Predicting the Severity of Postoperative Pancreatitis and Postoperative Pancreatic Fistulae following Major Pancreatic Resection

Juanita Noeline Chui *BSc(Adv) MD*

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STATEMENT OF ORIGINALITY

This is to certify that to the best of my knowledge the content of this thesis is my own work. I certify that all the assistance received in preparing this thesis and sources have been appropriately acknowledged. I declare that I have not submitted this material in full or in part to any other institution for consideration of a degree.

Juanita Noeline Chui

AUTHOR CONTRIBUTION STATEMENT

Chapter 1 of this thesis has been published as Chui *et al.* "Postoperative Pancreatitis and Postoperative Pancreatic Fistulae: A Review of Current Evidence". I performed the literature review, synthesised the data, and wrote the manuscript.

Chapter 2 of this thesis has been submitted for publication as Chui *et al.* "Lipase-to-Amylase Ratio for the Prediction of Clinically Relevant Postoperative Pancreatic Fistula Following Pancreaticoduodenectomy". I was responsible for the study design, data collection, statistical analysis, and writing the manuscript.

Chapter 3 of this thesis has been published as Chui *et al*. "Clinical Validation of the International Study Group of Pancreatic Surgery (ISGPS) Definition for Post-Pancreatectomy Acute Pancreatitis". I designed the study, collected the data, performed the statistical analyses, and wrote the manuscript.

Appendix 1 of this thesis contains material published in Lim *et al.* "Construction of a Pancreatojejunostomy with an External Stent: A Technical Description". *Figure 1* and *Figure 2* within the section, *2. Technique*, are my illustrations of the described reconstruction technique. The section, *3.2 Preliminary results from our unit*, presents the results of an internal audit, which I performed.

Juanita Noeline Chui

SUPERVISOR STATEMENT

As the primary supervisor for the candidature upon which this thesis is based, I confirm that the authorship attribution statements above are correct.

A/Prof Anubhav Mittal

THESIS ABSTRACT

Postoperative pancreatic fistula (POPF) represents the leading cause of morbidity and mortality following partial pancreatectomy. Emerging evidence has challenged the traditional understanding of POPF and redefined its relationship to postoperative pancreatitis. The aim of this thesis was to synthesize the recent evidence, characterise the risk factors for postoperative pancreatitis and POPF, and to identify a prognostic indicator to facilitate the risk stratification of patients undergoing major pancreatic resection.

Firstly, we evaluate established risk factors, including parameters that comprise the Fistula Risk Score. We then evaluate the utility of pancreatic enzyme concentrations in postoperative drain fluid to predict for clinically relevant fistulae. From these results, we propose lipase-to-amylase ratio as a novel predictor for clinically relevant POPF and demonstrate its correlation with acinar cell density at the pancreatic resection margin.

Post-pancreatectomy acute pancreatitis (PPAP) has recently been recognized as a distinct postoperative complication. The second part of this thesis presents a validation study for the 2021 *International Study Group on Pancreatic Surgery* (ISGPS) consensus criteria for diagnosis and grading. Our results support the utility of a universally accepted definition and the need for future studies to better characterise PPAP as a clinical entity.

This work highlights key areas warranting further research and provides important insights into PPAP and POPF pathophysiology that should interest future investigators. Our findings lend further evidence to the importance of acinar cell density as an intrinsic risk factor, the role of PPAP in driving POPF pathogenesis, and the mechanisms of iatrogenic acinar cell injury that represent new targets for risk mitigation strategies.

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PUBLICATIONS ARISING FROM THIS THESIS

Journal articles

The following manuscripts have been accepted or submitted for publication from the work embodied in this thesis:

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Additional publications relevant to this thesis but not forming part of it

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CHAPTER I.

Introduction

1. Background

Postoperative pancreatic fistula (POPF) represents one of the most severe complications after pancreatic resection, associated with significant morbidity and mortality[1-3]. Traditionally regarded as the leakage of pancreatic secretions arising from the breakdown of an anastomosis or a transection line, risk mitigation strategies have largely focused on optimising surgical technique or anastomotic reinforcement. Despite decades of research, the clinical burden of POPF persists and the understanding of its pathogenesis remains poor[4].

