175          | 111                      | 31                              | 15                   | 18                   | 0.080   |
| R1                        | 33           | 28                       | 3                               | 1                    | 1                    |         |
| **Sirius red global score**|            |                          |                                 |                      |                      |         |
| A                         | 85           | 76                       | 7                               | 1                    | 1                    | <0.001  |
| B                         | 81           | 36                       | 21                              | 11                   | 13                   |         |
| C                         | 12           | 1                        | 1                               | 6                    | 4                    |         |

(Continued)
### Table 2. (Continued)

|                                      | Total n = 340 | w/o POPF n = 220 (64.7%) | Biochemical leak n = 59 (17.4%) | POPF B n = 30 (8.8%) | POPF C n = 31 (9.1%) | p-value     |
|--------------------------------------|---------------|--------------------------|---------------------------------|----------------------|---------------------|-------------|
| **Stay in ICU/IMC (d)**              |               |                          |                                 |                      |                     |             |
| Median [range]                       | 6 (0–147)     | 5 (0–34)                 | 6 (1–39)                        | 8.5 (2–48)           | 25 (3–147)          | <0.001      |
| <6                                   | 163           | 130                      | 25                              | 7                    | 1                   |             |
| ≥6                                   | 177           | 90                       | 34                              | 23                   | 30                  |             |
| **Total stay in hospital (d)**       |               |                          |                                 |                      |                     |             |
| Median [range]                       | 20 (2–377)    | 16 (2–67)                | 20 (11–58)                      | 30 (9–74)            | 46 (13–377)         | <0.001      |
| <21                                  | 186           | 150                      | 31                              | 3                    | 2                   |             |
| ≥21                                  | 154           | 70                       | 28                              | 27                   | 29                  |             |
| **Post-op S-Amylase (U/l)**          |               |                          |                                 |                      |                     |             |
| Median [range]                       | 57 (0–903)    | 93.5 (15–825)            | 56.5 (10–340)                   | 114 (18–1035)        | 0.069               |             |
| <40                                  | 196           | 142                      | 24                              | 18                   | 12                  |             |
| ≥40                                  | 144           | 78                       | 35                              | 12                   | 19                  |             |
| **Amylase in drain fluid (U/l)**     |               |                          |                                 |                      |                     |             |
| Median [range]                       | 25 (2–118)    | 674 (142–4500)           | 977 (202–25700)                 | 1102 (138–52284)     | <0.001              |             |
| <120                                 | 220           | 220                      | 0                               | 0                    | 0                   |             |
| ≥120                                 | 120           | 0                        | 59                              | 30                   | 31                  |             |
| **Post-op S-Lipase (U/l)**           |               |                          |                                 |                      |                     |             |
| Median [range]                       | 31.5 (0–1088) | 62 (1–1075)              | 51 (14–2648)                    | 167.5 (4–1099)       | <0.001              |             |
| <60                                  | 204           | 151                      | 30                              | 16                   | 7                   |             |
| ≥60                                  | 136           | 69                       | 29                              | 14                   | 24                  |             |
| **Lipase in drain fluid (U/l)**      |               |                          |                                 |                      |                     |             |
| Median [range]                       | 17 (1–198)    | 2111.5 (231–18940)       | 3299.5 (400–33240)              | 4523.5 (138–80561)   | <0.001              |             |
| <180                                 | 220           | 220                      | 0                               | 0                    | 0                   |             |
| ≥180                                 | 120           | 0                        | 59                              | 30                   | 31                  |             |
| **Post-op S-CRP (mg/dl)**            |               |                          |                                 |                      |                     |             |
| Median [range]                       | 7.9 (0.5–45)  | 7.7 (0.5–27.6)           | 8.4 (1.3–27.5)                  | 12.1 (1.3–38.5)      | 0.262               |             |
| <8                                   | 166           | 108                      | 32                              | 15                   | 11                  |             |
| ≥8                                   | 174           | 112                      | 27                              | 15                   | 20                  |             |
| **Post-op chemotherapy**             |               |                          |                                 |                      |                     |             |
| No                                   | 113           | 58                       | 21                              | 14                   | 20                  | <0.001      |
| Yes                                  | 112           | 86                       | 18                              | 6                    | 2                   |             |
| **Tumor recurrence**                 |               |                          |                                 |                      |                     |             |
| No                                   | 112           | 69                       | 25                              | 11                   | 7                   | 0.392       |
| Yes                                  | 113           | 75                       | 14                              | 9                    | 15                  |             |
| **Tumor related death**              |               |                          |                                 |                      |                     |             |
| No                                   | 118           | 72                       | 26                              | 13                   | 7                   | 0.498       |
| Yes                                  | 107           | 72                       | 13                              | 7                    | 15                  |             |
| **Insufficiency of pancreatojejunostomy**|          |                          |                                 |                      |                     |             |
| No                                   | 220           | 220                      | 0                               | 0                    | 0                   | <0.001      |
| Yes                                  | 120           | 0                        | 59                              | 30                   | 31                  |             |
| **Hemorrhage**                       |               |                          |                                 |                      |                     |             |
| No                                   | 310           | 212                      | 54                              | 26                   | 18                  | <0.001      |
| Yes                                  | 30            | 8                        | 5                               | 4                    | 13                  |             |

(Continued)
## Table 2. (Continued)

| Complication Grade (Clavien-Dindo) | Total n = 340 | w/o POPF n = 220 (64.7%) | Biochemical leak n = 59 (17.4%) | POPF B n = 30 (8.8%) | POPF C n = 31 (9.1%) | p-value |
|-----------------------------------|--------------|---------------------------|-------------------------------|----------------------|----------------------|---------|
| No                                | 313          | 214                       | 56                           | 21                   | 22                   | <0.001  |
| Yes                               | 27           | 6                         | 3                            | 9                    | 9                    |         |
| Bile leak                         |              |                           |                               |                      |                      |         |
| No                                | 324          | 218                       | 58                           | 30                   | 18                   | <0.001  |
| Yes                               | 16           | 2                         | 1                            | 0                    | 13                   |         |
| Insufficiency of duodenojejunostomy|              |                           |                               |                      |                      |         |
| No                                | 321          | 216                       | 58                           | 30                   | 17                   | <0.001  |
| Yes                               | 19           | 4                         | 1                            | 0                    | 14                   |         |
| Lymphatic fistula                 |              |                           |                               |                      |                      |         |
| No                                | 325          | 213                       | 57                           | 29                   | 26                   | 0.035   |
| Yes                               | 15           | 7                         | 2                            | 1                    | 5                    |         |
| Pneumonia                         |              |                           |                               |                      |                      |         |
| No                                | 287          | 208                       | 52                           | 19                   | 8                    | <0.001  |
| Yes                               | 53           | 12                        | 7                            | 11                   | 23                   |         |
| Urinary tract infection           |              |                           |                               |                      |                      |         |
| No                                | 306          | 204                       | 54                           | 26                   | 22                   | 0.003   |
| Yes                               | 34           | 16                        | 5                            | 4                    | 9                    |         |
| Wound infection                   |              |                           |                               |                      |                      |         |
| No                                | 270          | 194                       | 52                           | 18                   | 6                    | <0.001  |
| Yes                               | 70           | 26                        | 7                            | 12                   | 25                   |         |
| Delayed gastric emptying          |              |                           |                               |                      |                      |         |
| No                                | 235          | 175                       | 42                           | 16                   | 2                    | <0.001  |
| Yes                               | 105          | 45                        | 17                           | 14                   | 29                   |         |
| Thrombosis                        |              |                           |                               |                      |                      |         |
| No                                | 329          | 216                       | 58                           | 27                   | 28                   | 0.006   |
| Yes                               | 11           | 4                         | 1                            | 3                    | 3                    |         |
| Pulmonary embolism                |              |                           |                               |                      |                      |         |
| No                                | 327          | 216                       | 56                           | 28                   | 27                   | 0.016   |
| Yes                               | 13           | 4                         | 3                            | 2                    | 4                    |         |
| Heart attack                      |              |                           |                               |                      |                      |         |
| No                                | 336          | 218                       | 59                           | 29                   | 30                   | 0.149   |
| Yes                               | 4            | 2                         | 0                            | 1                    | 1                    |         |

Bold values indicate significance (p ≤ 0.05, Fisher’s exact test Chi-Quadrat-test).

* ASA, American Society of Anesthesiologists; CA-19-9, Cancer Antigen 19–9; CEA, Carcinoembryonic antigen; CRP, C-reactive protein; d, day; Gamma-GT, Gamma-glutamyl transferase; ICU, intensive care unit; IMC, intermediate care unit; IPMN, intraductal papillary mucinous neoplasm; n, number of patients; NET, Neuroendocrine tumor; NYHA, New York Heart Association; op, operative; P, Plasma; S, Serum; UICC, Union for international cancer control.