Recently, there has been evidence to suggest that postoperative pancreatitis plays a critical role in the development of POPF[5-7]. With the growing recognition of its clinical relevance, post-pancreatotomy acute pancreatitis (PPAP) was established as a distinct complication entity in 2021 by international consensus[8]. PPAP refers to the acute inflammatory process that occurs in the pancreatic remnant following partial resection. It is proposed to arise from iatrogenic acinar cell injury, resulting in the release and activation of pancreatic enzymes. The severity of this inflammatory response is therefore thought to depend on the acinar cell content of the pancreatic remnant.

2. Hypothesis and Aims

The recent evidence on postoperative pancreatitis has led to a paradigm shift in our understanding of POPF. It is proposed that in the absence of technical failure, PPAP contributes to the development of POPF and that the two entities are driven by common mechanisms of acinar cell injury. The histologic composition of the remnant pancreas – specifically, the acinar cell density – should therefore represent the key predictor and determinant of severity for PPAP and POPF.

The work embodied in this thesis aims to provide a contemporary evaluation of post-operative pancreatitis and pancreatic fistula as distinct but related complication entities. Specifically, the objectives were: i) to review the concept of the clinically relevant POPF and its relationship with PPAP, ii) to apply the newly proposed international consensus criteria for PPAP diagnosis and grading to an external cohort, and iii) to identify the independent predictors of POPF using routine clinical, serologic, and histologic parameters to facilitate the risk stratification of patients undergoing pancreatic resection. It is hoped that this work will further our understanding of PPAP and POPF, to inform future research into their pathogenesis and provide insight into strategies to improve the perioperative care of patients undergoing pancreatic surgery.

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CHAPTER II.

A Review of Current Evidence

Postoperative Pancreatitis and Pancreatic Fistulae: A Review of Current Evidence

Juanita Noeline Chui[1,2] MD, Sumit Sahni[2,3] PhD, Jaswinder S Samra[1,2,4], DPhil (Oxon) FRACS, Anubhav Mittal[1,2,4,5] PhD FRACS

[1] Department of Upper Gastrointestinal Surgery, Royal North Shore Hospital, Sydney, Australia

[2] Faculty of Medical and Health Sciences, The University of Sydney, Sydney, Australia

[3] Kolling Institute of Medical Research, University of Sydney, Sydney, Australia

[4] Australian Pancreatic Centre, Sydney, Australia

[5] School of Medicine, The University of Notre Dame, Sydney, Australia

ABSTRACT (201 words)

OBJECTIVES: Postoperative pancreatic fistula (POPF) represents one of the most severe complications following pancreatic surgery. Despite being a leading cause of morbidity and mortality, its pathophysiology is poorly understood. In recent years, there has been growing evidence to support the role of postoperative or post-pancreatectomy acute pancreatitis (PPAP) in the development of POPF. This article reviews the contemporary literature on POPF pathophysiology, risk factors, and prevention strategies.

METHODS: A literature search was conducted using electronic databases, including Ovid Medline, EMBASE, and Cochrane Library, to retrieve relevant literature published between 2005-2023. A narrative review was planned from the outset.

RESULTS: A total of 104 studies fulfilled criteria for inclusion. Forty-three studies reported on technical factors predisposing to POPF, including resection and reconstruction technique and adjuncts for anastomotic reinforcement. Thirty-four studies reported on POPF pathophysiology. There is compelling evidence to suggest that PPAP plays a critical role in the development of POPF. The acinar component of the remnant pancreas should be regarded as an intrinsic risk factor; meanwhile, operative stress, remnant hypoperfusion, and inflammation represent common mechanisms for acinar cell injury.

CONCLUSIONS: The evidence base for PPAP and POPF is evolving. Future POPF prevention strategies should look beyond anastomotic reinforcement and target underlying mechanisms of PPAP development.