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Pancreatic duct drainage was performed internally in 92.5% (n = 74) and externally in 7.5% (n = 6). The leading indication for surgery was malignancy (n = 225; 66.2%) including pancreatic ductal adenocarcinoma (PDAC) (n = 126; 56%), neuroendocrine carcinoma (n = 36; 16%), cancer of the ampulla of Vater (n = 36; 16%), distal bile duct cancer (n = 19; 8.4%), duodenal adenocarcinoma (n = 3; 1.3%), and other tumors (n = 5; 2.2%). A minority of the study cohort underwent surgery because of chronic pancreatitis (n = 68; 20%), IPMN (n = 12; 3.5%), or adenoma (n = 35; 10.3%). Median operative time was 390 min (range 210–690 min) for PD, 240 min (range 120–720 min) for DP, and 270 min (range 90–450 min) for DPPHR, respectively. A total of 100 patients (29.4%) received an intraoperative blood transfusion. 100 μg of Octreotide was subcutaneously administered TDS for five days postoperatively in all patients. The mean length of hospital and intensive care unit stay was 20 and 6 days, respectively. The 30 days mortality rate was 5% (n = 17) in total, 6.3% (n = 14) in the PD, 3.4% (n = 3) in the DP, and 0% in the DPPHR group.

**Morbidity risk factors**

The overall morbidity rate was 65.6% (n = 223) in total, with 67.6% (n = 150) in the PD, 72.4% (n = 63) in the DP, and 32.3% (n = 10) in the DPPHR group. Postoperative minor complications defined as grades I–IIa according to Clavien-Dindo were identified in 149 cases (43.8%) with 41.0% (n = 91) in the PD, 57.5% (n = 50) in the DP, and 25.8% (n = 8) in the DPPHR group. Delayed gastric emptying was detected in 70.5% (n = 105), abdominal wound infection in 47.0% (n = 70), pneumonia in 35.6% (n = 53), urinary tract infection in 22.8% (n = 34), thrombosis in 7.4% (n = 11), pulmonary embolism in 8.7% (n = 13), and heart attack in 4 cases (2.7%). Postoperative major complications defined as grades IIIb–V according to Clavien-Dindo were observed in 74 patients (21.8%) with 26.6% (n = 59) in the PD, 14.9% (n = 13) in the DP, and 6.5% (n = 2) in the DPPHR group. Bile leakage was detected in 21.6% (n = 16), POPF in 82.4% (n = 61), insufficiency of the duodenojejunostomy in 25.7% (n = 19), chyle leak in 20.3% (n = 15), and abdominal bleeding in 40.5% (n = 30). A total of 13 patients (3.8%) underwent a second operation due to abdominal bleeding associated with POPF. Of all patients, 59 (17.4%) were affected by BL, 30 (8.8%) by POPF grade B, and 31 (9.1%) by POPF grade C. The overall incidence of clinically relevant POPF was 17.9% (n = 61).

In the univariable logistic regression analysis of main perioperative patient characteristics, age ≥60 years (p = 0.028), increased BMI (p = 0.005), ASA 3–4, (p = 0.048), Charlson comorbidity index ≥3 (p<0.001), cardiac and pulmonary comorbidity (p = 0.009; p = 0.007), absence of preoperative chronic pancreatitis (p = 0.002), preoperative elevated levels of bilirubin (p = 0.035) and CRP in blood serum (p = 0.036), plasma haemoglobin levels <11.5 or >17 g/dl (p = 0.004), intraoperative blood transfusion (p = 0.001), prolonged anaesthesia time ≥7 hours (p = 0.021), no use of internal or external pancreatic duct drainage (p = 0.042), PD as surgical procedure (p = 0.039), soft pancreatic tissue (p = 0.012), high Picrosirius red histochemical staining score (p = 0.001), prolonged stay at hospital and at ICU (p<0.001), and elevated levels of postoperative amylase and lipase in drainage fluid and blood serum (each p<0.03) were associated with an increased probability of major postoperative complications (Table 3). Multivariable logistic regression analysis indicated a correlation between major complications after pancreatic surgery and pulmonary comorbidity (p = 0.005), high BMI (p = 0.023), preoperative CRP of ≥8 mg/l (p = 0.04), plasma haemoglobin levels <11.5 or >17 g/dl (p = 0.041), prolonged anaesthesia time (p = 0.018), stay at ICU ≥7 days (p = 0.008), and elevated amylase and lipase activity in the drainage fluid (each p<0.001).

In addition univariable logistic regression analysis showed a correlation between POPF (grade B/C) incidence and high BMI (p = 0.001), elevated ASA score (p = 0.028), Charlson
Table 3. Uni- and multivariable logistic regression analysis of risk factors for major (grade IIIb to V) postoperative complications.

| Variable                        | Subset                                | Univariate analysis | Multivariate analysis |
|---------------------------------|---------------------------------------|---------------------|-----------------------|
|                                 |                                       | OR [95% CI]         | p                     |
| Age                             | <60 vs ≥60 years                      | 0.540 (0.312–0.935) | 0.028                 |
| Gender                          | Female vs male                        | 0.859 (0.511–1.444) | 0.567                 |
| Body mass index                 | <18 or >25 vs 18–25 kg/m²             | 2.178 (1.271–3.731) | 0.005                 |
| Smoking                         | Yes vs No                             | 1.505 (0.761–2.976) | 0.240                 |
| Alcohol                         | No vs Yes                             | 0.527 (0.178–1.562) | 0.248                 |
| Weight loss                     | No vs Yes                             | 0.576 (0.193–1.716) | 0.322                 |
| ASA                             | 1–2 vs 3–4                            | 0.574 (0.332–0.995) | 0.048                 |
| NYHA                            | 1–2 vs 3–4                            | 0.572 (0.332–0.995) | 0.048                 |
| Cardiac morbidity               | No vs Yes                             | 0.488 (0.285–0.836) | 0.009                 |
| Pulmonary morbidity             | Yes vs No                             | 2.272 (1.308–5.665) | 0.007                 |
| Pre-op diabetes mellitus        | No vs Yes                             | 0.780 (0.441–1.380) | 0.394                 |
| Pre-op acute pancreatitis       | No vs Yes                             | 1.855 (0.614–5.606) | 0.273                 |
| Pre-op chronic pancreatitis     | No vs Yes                             | 3.333 (1.585–7.011) | 0.002                 |
| Pre-op septic cholangitis       | No vs Yes                             | 1.077 (0.289–4.020) | 0.912                 |
| Pre-op ascites                  | Yes vs No                             | 7.361 (0.658–82.332) | 0.105                 |
| Charlson comorbidity index      | ≥3 vs <3                              | 1.365 (1.133–1.617) | <0.001                |
| Pre-op S-CEA                    | <4.6 vs ≥4.6 ng/ml                    | 1.977 (0.669–5.841) | 0.218                 |
| Pre-op S-CA.19-9                | <37 vs ≥37 U/l                        | 1.021 (0.608–1.714) | 0.937                 |
| Pre-op S-Creatinine             | <1.25 vs ≥1.25 mg/dl                  | 0.814 (0.378–1.752) | 0.598                 |
| Pre-op S-Bilirubin (mg/dl)      | <1.1 vs ≥1.1 mg/dl                    | 0.549 (0.315–0.958) | 0.035                 |
| Pre-op S-Gamma-GT               | <28 U/l vs ≥28 U/l                    | 0.660 (0.352–1.235) | 0.192                 |
| Pre-op S-Alkaline phosphatase   | <175 vs ≥175 U/l                      | 1.091 (0.651–1.829) | 0.740                 |
| Pre-op S-Amylase                | <40 vs ≥40 U/l                        | 0.983 (0.576–1.679) | 0.950                 |
| Pre-op S-Lipase                 | <60 vs ≥60 U/l                        | 1.120 (0.647–1.937) | 0.685                 |
| Pre-op S-CRP                    | <8 vs ≥8 mg/dl                        | 0.410 (0.177–0.945) | 0.036                 |
| Pre-op P-Leukocytes             | 10.000 vs ≥10.000 /μl                 | 0.814 (0.378–1.752) | 0.598                 |
| Pre-op P-Hemoglobin             | 11.5–17 vs <11.5 or >17 g/dl          | 0.459 (0.269–0.783) | 0.004                 |
| Pre-op systolic blood pressure  | 110–140 vs <110 or >140 mmHg          | 0.858 (0.496–1.484) | 0.584                 |
| Pre-op heart rate               | 60–100 vs <60 or >100/min             | 1.820 (0.682–4.860) | 0.232                 |
| Intra-op blood transfusion      | Yes vs No                             | 2.576 (1.508–4.399) | 0.001                 |
| Pre-op hospital stay            | ≤5 vs >5                              | 0.596 (0.305–1.164) | 0.129                 |
| Anesthesia time                 | ≤7 vs >7                              | 0.489 (0.266–0.897) | 0.021                 |
| Operating time                  | ≤3.5 vs >3.5                          | 0.549 (0.298–1.009) | 0.053                 |
| Epidural anesthesia             | No vs Yes                             | 0.701 (0.374–1.314) | 0.268                 |
| Spleen resection                | No vs Yes                             | 1.026 (0.568–1.852) | 0.932                 |
| Internal/external drainage      | No vs Yes                             | 1.059 (0.319–0.980) | 0.042                 |
| Operation procedure             | Left resection vs Whipple/TLM         | 0.485 (0.251–0.939) | 0.032                 |
|                               | Drainage operation vs Whipple/TLM     | 0.191 (0.044–0.823) | 0.026                 |
| Pancreateojunostomy             | Yes vs No                             | 2.029 (1.035–3.978) | 0.039                 |
| Main pancreatic duct           | ≤2 vs >2 mm                           | 0.692 (0.410–1.167) | 0.168                 |
| Pancreatic tissue               | Soft vs hard                          | 1.990 (1.161–3.409) | 0.012                 |
| Histological diagnosis         | Malignant vs benign                   | 1.500 (0.847–2.655) | 0.164                 |
|                               | PDAC vs Chronic pancreatitis          | 1.862 (710–4.881)   | 0.206                 |
| Tumor size                      | <3 vs >3 mm                           | 1.088 (0.588–2.011) | 0.789                 |
| UICC                            | 3–4 vs 1–2                            | 1.105 (0.580–2.107) | 0.761                 |
| T                               | T3–4 vs T1–2                          | 1.825 (0.935–3.565) | 0.078                 |