1. INTRODUCTION

Postoperative pancreatic fistula (POPF) is a major complication in pancreatic surgery[1], contributing to significant postoperative morbidity and mortality. POPF is a challenge in even high-volume institutions and is estimated to occur in up to 41% of all pancreatic resections. Despite extensive research into risk mitigation strategies, there has been no significant reduction in the incidence or severity of POPF in over two decades, owing to the fundamental lack of understanding of its pathogenesis.

A consensus definition for POPF was established in 2005 by the *International Study Group on Pancreatic Surgery* (ISGPS)[2]. It is presently defined as an external fistula with abdominal drain outputs containing an amylase concentration greater than three times the upper limit of institutional normal serum levels, on or after the third postoperative day. In 2016, the notion of clinical relevance was introduced by the ISGPS to grade POPF presentations on a spectrum of severity[1]. Presently, the benign "biochemical leak" of no clinical importance (previously known as Grade A), is distinguished from the clinically relevant fistula (CR-POPF), defined as those requiring minimally invasive intervention (Grade B) or resulting in critical illness, organ failure, surgical intervention, or death (Grade C). Patients developing CR-POPF face potentially lethal sequalae related to intraabdominal sepsis and haemorrhage and a mortality risk of up to 25%[3]. Despite its clinical burden, the precise aetiologic mechanisms leading to the development of CR-POPF remain poorly understood.

In recent years, there has been substantial evidence to suggest that postoperative acute pancreatitis (PPAP) plays a critical role in the development of POPF[4]. These findings have challenged the traditional concept of POPF as arising from the breakdown of the pancreato-enteric anastomosis or a mechanical leak from a transection line. Of note, the acinar cell density

of the pancreatic remnant has been identified as a key determinant of intrinsic susceptibility to developing POPF[5-10]. Furthermore, potentiating factors related to iatrogenic acinar cell injury represent new targets for risk assessment and prevention.

Our understanding of POPF pathophysiology is rapidly evolving. In recent years, there have been major changes to clinical practice and revisions to international consensus criteria. Meanwhile, postoperative acute pancreatitis is becoming increasingly accepted as a distinct post-pancreatectomy complication. This article aims to provide a comprehensive overview of the contemporary literature on POPF pathophysiology, with a focus on its relationship with postoperative pancreatitis and the implications for prognostication and management.

2. Methods

A systematic literature search was conducted using electronic databases, including Ovid Medline, EMBASE, and Cochrane Library, to retrieve all articles relevant to pathophysiology of POPF and PPAP. Keywords included “pancreatectomy”, “pancreaticoduodenectomy”, “acute pancreatitis”, “postoperative”, and “fistula”. Terms were searched in isolation and in combination to identify relevant studies. The literature search was limited to studies published since the ISGPS consensus statement on POPF, from July 2005 to January 2023, those written in the English language, and conducted on human subjects. Articles published in abstract form only, relating to animal work, case reports, and series with fewer than 10 patients were excluded. After screening for duplicates and eligibility, reference lists of full articles were hand-searched for studies eligible for inclusion. A narrative review was planned from the outset with no attempt at quantitative meta-analysis.

3. Insights into Pathophysiology of POPF

While there are relatively few primary studies on the pathophysiology of POPF, there have been extensive research into its risk factors. These are presented here according to the proposed mechanism, including: i) loss of anastomotic integrity, ii) intrinsic predisposition, as determined by the histologic composition of the remnant gland, and iii) iatrogenic acinar cell injury, resulting in post-pancreatectomy pancreatitis.

3.1 POPF as a "Pancreatic Leak" – Loss of anastomotic Integrity

Colloquially regarded as a "pancreatic leak", POPF has traditionally been thought to arise from the failure of a pancreato-enteric anastomosis, in the context of pancreaticoduodenectomy, or leakage from the cut surface of the parenchyma, in other partial pancreatic resections. Until recently, the focus has been on meticulous surgical technique.