(Continued)
comorbidity index $\geq 3$ ($p = 0.047$), cardiac comorbidity ($p = 0.009$), absence of preoperative chronic pancreatitis ($p = 0.001$), elevated levels of preoperative bilirubin ($p = 0.023$), preoperative tachycardia ($p = 0.034$), no use of internal or external pancreatic duct drainage ($p < 0.001$), soft pancreatic tissue ($p < 0.001$), high histochemical staining score ($p < 0.001$), prolonged stay in ICU and hospital (each $p < 0.001$), and elevated postoperative levels of amylase and lipase in drain fluid (each $p < 0.001$) (Table 4). Moreover, BMI ($p = 0.025$), high Picrosirius red global score ($p = 0.049$), prolonged stay in hospital (each $p < 0.001$), and elevated postoperative levels of amylase and lipase in drain fluid ($p \leq 0.001$) were identified as potential independent risk factors of POPF in multivariable logistic regression analysis.

Further multivariable logistic regression analyses showed a correlation of clinically relevant POPF and the postoperative major and minor complications, such as bile leakage ($p = 0.003$), abdominal haemorrhage and abscess (each $p < 0.001$), pneumonia ($p < 0.001$), and wound infection ($p = 0.003$) (Table 5).

Among patients that developed a POPF grade B ($n = 30$; 8.8%), a total of 28 (93.3%) were treated by computed tomography guided percutaneous drainage for infected intra-abdominal fluid collections and 3 angiographic procedures with successful coil embolization of a ruptured gastroduodenal artery pseudoaneurysm were performed. Surgical reoperations were required in 31 patients with POPF grade C (9.1%). In detail, 12 cases (38.7%) with recreation of the pancreaticojejunostomy, 4 cases (12.9%) with recreation of the pancreaticojejunostomy as well as the biliodigestive anastomosis, 7 cases (22.6%) with recreation of the pancreaticojejunostomy as well as the biliodigestive anastomosis followed by relaparotomy with resection of the pancreatic stump, 3 cases (9.7%) with primary resection of the pancreatic stump, 4 cases (12.9%) with intra-abdominal haematoma removal and resection of the bleeding pseudoaneurysm of the gastroduodenal artery stump, and 1 case (0.9%) with relaparotomy and removal of abdominal abscess formation were performed as postoperative complication management.
Table 4. Uni- and multivariable logistic regression analysis of risk factors for postoperative pancreatic fistula (B/C).

| Variable                                      | Subset                | Univariate analysis | Multivariate analysis |
|------------------------------------------------|-----------------------|---------------------|-----------------------|
|                                                 |                       | OR [95% CI]          | p                     | OR [95% CI]          | p         |
| Age                                             | <60 vs ≥60 years      | 0.765 (0.434–1.349) | 0.355                 |                      |           |
| Sex                                             | Female vs male        | 1.115 (0.649–1.934) | 0.699                 |                      |           |
| Body mass index                                 | <18 or >25 vs 18–25 kg/m² | 2.743 (1.510–4.982) | **0.001**            | 2.892 (1.143–7.318) | **0.025** |
| Smoking                                         | Yes vs No             | 1.147 (0.574–2.291) | 0.698                 |                      |           |
| Alcohol                                         | No vs Yes             | 0.473 (0.139–1.611) | 0.231                 |                      |           |
| Weight loss                                      | No vs Yes             | 0.515 (0.150–1.762) | 0.290                 |                      |           |
| ASA                                             | 1–2 vs 3–4            | 0.523 (0.293–0.933) | **0.028**            | 0.212 (0.043–1.037) | 0.055     |
| Cardiac comorbidity                             | No vs Yes             | 0.463 (0.259–0.828) | **0.009**            | 0.597 (0.223–1.603) | 0.306     |
| Pulmonary comorbidity                           | Yes vs No             | 1.946 (0.882–4.296) | 0.099                 |                      |           |
| Pre-op diabetes mellitus                        | No vs Yes             | 0.747 (0.408–1.369) | 0.345                 |                      |           |
| Pre-op acute pancreatitis                       | No vs Yes             | 0.309 (0.040–2.396) | 0.261                 |                      |           |
| Pre-op chronic pancreatitis                     | No vs Yes             | 4.251 (1.765–10.242)| **0.001**            | 1.725 (0.373–7.976) | 0.485     |
| Pre-op septic cholangitis                       | No vs Yes             | 0.806 (0.174–3.732) | 0.783                 |                      |           |
| Pre-op ascites                                  | Yes vs No             | 2.262 (0.202–25.351)| 0.508                 |                      |           |
| Charlson comorbidity index                      | ≥3 vs <3              | 1.162 (1.002–1.348) | **0.047**            | 0.872 (0.567–1.343) | 0.534     |
| Pre-op S-CEA                                    | <4.6 vs ≥4.6 ng/ml    | 1.176 (0.433–3.195) | 0.750                 |                      |           |
| Pre-op S-CA.19-9                                | <37 vs ≥37 U/ml       | 0.992 (0.571–1.725) | 0.977                 |                      |           |
| Pre-op S-Creatinine                             | <1.25 vs ≥1.25 mg/dl  | 1.058 (0.445–2.517) | 0.898                 |                      |           |
| Pre-op S-Bilirubin (mg/dl)                       | <1.1 vs ≥1.1 mg/dl    | 0.507 (0.282–0.911) | **0.023**            | 1.112 (0.372–7.976) | 0.849     |
| Pre-op S-Gamma-GT                               | < 28 U/l vs ≥ 28 U/l | 0.533 (0.274–1.116) | 0.098                 |                      |           |
| Pre-op S-Alkaline phosphatase                   | <175 vs ≥175 U/l      | 1.044 (0.601–1.812) | 0.879                 |                      |           |
| Pre-op S-Amylase                                | <40 vs ≥40 U/l        | 0.630 (0.361–1.102) | 0.105                 |                      |           |
| Pre-op S-Lipase                                 | <60 vs ≥60 U/l        | 0.866 (0.489–1.535) | 0.623                 |                      |           |
| Pre-op S-CRP                                    | <8 vs ≥8 mg/l         | 1.246 (0.414–3.754) | 0.696                 |                      |           |
| Pre-op P-Leukocytes                             | 10.000 vs ≥10.000 /μl | 2.157 (0.738–6.301) | 0.160                 |                      |           |
| Pre-op P-Hemoglobin                             | 11.5–17 vs <11.5 or >17 g/dl | 1.268 (0.730–2.203) | 0.400                 |                      |           |
| Pre-op systolic blood pressure                  | 110–140 vs <110 or >140 mmHg | 1.031 (0.568–1.872) | 0.920                 |                      |           |
| Pre-op heart rate                               | 60–100 vs <60 or >100/min | 0.114 (0.015–0.847) | **0.034**            | 0.112 (0.012–1.050) | 0.055     |
| Intra-op blood transfusion                      | Yes vs No             | 1.075 (0.591–1.956) | 0.814                 |                      |           |
| Pre-op hospital stay                            | <5 vs ≥5d             | 1.201 (0.533–2.705) | 0.658                 |                      |           |
| Anesthesia time                                 | <7 vs ≥7 h            | 0.879 (0.485–1.593) | 0.671                 |                      |           |
| Operating time                                  | <3.5 vs ≥3.5 h        | 0.739 (0.396–1.376) | 0.340                 |                      |           |
| Epidural anesthesia                             | No vs Yes             | 0.637 (0.329–1.234) | 0.181                 |                      |           |
| Spleen resection                                | No vs Yes             | 0.678 (0.342–1.346) | 0.267                 |                      |           |
| Internal/external drainage                      | No vs Yes             | 0.306 (0.172–0.547) | <0.001               | 0.668 (0.251–1.780) | 0.419     |
| Operation procedure                             | Left resection vs Whipple/TLM | 0.627 (0.343–1.317) | 0.247                 |                      |           |
| Drainage operation vs Whipple/TLM               | 0.410 (0.119–1.408)   | 0.157                 |                      |           |
| Pancreateojunostomy                             | Yes vs No             | 1.992 (0.964–4.117) | 0.063                 |                      |           |
| Main pancreatic duct                            | ≤2 vs >2 mm           | 0.590 (0.335–1.039) | 0.068                 |                      |           |
| Pancreatic tissue                               | Soft vs hard          | 3.045 (1.646–5.636) | <0.001               | 1.400 (0.382–5.125) | 0.611     |
| Histological diagnosis                          | Malignant vs benign   | 1.058 (0.587–1.908) | 0.850                 |                      |           |
| PDAC vs Chronic pancreatitis                    | 1.763 (0.522–5.630)   | 0.399                 |                      |           |
| Tumor size                                       | ≤3 vs >3mm            | 1.590 (0.783–3.229) | 0.199                 |                      |           |
| UICC                                            | 3–4 vs 1–2            | 1.204 (0.582–2.494) | 0.616                 |                      |           |
| T                                              | T3–4 vs T1–2          | 1.845 (0.875–3.890) | 0.107                 |                      |           |

(Continued)
Overall survival and tumor recurrence

Univariable Kaplan–Meier survival analysis showed a decreased overall survival ($p = 0.002$) and recurrence-free survival ($p<0.001$) in curatively treated PDAC patients UICC Stage I to III ($n = 126$) with POPF grade B or C versus PDAC patients without POPF or BL (Fig 2A and 2B). Decreased overall survival ($p<0.001$) and recurrence free survival ($p = 0.001$) was

Table 4. (Continued)

| Variable       | Subset                        | Univariate analysis | Multivariate analysis |
|----------------|-------------------------------|---------------------|-----------------------|
|                |                               | OR [95% CI]         | $p$                   | OR [95% CI]         | $p$                   |
| N              | N1-2 vs N0                    | 1.190 (0.575–2.462) | 0.640                 |                      |                      |
| G              | G3-4 vs G1-2                  | 1.009 (0.467–2.181) | 0.981                 |                      |                      |
| R              | R1-2 vs R0                    | 0.774 (0.362–1.652) | 0.507                 |                      |                      |
| L              | L1 vs L0                      | 3.707 (0.844–16.274)| 0.083                 |                      |                      |
| V              | V1 vs V0                      | 1.641 (0.538–5.006) | 0.385                 |                      |                      |
| M              | M1 vs M0                      | 1.444 (0.172–12.131)| 0.735                 |                      |                      |
| Stay in ICU/IMC| <7 vs ≥7d                     | 0.137 (0.065–0.288) | $<0.001$              | 0.347 (0.113–1.063)  | 0.064                 |
| Total stay in hospital| <21 vs ≥21d | 0.047 (0.018–0.121) | $<0.001$              | 0.123 (0.040–0.383)  | $<0.001$              |
| Post-op S-Amylase| <40 vs ≥40 U/l               | 0.633 (0.364–1.099) | 0.104                 |                      |                      |
| Amylase in drain fluid| <120 vs ≥120 U/l            | 0.005 (0.001–0.034) | $<0.001$              | 0.177 (0.034–0.107)  | 0.001                 |
| Lipase in drain fluid| <180 vs ≥180 U/l            | 0.004 (0.001–0.033) | $<0.001$              | 0.005 (0.001–0.044)  | $<0.001$              |
| Post-op S-CRP   | <8 vs ≥ 8 mg/l                | 0.712 (0.408–1.242) | 0.231                 |                      |                      |
| Sirius red global score| A vs B/C       | 0.042 (0.010–0.181) | $<0.001$              | 0.138 (0.019–0.991)  | 0.049                 |

Bold values indicate significance ($p \leq 0.05$). ASA, American Society of Anesthesiologists; CA-19-9, Cancer Antigen 19–9; CEA, Carcinoembryonic antigen; CI, confidence interval; CRP, C-reactive protein; d, day; Gamma-GT, Gamma-glutamyl transferase; OR, Odds ratio; ICU, intensive care unit; IMC, intermediate care unit; NYHA, New York Heart Association; op, operative; P, Plasma; S, Serum; UICC, Union for International cancer control.

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Overall survival and tumor recurrence

Univariable Kaplan–Meier survival analysis showed a decreased overall survival ($p = 0.002$) and recurrence-free survival ($p<0.001$) in curatively treated PDAC patients UICC Stage I to III ($n = 126$) with POPF grade B or C versus PDAC patients without POPF or BL (Fig 2A and 2B). Decreased overall survival ($p<0.001$) and recurrence free survival ($p = 0.001$) was

Table 5. Uni- and multivariable logistic regression analysis of the association between postoperative pancreatic fistula (B/C) and other post-surgical complications.

| Variable                                | Subset                        | Univariate analysis | Multivariate analysis |
|-----------------------------------------|-------------------------------|---------------------|-----------------------|
|                                        |                               | OR [95% CI]         | $p$                   | OR [95% CI]         | $p$                   |
| Hemorrhage                              | No vs Yes                     | 0.078 (0.037–0.164) | $<0.001$              | 0.136 (0.049–0.373)  | $<0.001$              |
| Abscess                                 | No vs Yes                     | 0.082 (0.035–0.194) | $<0.001$              | 0.087 (0.030–0.255)  | $<0.001$              |
| Bile Leak                               | No vs Yes                     | 0.041 (0.011–0.150) | $<0.001$              | 0.092 (0.019–0.456)  | 0.003                 |
| Insufficiency of duodenojejunostomy      | No vs Yes                     | 0.063 (0.022–0.182) | $<0.001$              | 0.414 (0.033–3.214)  | 0.399                 |
| Lymphatic fistula                       | No vs Yes                     | 0.312 (0.107–0.913) | 0.033                 | 2.261 (0.364–14.034) | 0.381                 |
| Pneumonia                               | No vs Yes                     | 0.060 (0.030–0.120) | $<0.001$              | 0.151 (0.061–0.372)  | $<0.001$              |
| Urinary tract infection                 | No vs Yes                     | 0.308 (0.145–0.656) | 0.002                 | 0.574 (0.162–2.029)  | 0.389                 |
| Wound infection                         | No vs Yes                     | 0.082 (0.044–0.154) | $<0.001$              | 0.272 (0.115–0.647)  | 0.003                 |
| Delayed gastric emptying                | No vs Yes                     | 0.155 (0.062–0.213) | $<0.001$              | 0.865 (0.312–2.393)  | 0.779                 |
| Thrombosis                              | No vs Yes                     | 0.171 (0.050–0.580) | 0.005                 | 1.021 (0.141–7.386)  | 0.984                 |
| Pulmonary embolism                      | No vs Yes                     | 0.241 (0.078–0.745) | 0.013                 | 1.024 (0.185–5.675)  | 0.979                 |
| Heart attack                            | No vs Yes                     | 0.217 (0.030–1.574) | 0.131                 | 0.122 (0.006–2.345)  | 0.164                 |
| Complication grade (Clavien-Dindo)      | <3b vs ≥3b                    | 0.062 (0.032–0.188) | $<0.001$              | 0.461 (0.160–1.329)  | 0.152                 |

Bold values indicate significance ($p \leq 0.05$). ASA, American Society of Anesthesiologists; CA-19-9, Cancer Antigen 19–9; CEA, Carcinoembryonic antigen; CI, confidence interval; CRP, C-reactive protein; d, day; Gamma-GT, Gamma-glutamyl transferase; HR, hazard ratio; ICU, intensive care unit; IMC, intermediate care unit; NYHA, New York Heart Association; op, operative; P, Plasma; S, Serum; UICC, Union for International cancer control.

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associated with the occurrence of major postoperative complications of Clavien-Dindo ≥3b in curatively treated PDAC patients as well (Fig 2C and 2D).

Univariable Cox regression analysis indicated improved overall and recurrence-free survival in PDAC patients with early UICC stage (p = 0.035; p = 0.048), low histological grading (p = 0.010; p = 0.002), low depth of invasion (p = 0.012; p = 0.013), and nodal invasion (p = 0.035; p = 0.048) as well as low NYHA score (p = 0.017; p = 0.049), elevated levels of presurgical tumor marker CA.19-9 (p = 0.012; p = 0.024), incidence of POPF grades B and C (p = 0.002; p < 0.001), and postoperative major complications according to Clavien-Dindo ≥3b (p < 0.001; p = 0.001) (Tables 6 and 7). In the multivariable Cox analysis NYHA (p = 0.004), UICC stage (p = 0.012), tumor differentiation (p = 0.001), depth of invasion (p < 0.001), nodal invasion (p = 0.044), and the incidence of postoperative major complications (p = 0.025) remained independent risk factors for overall survival. Prognostic markers of recurrence-free survival were UICC stage (p < 0.001), tumor differentiation (p < 0.001), depth of invasion (p = 0.001), nodal invasion (p = 0.001), postoperative major complications (p < 0.001), and the incidence of POPF grade B and C (p = 0.006).

The incidence of clinically relevant POPF (HR no vs yes [CI]: 0.100 [0.034–0.291]; p < 0.001) and major postoperative complications (HR no vs yes [CI]: 0.148 [0.041–0.530]; p = 0.003) correlated with early tumor recurrence within 6 months.

Univariable Cox regression analysis revealed improved overall and recurrence-free survival in NET patients with adjuvant chemotherapy (each p = 0.003). Decreased recurrence-free survival was associated with major postoperative complications of Clavien-Dindo ≥3b
(p = 0.022) in curatively treated NET patients. In patients with curatively treated papillary carcinoma, decreased overall and recurrence-free survival were associated with smoking (p = 0.002; p = 0.004), high histological grading (p = 0.036; p = 0.026), and no adjuvant chemotherapy (p = 0.031; p = 0.04). Multivariable Cox regression analyses revealed no significant risk factor for overall and recurrence-free survival in patients with NET or papillary carcinoma.