Type of resection. POPF rates have been observed to vary with the type of resection, estimated to occur in 5-30% in pancreaticoduodenectomy (PD)[11-13], 20-31% in distal pancreatectomy (DP)[5, 14, 15] and 41% in central pancreatectomy (CP)[16, 17]. In patients undergoing PD, the prevailing mechanism by which POPF arises is the loss of integrity of the pancreatoenteric anastomosis. In DP, the leakage of pancreatic secretions arises from the remnant pancreatic stump, either oversewn or from a staple line. The higher POPF rates associated with CP have been attributed to the presence of two potential sites of failure, where reconstruction involves both a pancreatic stump and a pancreato-enteric anastomosis. The risk of POPF is even greater in enucleations for pancreatic tumours, due to the risk of ductal injury[18, 19]. The prevention of POPF

has been the subject of extensive research. Most studies have been in the context of PD given the associated morbidity and mortality of this procedure. The following sections describe the technical factors contributing to POPF in the context of PD accordingly.

Pancreato-enteric anastomosis and reconstruction. The pancreato-enteric anastomosis has been described as the Achilles heel of PD for its association with POPF[20]. The two most widely performed methods are the pancreaticojejunostomy (PJ) (88.7%) and the pancreaticogastrostomy (PG) (9.7%)[21]. In comparison to the more traditional PJ, PG was proposed to confer the benefit of proteolytic enzyme deactivation in the acidic environment of the stomach and lack of enterokinase for the activation of pancreatic trypsin[22]. However, the RECOPANC study, published in 2016 as the largest multicentre randomized controlled trial (RCT) to compare outcomes of PG and PJ, reported no significant difference between the two methods in 440 patients across 14 high-volume centres, with respect to POPF incidence and severity[23]. Subsequent meta-analyses of RCTs have similarly reported no difference between PJ and PG in associated POPF rates[24, 25].

Variations in anastomotic techniques have similarly been explored. Among patients undergoing PD with construction of a PJ, common approaches include the duct-to-mucosa end-to-side anastomosis, followed by the invaginating ("dunking") end-to-side or end-to-end anastomosis, and the end-to-side PG anastomosis, which can also be formed using an invaginating or duct-to-mucosa anastomosis. A meta-analysis of six RCTs comparing variations in PJ anastomotic techniques showed no significant difference in POPF morbidity or mortality[26]. Other methods have been described[27-29] with a recent systematic review identifying 61 variants in the literature, yet no consensus exists on a superior technique[30].

A soft pancreatic gland texture and small main pancreatic duct diameter are widely accepted risk factors for POPF development following PD[31]. These intraoperative factors are thought to contribute to the technical difficulty of creating an anastomosis. A more dilated main pancreatic duct, as seen in cases of proximal obstruction, has been associated with greater ease of anastomosis and thereby lower rates of failure; meanwhile, a soft gland has been associated with reduced suture holding capacity, contributing to higher fistula rates postoperatively[32, 33]. The combination of a soft gland and a small duct diameter (<3mm) has been reported to increase the risk for POPF formation by magnitudes. Finally, suture type has also been recognized as a contributing factor, with non-absorbable sutures being suggested to reduce the risk and severity of POPF[34].

Stents. Pancreatic duct stents are widely used as a mitigation strategy for POPF. In the context of PD, trans-anastomotic pancreatic stents may be internal or external. Internal stents are introduced into the main pancreatic duct, over which the anastomosis is constructed. Their use was introduced to achieve diversion of pancreatic secretions, while maintaining duct patency by facilitating precise placement of sutures in the creation of the anastomosis. However, results from large retrospective studies and RCTs demonstrate no benefit with the placement of internal stents and have in fact raised concern for increased POPF and overall postoperative complication rates, including a high propensity for stent migration[35, 36]. External stents are exteriorized through the proximal jejunum via an enterotomy and through the abdominal wall to drain extracorporeally. These have shown greater promise in reducing POPF rates[37-42]. However, the supporting evidence derives predominantly from small RCTs and cohort studies.