### Picrosirius red staining score

Histochemical staining by Picrosirius Red and its grading of fibrosis, inflammation, and steatosis were performed in pancreatic tissue sections of the resection margins of 178 consecutive study patients with PD since 2009. **Table 8** shows the distribution of the Picrosirius red global score and each of the three morphological features the score is based on in stained tissue samples by POPF grading. Low-grade fibrosis and high-grade inflammation or steatosis of the pancreatic remnant were associated with higher POPF grades (each p < 0.01). A correlation between the Picrosirius red staining score and the incidence of severe postoperative complications and clinically relevant POPF was also found in the univariable logistic regression analyses (p ≤ 0.001) (**Tables 3 and 4**). In multivariable logistic regression analyses, the Picrosirius red staining score correlated only with POPF (p = 0.049). Cox regression analysis for overall and

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**Table 6. Uni- and multivariable Cox regression analysis of overall survival in PDAC patients.**

| Variable                  | Subset | Univariate analysis | Multivariate analysis |
|---------------------------|--------|---------------------|-----------------------|
| Age (years)               | <60/≥60| 0.900 (0.572–1.415) | 0.647                 |
| Gender                    | Female/male | 1.026 (0.656–1.606) | 0.911                 |
| Body mass index (kg/m²)   | <18,≥18-25 | 1.053 (0.676–1.642) | 0.818                 |
| ASA                       | 1-2/3-4 | 0.718 (0.477–1.081) | 0.113                 |
| NYHA                      | 1-2/3-4 | 0.453 (0.236–0.870) | 0.017; 0.348 (0.170–0.713) | 0.004 |
| Smoker                    | No/Yes  | 0.681 (0.392–1.181) | 0.171                 |
| Alcohol                   | No/Yes  | 0.644 (0.203–2.043) | 0.644                 |
| Pre-surgical diabetes     | No/Yes  | 0.991 (0.605–1.622) | 0.971                 |
| Pre-surgical pancreatitis | No/Yes  | 0.754 (0.387–1.469) | 0.407                 |
| Pre-surgical CA 19-9 (U/l)| <37/≥37 | 0.514 (0.306–0.866) | 0.012; 0.738 (0.478–1.141) | 0.172 |
| Pre-surgical CEA (ng/ml)  | <4.6/≥4.6 | 0.833 (0.414–1.676) | 0.609                 |
| UICC stage                | I-II/III-IV | 0.598 (0.371–0.965) | 0.035; 0.107 (0.019–0.617) | 0.012 |
| Grading                   | G1-2/G3-4 | 0.559 (0.359–0.871) | 0.010; 0.497 (0.335–0.739) | 0.001 |
| T-stage                   | T1-2/T3-4 | 0.392 (0.188–0.817) | 0.012; 0.352 (0.202–0.613) | <0.001 |
| Nodal invasion            | N0/N1-2 | 0.598 (0.371–0.965) | 0.035; 5.899 (1.046–33.264) | 0.044 |
| Lymphatic invasion        | L0/L1    | 0.709 (0.455–1.104) | 0.128                 |
| Vene invasion             | V0/V1    | 1.184 (0.640–2.191) | 0.590                 |
| Resection margin          | R0/R1    | 0.931 (0.550–1.575) | 0.790                 |
| Surgery                   | left/head | 0.995 (0.581–1.704) | 0.986                 |
| Chemotherapy              | No/Yes   | 0.962 (0.568–1.630) | 0.886                 |
| Pancreatic fistula (B/C)  | No/Yes   | 0.362 (0.190–0.689) | 0.002; 0.779 (0.431–1.407) | 0.408 |
| Sirius red global score   | A/B/C    | 1.077 (0.600–1.933) | 0.805                 |
| Clavian-Dindo             | <3b/≥3b  | 0.283 (0.154–0.521) | <0.001; 0.592 (0.374–0.937) | 0.025 |

Bold values indicate significance (p ≤ 0.05).

1 HR, hazard ratio
2 CI, confidence interval.

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(p = 0.022) in curatively treated NET patients. In patients with curatively treated papillary carcinoma, decreased overall and recurrence-free survival were associated with smoking (p = 0.002; p = 0.004), high histological grading (p = 0.036; p = 0.026), and no adjuvant chemotherapy (p = 0.031; p = 0.04). Multivariable Cox regression analyses revealed no significant risk factor for overall and recurrence-free survival in patients with NET or papillary carcinoma.
Table 7. Uni- and multivariable Cox regression analysis of recurrence-free survival in PDAC patients.

| Variable                      | Subset       | Univariate analysis | Multivariate analysis |
|-------------------------------|--------------|---------------------|-----------------------|
|                              |              | HR [95% CI]         | p                     | HR [95% CI]         | p          |
| Age (years)                   | <60/>60      | 1.092 (0.699–1.706) | 0.698                 |                       |            |
| Gender                        | female/male  | 1.061 (0.681–1.655) | 0.793                 |                       |            |
| Body mass index               | <18, >25/18-25 | 1.020 (0.966–1.076) | 0.478                 |                       |            |
| ASA                           | 1-2/3-4      | 0.779 (0.520–1.166) | 0.255                 |                       |            |
| NYHA                          | 1-2/3-4      | 0.521 (0.272–0.997) | **0.049**             | 0.497 (0.279–1.172)  | 0.227      |
| Smoker                        | No/Yes       | 0.713 (0.417–1.220) | 0.217                 |                       |            |
| Alcohol                       | No/Yes       | 0.744 (0.283–2.117) | 0.618                 |                       |            |
| Pre-surgical diabetes         | No/Yes       | 1.072 (0.656–1.752) | 0.782                 |                       |            |
| Pre-surgical pancreatitis     | No/Yes       | 0.896 (0.461–1.740) | 0.745                 |                       |            |
| Pre-surgical CA,19-9 (U/l)    | <37/>37      | 0.555 (0.333–0.924) | **0.024**             | 0.672 (0.441–1.024)  | 0.064      |
| Pre-surgical CEA (ng/ml)      | <1.6/>1.6     | 1.057 (0.527–2.122) | 0.875                 |                       |            |
| UICC stage                    | I-II/III-IV  | 0.614(0.379–0.995)  | **0.048**             | 0.483 (0.293–0.781)  | <0.001     |
| Grading                       | G1-2/G3-4    | 0.503 (0.322–0.785) | **0.002**             | 0.484 (0.329–0.731)  | <0.001     |
| T-stage                       | T1-2/T3-4    | 0.395 (0.190–0.823) | **0.013**             | 0.417 (0.243–0.715)  | 0.001      |
| Nodal invasion                | N0/N1-2      | 0.614 (0.379–0.995) | **0.048**             | 19.375 (3.383–110.960)| 0.001      |
| Lymphatic invasion            | L0/L1        | 0.794 (0.510–1.236) | 0.307                 |                       |            |
| Vene invasion                 | V0/V1        | 1.316 (0.695–2.488) | 0.399                 |                       |            |
| Resection margin              | R0/R1        | 1.030 (0.594–1.785) | 0.916                 |                       |            |
| Surgery                       | left/head    | 0.894 (0.529–1.511) | 0.676                 |                       |            |
| Chemotherapy                  | No/Yes       | 1.031 (0.608–1.747) | 0.910                 |                       |            |
| Pancreatic fistula (B/C)      | No/Yes       | 0.263 (0.138–0.501) | **<0.001**            | 0.533 (0.339–0.837)  | **0.006**  |
| Sirius red global score       | A/B,C        | 0.915 (0.515–1.628) | 0.763                 |                       |            |
| Clavien-Dindo                 | <3b/>3b      | 0.340 (0.182–0.634) | **0.001**             | 0.246 (0.138–0.504)  | **<0.001** |

Bold values indicate significance (p ≤ 0.05).

1 HR, hazard ratio
2 CI, confidence interval.

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Discussion

In the last decades, advances in surgical techniques and in perioperative care, in addition to careful patient selection, have improved the outcomes in pancreatic surgery with considerable decrease of mortality rates below 5%. However, morbidity remains close to 50% even in high-volume centres, where clinically relevant POPF in 12 to 20% of patients continues to be the major cause of subsequent severe complications [2,3,34]. Numerous studies have identified a number of risk factors associated with an increased incidence of POPF including age, gender, BMI, pancreatic duct diameter, pancreas texture, operative time, and anastomosis technique [3,19,20,22,33]. Logistic regression analysis of clinicopathological parameters in our study cohort could confirm increased risk of major complications post pancreatic surgery in patients with advanced age, increased BMI and ASA, coexisting cardiopulmonary diseases, absence of preoperative chronic pancreatitis, intraoperative blood transfusion, prolonged operative and anesthesia time, PD procedure, soft pancreatic tissue, and absence of internal or external pancreatic duct stenting. Furthermore, preoperative anemia and elevated serum levels of bilirubin and CRP were associated with postoperative complications. The multivariate correlation of recurrence-free survival revealed no prognostic impact of Picrosirius red global score in the study group (Tables 6 and 7).
postoperative major complications and elevated preoperative serum levels of CRP \( \geq 8 \text{ mg/l} \) reflects the systemic inflammatory response of the study patients with predominantly malignant and chronic inflammatory pancreatic diseases. However, the results suggest that even a subtle rise in preoperative CRP may stratify patients into a higher risk group for postoperative major complications. A recent study by Oehme et al. identified preoperative anemia as a risk factor for postoperative complications greater than grade 2 according to Clavien-Dindo and as an independent prognostic factor for shorter overall survival in patients undergoing surgical procedures for pancreatic malignancies [35]. A preoperative serum bilirubin level of >3 mg/dl was the most significant risk factor for clinically relevant POPF in a retrospective analysis by Rungsakulkij et al. [36]. Univariable regression analysis revealed that the rise in the serum levels of amylase and lipase in the early postoperative period was associated with postoperative major complications suggesting manifestation of intraoperatively induced self-resolving pancreatitis [37]. In line with a multitude of reports, elevated amylase activity in the drainage fluid on POD 3 correlated with postoperative major complications, particularly with POPF [2,5,7,8,18,38,39]. The POPF definition by the ISGPS using amylase drain concentration is widely used. However, in accordance with our findings Tzedakis et al. could show that lipase is as effective as amylase drain concentration to define POPF [40]. Consequently, ICU and hospital stay were prolonged in patients with postoperative complications. In particular, the incidence of clinically relevant POPF correlated with a high score of histological Picrosirius red staining in multivariable analysis. The used Picrosirius red F3BA is a strong, linear anionic dye comprising six sulfonate groups that can associate along cationic collagen fibres. Picrosirius red staining developed by Junqueira et al. [31] provides a simple, specific, and sensitive method for localizing fibrillar collagen in tissue sections. In addition to the surgeon’s judgment about pancreatic texture by palpation, this histological tool allows a more objective assessment of the pancreatic remnant before reconstruction. There is broad agreement that fibrosis and low-grade fatty or inflammatory cell infiltration of the pancreatic parenchyma are POPF protective factors. It could be demonstrated that increased pancreatic fibrosis e.g. due to chronic