In a recent meta-analysis of seven RCTs and nonrandomised studies, analysing data from 847 patients, no significant reduction in POPF rates were observed between treatment with and without pancreatic duct stents in patients undergoing PD[43]. However, subgroup analyses favoured the use of external stents over internal stents for reduction in POPF rates. Overall, there remains a need for large-scale RCTs to clarify the efficacy of pancreatic duct stents for the prevention of POPF.

Pharmacological prophylaxis. The use of somatostatin analogues has been subject to considerable debate[44]. They were initially proposed to reduce POPF rates by the inhibition of pancreatic exocrine secretions; however, there have been conflicting results in the literature to date. A 2010 Cochrane review comprising of data from seventeen RCTs, including 2143 patients, demonstrated a reduction in overall fistula rates after pancreatic surgery. However, there was no significant difference in rates of clinically relevant fistulas[45]. Pasireotide has a higher receptor affinity and a longer half-life than most other clinically used somatostatin analogues. The results of an early single-centre RCT led to initial promise of perioperative treatment with Pasireotide for the reduction POPF incidence and severity[46]. These results were not reproduced in subsequent studies[47]. In fact, recent studies have questioned the safety of prophylactic somatostatin analogue use. In particular, octreotide has been associated with higher CR-POPF incidence, impaired wound healing, and poor splanchnic flow[48, 49]. There has been no benefit to overall morbidity or mortality in PD and many studies now propose to abandon its use.

Adjuncts for anastomotic reinforcement. These include use of fibrin sealants[50], patches[51], and mesh[52], as well as wrapping of the anastomosis with omentum or

falciform ligament[53]. Results are inconsistent between studies, which are predominantly based on single-centre and heterogenous data. There is presently no proven benefit with their use for the reduction of POPF risk.

Despite the developments in surgical technique and extensive research into strategies to preserve the integrity of the pancreatoenteric anastomosis, there has been no significant reduction in POPF incidence or associated morbidity in decades[4]. It is becoming apparent that POPF is driven by more complex underlying pathophysiology.

3.2 Acinar cell density - Intrinsic predisposition of remnant pancreas

There is growing evidence to suggest that the intrinsic predisposition of the remnant pancreas to the development of a clinically relevant fistula depends on its histologic composition – specifically, the acinar cell content relative to collagen and fat[6, 54, 55]. Higher acinar cell densities at the pancreatic resection margin have been associated with increased POPF risk and higher grades of severity[6, 8, 9, 56].

The role of the acinar cell component as an inherent determinant of POPF risk may in part explain the lower rates associated with states of reduced exocrine function. It is well established that chronic pancreatitis and pancreatic ductal adenocarcinoma result in fibrotic change, fatty infiltration, and reduced acinar cell density[57, 58]. These diagnoses have consistently been shown to predict for a lower risk of POPF[59]. Similarly, lower rates of POPF have been reported for patients receiving neoadjuvant chemoradiotherapy compared to those proceeding to upfront resection in cohorts undergoing distal pancreatectomy[60] and pancreaticoduodenectomy[61]. Neoadjuvant chemo-radiation induces fibrotic changes in pancreatic tissue similar to those seen in chronic pancreatitis, resulting in a reduced acinar compartment[61].

In addition to its association with POPF, acinar cell density has been associated with post-pancreatectomy pancreatitis. Nahm et al. reported that patients with higher acinar cell densities at the transection margin on frozen sections not only experienced higher rates of POPF, but demonstrated elevated markers of acute pancreatitis intraoperatively, including serum amylase and urinary trypsinogen-2[6]. It has also been shown that elevated amylase in the peripancreatic fluid sampled after resection predicts for POPF development in patients undergoing PD[62]. PPAP has therefore been proposed to play a critical role in the pathogenesis of POPF and postulated to arise from common mechanisms of acinar cell injury. Table 2 summarises the recent evidence on acinar cell density as a predictor for PPAP and POPF.