| Grading | Total | w/o POPF n = 113 (63.5%) | Biochemical leak n = 29 (16.3%) | POPF B n = 18 (10.1%) | POPF C n = 18 (10.1%) | \( p \)-value |
|---------|-------|----------------------|----------------------|----------------------|----------------------|------------------|
| **Fibrosis** | | | | | | |
| 1 (\(>20\%\)) | 93 (52.2%) | 81 (71.6%) | 8 (27.5%) | 1 (5.5%) | 3 (16.6%) | \(<0.001\) |
| 2 (11–20%) | 39 (21.9%) | 15 (13.2%) | 10 (34.3%) | 10 (55.5%) | 4 (22.2%) | |
| 3 (0–10%) | 46 (25.8%) | 17 (15%) | 11 (37.9%) | 7 (38.3%) | 11 (61.1%) | |
| **Inflammation** | | | | | | |
| 1 (0–10%) | 175 (98.3%) | 113 (100%) | 29 (100%) | 16 (88.8%) | 17 (94.4%) | \(0.008\) |
| 2 (11–20%) | 1 (0.5%) | 0 (0%) | 0 (0%) | 1 (5.5%) | 0 (0%) | |
| 3 (>20%) | 2 (1.1%) | 0 (0%) | 0 (0%) | 1 (5.5%) | 1 (5.5%) | |
| **Fat** | | | | | | |
| 1 (0–10%) | 69 (38.7%) | 54 (47.4%) | 8 (27.5%) | 1 (5.5%) | 6 (33.3%) | \(0.003\) |
| 2 (11–20%) | 74 (41.5%) | 44 (38.9%) | 15 (51.7%) | 8 (44.4%) | 7 (38.8%) | |
| 3 (>20%) | 35 (19.6%) | 15 (13.2%) | 6 (20.6%) | 9 (50%) | 5 (27.7%) | |
| **Global Score** | | | | | | |
| A (3–4) | 85 (47.7%) | 76 (67.2%) | 7 (24.1%) | 1 (5.5%) | 1 (5.5%) | \(<0.001\) |
| B (5–6) | 81 (45.5%) | 36 (31.8%) | 21 (72.4%) | 11 (61.1%) | 13 (72.2%) | |
| C (7–9) | 12 (6.7%) | 6 (5.3%) | 1 (3.4%) | 6 (33.3%) | 4 (22.2%) | |

Bold values indicate significance (\(p \leq 0.05\), Chi-Quadrat-test). POPF, postoperative pancreatic fistula; w/o, without.

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pancreatitis not only enables a more secure anastomosis with solid fixing sutures but was also associated with decreased exocrine activity with reduced pancreatic juice output [32,33,41–43]. Therefore, intraoperative knowledge about the pancreatic texture in addition to the dignity of the resection margin of the pancreatic stump could help surgeons to adjust their surgical procedure. Besides the choice of the anastomotic technique, the use of intraoperative internal or external pancreatic duct stenting or even total pancreatectomy to prevent devastating POPF could be the consequence. We believe that further prospective studies evaluating the impact of intraoperative frozen section histology on anastomotic technique and outcome could help pancreatic surgeons prevent clinically relevant POPF.

In addition to patient characteristics and blood tests, the preoperative risk assessment of non-invasive cross-sectional imaging related to POPF has been the focus of several research groups [44,45]. A systematic review and meta-analysis of POPF prediction using preoperative computed tomography (CT) scan by Yue et al. revealed a significant increase in the incidence of clinically relevant POPF in patients with visceral obesity and sarcopenic obesity [46]. Furthermore, a narrow pancreatic duct assessed by preoperative CT images significantly related to POPF [47]. Multiparametric magnetic resonance imaging of the pancreas by Yoon et al. could enable accurate quantification of pancreatic fibrosis and steatosis, which have been shown to be associated with POPF [48]. The correlation of preoperative cross-sectional imaging with the surgeon’s judgment about pancreatic texture by palpation and intraoperative histological findings may allow a more objective assessment of the pancreatic remnant to prevent POPF and should be evaluated in future studies.

Although high grade POPF is universally regarded as a major source of early postoperative morbidity and mortality, its role in oncological outcome remains uncertain. Indeed, only a few studies have investigated the impact of clinically relevant POPF on PDAC specific survival and recurrence with contradictory results [6,49–51]. However, recent reports have demonstrated that early initiation of adjuvant chemotherapy was an important prognostic factor in patients with PDAC as severe POPF significantly prolonged initiation of adjuvant chemotherapy after primary surgery [52,53]. In curative resected PDAC patients of our study, Cox regression analyses indicated a survival benefit for low UICC and NYHA stages, low grading, low tumor invasion, absence of nodal invasion, low preoperative serum level of CA.19-9, and low incidence of postoperative major complications according to Clavien-Dindo. Advanced UICC stages and tumor differentiation, tumor invasion, nodal invasion, high preoperative serum level of CA.19-9, and the incidence of major complications, in particular clinically relevant POPF were identified as potentially favorable factors for tumor recurrence. Moreover, high grade POPF correlated with recurrence-free survival in multivariable analysis. Our results are in line with the negative influence of anastomotic leakage on survival outcome with high incidence of local recurrence in other gastrointestinal carcinoma entities [54]. It is supposed that anastomotic leakage leads to inflammation with the release of pro-inflammatory cytokines that alter host defense and promote growth of residual cancer cells [55]. In PDAC, Nagai et al. were able to demonstrate that clinically relevant POPF was an independent prognostic factor for peritoneal tumor recurrence [51]. Interestingly, even 28% of R0 curative resected patients with invasive PDAC revealed postoperatively higher cytology-positive rates in the drained fluid from the pancreatic bed with subsequent development of local recurrence [56].

Taken together, in addition to known risk factors the Picrosirius red staining comes along with a high diagnostic potential in the risk management of POPF that correlates with tumor recurrence in this study cohort. Further multicenter studies with a larger number of PDAC patients are required to reevaluate the value of intraoperatively extended histological diagnostic as well as the impact of high grade POPF as an independent survival predictor.
Conclusion
This study indicates the high potential of pre-surgical risk stratification of known clinical risk factors and the intraoperative histopathological diagnostic of fibrosis, fatty and inflammatory cell infiltration in the resection margins of pancreatic stumps of curatively resected PDAC patients to prevent devastating POPF. Its prevention is urgently needed as clinically relevant POPF seems to be a prognostic factor of tumor recurrence in PDAC.

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Author Contributions
Conceptualization: Sameer A. Dhayat, Norbert Senninger.
Data curation: Sameer A. Dhayat, Ahmad N. J. Tamim, Marius Jacob, Georg Ebeling, Iyad Kabar, Norbert Senninger.
Formal analysis: Sameer A. Dhayat, Ahmad N. J. Tamim, Laura Kerschke.
Funding acquisition: Sameer A. Dhayat, Iyad Kabar, Norbert Senninger.
Investigation: Sameer A. Dhayat, Ahmad N. J. Tamim, Marius Jacob, Georg Ebeling, Iyad Kabar.
Methodology: Sameer A. Dhayat, Ahmad N. J. Tamim, Laura Kerschke.
Project administration: Sameer A. Dhayat, Norbert Senninger.
Resources: Sameer A. Dhayat, Norbert Senninger.
Software: Sameer A. Dhayat, Ahmad N. J. Tamim, Laura Kerschke.
Supervision: Sameer A. Dhayat, Laura Kerschke.
Validation: Sameer A. Dhayat, Ahmad N. J. Tamim, Georg Ebeling, Laura Kerschke.
Visualization: Sameer A. Dhayat, Ahmad N. J. Tamim.
Writing – original draft: Sameer A. Dhayat.
Writing – review & editing: Sameer A. Dhayat, Ahmad N. J. Tamim, Georg Ebeling, Laura Kerschke, Iyad Kabar, Norbert Senninger.

References
1. Grobmyer SR, Pieracci FM, Allen PJ, Brennan MF, Jaques DP (2007) Defining morbidity after pancreatoduodenectomy: use of a prospective complication grading system. Journal of the American College of Surgeons 204: 356–364. https://doi.org/10.1016/j.jamcollsurg.2006.11.017 PMID: 17324768
2. DeOliveira ML, Winter JM, Schafer M, Cunningham SC, Cameron JL, et al. (2006) Assessment of complications after pancreatic surgery: A novel grading system applied to 633 patients undergoing pancreatoduodenectomy. Annals of surgery 244: 931–937; discussion 937–939. https://doi.org/10.1097/01.sla.0000246856.03918.9a PMID: 17122618
3. Greenblatt DY, Kelly KJ, Rajamanickam V, Wan Y, Hanson T, et al. (2011) Preoperative factors predict perioperative morbidity and mortality after pancreatoduodenectomy. Annals of surgical oncology 18: 2126–2135. https://doi.org/10.1245/s10434-011-1594-6 PMID: 21336514
4. Buchler MW, Friess H, Wagner M, Kulli C, Wagener V, et al. (2000) Pancreatic fistula after pancreatic head resection. The British journal of surgery 87: 883–889. https://doi.org/10.1046/j.1365-2168.2000.01465.x PMID: 10931023

5. Kleeff J, Diener MK, Z’Graggen K, Hinz U, Wagner M, et al. (2007) Distal pancreatectomy: risk factors for surgical failure in 302 consecutive cases. Annals of surgery 245: 573–582. https://doi.org/10.1097/01.sla.0000251438.43135.fb PMID: 17414606

6. Murakami Y, Uemura K, Sudo T, Hashimoto Y, Kondo N, et al. (2013) Early initiation of adjuvant chemotherapy improves survival of patients with pancreatic carcinoma after surgical resection. Cancer chemotherapy and pharmacology 71: 419–429. https://doi.org/10.1007/s00280-012-2029-1 PMID: 23178955

7. Bassi C, Dervenis C, Butturini G, Fingerhut A, Yeo C, et al. (2005) Postoperative pancreatic fistula: an international study group (ISGPF) definition. Surgery 138: 8–13. https://doi.org/10.1016/j.surg.2005.05.001 PMID: 16003309

8. Bassi C, Marchegiani G, Dervenis C, Sarr M, Abu Hilal M, et al. (2017) The 2016 update of the International Study Group (ISGPF) definition and grading of postoperative pancreatic fistula: 11 Years After. Surgery 161: 584–591. https://doi.org/10.1016/surgery.2016.11.014 PMID: 28042557

9. Keck T, Wellner UF, Bahra M, Klein F, Sick O, et al. (2016) Pancreategastrostomy Versus Pancreatojejunostomy for RECONstruction After PANcreatoDudenoNeCtomy (RECONPD, DRKS 00000767): Perioperative and Long-term Results of a Multicenter Randomized Controlled Trial. Annals of surgery 263: 440–449. https://doi.org/10.1097/SLA.0000000000001240 PMID: 26135690

10. Yeo CJ, Cameron JL, Maher MM, Sauter PK, Zahurak ML, et al. (1995) A prospective randomized trial of pancreaticogastrostomy versus pancreateojejunostomy after pancreatoduodenectomy. Annals of surgery 222: 580–588; discussion 588–592. https://doi.org/10.1097/00000658-199510000-00014 PMID: 7574936

11. Bassi C, Falconi M, Molinari E, Mantovani W, Butturini G, et al. (2003) Duct-to-mucosa versus end-to-side pancreateojejunostomy reconstruction after pancreatoduodenectomy: results of a prospective randomized trial. Surgery 134: 766–771. https://doi.org/10.1016/s0039-6060(03)00345-3 PMID: 14639354

12. Yeo CJ, Cameron JL, Lillemoe KD, Sauter PK, Coleman J, et al. (2000) Does prophylactic ocreotide decrease the rates of pancreatic fistula and other complications after pancreaticoduodenectomy? Results of a prospective randomized placebo-controlled trial. Annals of surgery 232: 419–429. https://doi.org/10.1097/00000658-200009000-00014 PMID: 10973392

13. Berger AC, Howard TJ, Kennedy EP, Sauter PK, Bower-Cherry M, et al. (2009) Does type of pancreatojejunostomy after pancreatoduodenectomy decrease rate of pancreatic fistula? A randomized, prospective, dual-institution trial. Journal of the American College of Surgeons 208: 738–747; discussion 747–739. https://doi.org/10.1016/j.jamcollsurg.2008.12.031 PMID: 19476827

14. Lillemoe KD, Cameron JL, Kim MP, Campbell KA, Sauter PK, et al. (2004) Does fibrin glue sealant decrease the rate of pancreatic fistula after pancreaticoduodenectomy? Results of a prospective randomized trial. Journal of gastrointestinal surgery: official journal of the Society for Surgery of the Alimentary Tract 8: 766–772; discussion 772–764. https://doi.org/10.1016/j.jgassur.2004.06.011 PMID: 15531229

15. Winter JM, Cameron JL, Campbell KA, Chang DC, Riall TS, et al. (2006) Does pancreatic duct stenting decrease the rate of pancreatic fistula following pancreatoduodenectomy? Results of a prospective randomized trial. Journal of gastrointestinal surgery: official journal of the Society for Surgery of the Alimentary Tract 10: 1280–1290; discussion 1290. https://doi.org/10.1016/j.jgassur.2006.07.020 PMID: 17114014

16. Lowy AM, Lee JE, Pisters PW, Davidson BS, Fenoglio CJ, et al. (1997) Prospective, randomized trial of ocreotide to prevent pancreatic fistula after pancreaticoduodenectomy for malignant Annals of surgery 226: 632–641. https://doi.org/10.1097/00000658-199711000-00008 PMID: 9389397

17. Shrikhande SV, Sivasanker M, Vollmer CM, Friess H, Besselink MG, et al. (2017) Pancreatic anastomosis after pancreatoduodenectomy: A position statement by the International Study Group of Pancreatic Surgery (ISGPS). Surgery 161: 1221–1234. https://doi.org/10.1016/j.surg.2016.11.021 PMID: 28027816

18. Pedrazzoli S (2017) Pancreatoduodenectomy (PD) and postoperative pancreatic fistula (POPF): A systematic review and analysis of the POPF-related mortality rate in 60,739 patients retrieved from the English literature published between 1990 and 2015. Medicine 96: e6855. https://doi.org/10.1097/MD.000000000000251438.43135.fb PMID: 28489778

19. Uzunoglu FG, Reeh M, Vettorazzi E, Ruschke T, Hannah P, et al. (2014) Preoperative Pancreatic Resection (PREPARE) score: a prospective multicenter-based morbidity risk score. Annals of surgery 260: 857–863; discussion 863–854. https://doi.org/10.1097/SLA.0000000000000946 PMID: 25243549

20. Mungroop TH, van Rijssen LB, van Klaveren D, Smits FJ, van Woerden V, et al. (2019) Alternative Fistula Risk Score for Pancreatoduodenectomy (a-FRS): Design and International External Validation. Annals of surgery 269: 937–943. https://doi.org/10.1097/SLA.0000000000002620 PMID: 29240007
21. Miller BC, Christein JD, Behrman SW, Dreibin JA, Pratt WB, et al. (2014) A multi-institutional external validation of the fistula risk score for pancreatoduodenectomy. Journal of gastrointestinal surgery: official journal of the Society for Surgery of the Alimentary Tract 18: 172–179; discussion 179–180. https://doi.org/10.1007/s11605-013-2337-8 PMID: 24002771

22. Callery MP, Pratt WB, Kent TS, Chaikof EL, Voller CM Jr. (2013) A prospectively validated clinical risk score accurately predicts pancreatic fistula after pancreatoduodenectomy. Journal of the American College of Surgeons 216: 1–14. https://doi.org/10.1016/j.jamcollsurg.2012.09.002 PMID: 23122535

23. Neary WD, Heather BP, Earnshaw JJ (2003) The Physiological and Operative Severity Score for the enUmeration of Mortality and morbidity (POSSUM). The British journal of surgery 90: 157–165. https://doi.org/10.1002/bjs.4041 PMID: 12555290

24. Charlson ME, Pompei P, Ales KL, MacKenzie CR (1987) A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. Journal of chronic diseases 40: 373–383. https://doi.org/10.1016/0021-9681(87)90171-8 PMID: 3558716

25. Dindo D, Demartines N, Clavien PA (2004) Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Annals of surgery 240: 205–213. https://doi.org/10.1097/01.sla.0000130635.54934.ae PMID: 15273542

26. Clavien PA, Barkun J, de Oliveira ML, Vauthey JN, Dindo D, et al. (2009) The Clavien-Dindo classification of surgical complications: five-year experience. Annals of surgery 250: 187–196. https://doi.org/10.1097/SLA.0b013e3181e13ca2 PMID: 19638912

27. Koch M, Garden OJ, Padbury R, Rahbari NN, Adam R, et al. (2011) Bile leakage after hepatobiliary and pancreatic surgery: a definition and grading of severity by the International Study Group of Liver Surgery. Surgery 149: 680–688. https://doi.org/10.1016/j.surg.2010.12.002 PMID: 21316725

28. Besselink MG, van Rijssen LB, Bassi C, Dervenis C, Montorsi M, et al. (2017) Definition and classification of chyle leak after pancreatic operation: A consensus statement by the International Study Group on Pancreatic Surgery. Surgery 161: 365–372. https://doi.org/10.1016/j.surg.2016.06.058 PMID: 27692778

29. Wente MN, Veit JA, Bassi C, Dervenis C, Fingerhut A, et al. (2007) Postpancreatectomy hemorrhage (PPH): an International Study Group of Pancreatic Surgery (ISGPS) definition. Surgery 142: 20–25. https://doi.org/10.1016/j.surg.2007.02.001 PMID: 17628996

30. Wente MN, Bassi C, Dervenis C, Fingerhut A, Gouma DJ, et al. (2007) Delayed gastric emptying (DGE) after pancreatic surgery: a suggested definition by the International Study Group of Pancreatic Surgery (ISGPS). Surgery 142: 761–768. https://doi.org/10.1016/j.surg.2007.05.005 PMID: 17981197

31. Junqueira LC, Bignolas G, Brentani RR (1979) Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. The Histochemical journal 11: 447–455. https://doi.org/10.1007/BF01002772 PMID: 91593

32. Ridolfi C, Angiolini MR, Gavazzi F, Spaggiari P, Tinti MC, et al. (2014) Morphohistological features of pancreatic stump are the main determinant of pancreatic fistula after pancreatoduodenectomy. BioMed research international 2014: 641239. https://doi.org/10.1155/2014/641239 PMID: 24900974

33. Gaujoux S, Cortes A, Couvelard A, Noullet S, Clavel L, et al. (2010) Fatty pancreas and increased body mass index are risk factors of pancreatic fistula after pancreatocoduodenectomy. Surgery 148: 15–23. https://doi.org/10.1016/j.surg.2009.12.005 PMID: 20138325

34. de Castro SM, Busch OR, van Gulik TM, Obertop H, Gouma DJ (2005) Incidence and management of pancreatic leakage after pancreatoduodenectomy. The British journal of surgery 92: 1117–1123. https://doi.org/10.1002/bjs.5047 PMID: 15931856

35. Oehme F, Hempel S, Knote R, Addai D, Distler M, et al. (2021) Perioperative Blood Management of Preoperative Anemia Determines Long-Term Outcome in Patients with Pancreatic Surgery. Journal of gastrointestinal surgery: official journal of the Society for Surgery of the Alimentary Tract. https://doi.org/10.1007/s11605-021-04917-2 PMID: 33575903

36. Rungsakulij N, Mingphruedhi S, Tangtawee P, Krutsri C, Muangkaew P, et al. (2017) Risk factors for Drain Fluid Amylase on Postoperative Day 1 for Predicting Clinically Relevant Fistula After Distal Pancreatectomy: A Multi-institutional Analysis and External Validation. Annals of surgery 269: 337–343. https://doi.org/10.1097/SLA.0000000000002532 PMID: 28938266

37. Connor S (2016) Defining post-operative pancreatitis as a new pancreatic specific complication following pancreatic resection. HPB: the official journal of the International Hepato Pancreateo Biliary Association 18: 642–651. https://doi.org/10.1016/j.hpb.2016.05.006 PMID: 27485058

38. Maggino L, Malleo G, Bassi C, Allegrini V, Beane JD, et al. (2019) Identification of an Optimal Cut-off for Drain Fluid Amylase on Postoperative Day 1 for Predicting Clinically Relevant Fistula After Distant Pancreatectomy: A Multi-institutional Analysis and External Validation. Annals of surgery 269: 337–343. https://doi.org/10.1097/SLA.0000000000002532 PMID: 28938266
39. Murakami Y, Uemura K, Hayasidani Y, Sudo T, Hashimoto Y, et al. (2008) A soft pancreatic remnant is associated with increased drain fluid pancreatic amylase and serum CRP levels following pancreato-duodenectomy. Journal of gastrointestinal surgery: official journal of the Society for Surgery of the Alimentary Tract 12: 51–56. https://doi.org/10.1007/s11605-007-0340-7 PMID: 17955317

40. Tzedakis S, Sauvanet A, Schiavone R, Razafirimananana M, Cauchi F, et al. (2020) What should we trust to define, predict and assess pancreatic fistula after pancreatoduodenectomy? Pancreatology: official journal of the International Association of Pancreatology 20: 1779–1785. https://doi.org/10.1016/j.pan.2020.10.036 PMID: 33077382

41. Hamanaka Y, Nishihara K, Harasaki T, Kawabata A, Yamamoto S, et al. (1996) Pancreatic juice output after pancreatoduodenectomy in relation to pancreatic consistency, duct size, and leakage. Surgery 119: 281–287. https://doi.org/10.1016/s0039-6060(96)80114-0 PMID: 869183

42. Ansurge C, Strommer L, Andreu-Sandberg A, Lundell L, Herrington MK, et al. (2012) Structured intraoperative assessment of pancreatic gland characteristics in predicting complications after pancreato-duodenectomy. The British journal of surgery 99: 1076–1082. https://doi.org/10.1002/bjs.8784 PMID: 22556164

43. Fniess H, Malfertheiner P, Isenmann R, Kuhne H, Beger HG, et al. (1996) The risk of pancreatico-intestinal anastomosis can be predicted preoperatively. Pancreas 13: 202–208. PMID: 8829190

44. Tranchart H, Gaujoux S, Rebours V, Vullierme MP, Dokmak S, et al. (2012) Preoperative CT scan helps to predict the occurrence of severe pancreatic fistula after pancreatoduodenectomy. Annals of surgery 256: 139–145. https://doi.org/10.1097/SLA.0b013e318256c32c PMID: 22609944

45. Roberts KJ, Sutcliffe RP, Marudananayagam R, Hodson J, Isaac J, et al. (2015) Scoring System to Predict Pancreatic Fistula After Pancreatoduodenectomy: A UK Multicenter Study. Annals of surgery 261: 1191–1197. https://doi.org/10.1097/SLA.0000000000000997 PMID: 25371115

46. Yue Y, Li M, Zhang X, Yu H, Song B (2020) Prediction of clinically relevant pancreatic fistula after pancreatic surgery using preoperative CT scan: A systematic review and meta-analysis. Pancreatology: official journal of the International Association of Pancreatology 20: 1558–1565. https://doi.org/10.1016/j.pan.2020.09.009 PMID: 32972935

47. Roberts KJ, Karkhanis S, Pitha Jmuthm M, Khan MS, Hodson J, et al. (2016) Comparison of preoperative CT-based imaging parameters to predict postoperative pancreatic fistula. Clinical radiology 71: 989–992. https://doi.org/10.1016/j.crad.2016.06.108 PMID: 27426676

48. Yoon JH, Lee JM, Lee KB, Kim SW, Kang MJ, et al. (2016) Pancreatic Steatosis and Fibrosis: Quantitative Assessment with Preoperative Multiparametric MR Imaging. Radiology 279: 140–150. https://doi.org/10.1148/radiol.2015142254 PMID: 26566228

49. Pugalenthi A, Protic M, Gonen M, Kingham TP, Angelica MI, et al. (2016) Postoperative complications and overall survival after pancreatoduodenectomy for pancreatic ductal adenocarcinoma. Journal of surgical oncology 113: 188–193. https://doi.org/10.1002/jso.24125 PMID: 26678549

50. Assifi MM, Zhang S, Leiby BE, Pequignot EC, Xia B, et al. (2013) Tumor recurrence is independent of pancreatic fistula in patients after pancreatoduodenectomy for pancreatic ductal adenocarcinoma. Journal of the American College of Surgeons 217: 621–627. https://doi.org/10.1016/j.jamcollsurg.2013.05.014 PMID: 23810574

51. Nagai S, Fujii T, Kodera Y, Kanda M, Sahin TT, et al. (2011) Recurrence pattern and prognosis of pancreatic cancer after pancreaticoduodenectomy. Annals of surgical oncology 18: 2329–2337. https://doi.org/10.1245/s10434-011-1604-8 PMID: 21327822

52. Oettle H, Post S, Neuhaus P, Gellert K, Langrehr J, et al. (2007) Adjuvant chemotherapy with gemcitabine vs observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized controlled trial. JAMA 297: 267–277. https://doi.org/10.1001/jama.2007.71 PMID: 17227978

53. Neoptolemos JP, Stocken DD, Bassi C, Ghanem P, Cunningham D, et al. (2010) Adjuvant chemotherapy with fluorouracil plus folinic acid vs gemcitabine following pancreatic cancer resection: a randomized controlled trial. JAMA 304: 1073–1081. https://doi.org/10.1001/jama.2010.1275 PMID: 20823433

54. Mirnezami A, Mirmezami R, Chandrakumaran K, Sasapu K, Sagar P, et al. (2011) Increased local recurrence and reduced survival from colorectal cancer following anastomotic leak: systematic review and meta-analysis. Annals of surgery 253: 890–899. https://doi.org/10.1097/SLA.0b013e3182128929 PMID: 21394013

55. McMillan DC, Canna K, McArdle CS (2003) Systemic inflammatory response predicts survival following curative resection of colorectal cancer. The British journal of surgery 90: 215–219. https://doi.org/10.1002/bjs.4038 PMID: 12555298

56. Ishikawa O, Wada H, Ohigashi H, Doki Y, Yokoyama S, et al. (2003) Postoperative cytology for drained fluid from the pancreatic bed after “curative” resection of pancreatic cancers: does it predict both the patient’s prognosis and the site of cancer recurrence? Annals of surgery 238: 103–110. https://doi.org/10.1097/01.SLA.0000074982.51763.d6 PMID: 12832972