3.3 Remnant ischemia and inflammation – Precipitating factors for postoperative pancreatitis

While not fully understood, several key mechanisms of acinar cell injury have been described in the literature, including remnant hypoperfusion and ischaemia, and focal pancreatitis[38]. Ansorge et al. analysed intraperitoneal metabolites and protease activation at the pancreatico-jejunal anastomosis[63]; compared to patients who proceeded to an uncomplicated recovery, those who developed POPF demonstrated higher glycerol levels, higher lactate-to-pyruvate ratios, and lower glucose levels, suggestive of tissue ischemia. This was observed in combination with higher typsinogen activation peptide concentrations, providing biochemical evidence for ischaemic pancreatitis. The exocrine pancreas is known to be sensitive to even transient hypoperfusion[64]. In the context of PD, vascular collaterals between the coeliac trunk and superior mesenteric arterial systems are found at the neck of the pancreas. Ischaemia in this region following resection can contribute to poor healing and anastomotic breakdown. Furthermore, intraoperative blood loss is a widely validated risk factor for POPF[59]. Several

studies have explored the use of surrogate markers for tissue ischaemia to predict for POPF risk with limited success. These include postoperative lactatemia[65], radiological evidence of remnant perfusion[66], and intraoperative inspection of blood flow at the cut surface to guide resection margins[67]. None of these methods have been widely accepted into practice.

Inflammation is also recognized as an important physiological mediator for POPF development. Early experimental models have demonstrated that acinar cells may behave like inflammatory cells[68]. Laaninen et al. observed that the inflammatory response was more significant in the acinar cell-rich pancreas[69], and proposed that intraoperative trauma may trigger an inflammation cascade within the pancreatic remnant. The same authors subsequently demonstrated in a RCT enrolling 155 patients that anti-inflammatory treatment with peri-operative hydrocortisone led to reduced post-pancreatectomy complications and a nonsignificant trend toward a reduction in POPF rates[70]. Several studies comparing laparoscopic to open approaches have suggested that laparoscopic surgery may be linked with lower POPF rates, potentially due to reduced intraoperative handling[71, 72]. Finally, there has been evidence for the use of urinary trypsin inhibitors in the prevention of POPF. The inhibition of enzyme activation has been proposed to limit the postoperative inflammatory response, although the precise mechanisms and their utility in practice have yet to be determined. These agents are presently used to treat acute and chronic pancreatitis and have been reported in recent RCTs to reduce POPF incidence[73, 74].

Overall, the emerging role of post-pancreatectomy pancreatitis has led to a paradigm shift in our understanding of POPF, providing new directions for risk mitigation strategies beyond conventional measures for the reinforcement of a pancreato-enteric anastomosis or transection line.

4. Prediction of POPF

Prediction scores. Multiple groups have sought to develop an accurate perioperative risk assessment tool for POPF. The Fistula Risk Score (FRS), developed by Callery et al. in 2013, is the most widely used and validated assessment tool for POPF risk in patients undergoing PD[59, 75]. The FRS is based on intraoperative variables of gland texture (firm or soft), underlying pathology (dichotomised to pancreatic adenocarcinoma or chronic pancreatitis and others), main pancreatic duct diameter (measured on the cut surface), and estimated volume of intraoperative blood loss.

External validation studies have since demonstrated conflicting results[76, 77], leading to the development of a number of FRS variants, each incorporating different risk factors and purporting to optimize POPF prediction. In the 2019 alternative FRS (a-FRS), Mungroop et al. proposed the inclusion of body mass index (BMI), in addition to soft pancreatic texture and small main pancreatic duct diameter[78]. The same authors subsequently proposed an updated a-FRS in 2021, which included male sex as an additional factor. This was validated in patients undergoing minimally invasive and open PD to demonstrate improved predictive capacity[79]. Meanwhile, the Modified FRS, developed using data from the ACS NSQUIP national database by Kantor et al., incorporates preoperative bilirubin, in addition to male sex, BMI, pancreatic duct diameter, and gland texture as significant risk factors for POPF[80]. External validation studies have demonstrated comparable predictive performance between FRS variants[81, 82].

Other patient factors used in scoring systems include perioperative nutritional status, old age, and smoking as indicators of healing capacity[83]. Obesity, concurrent sarcopenia[84, 85] and distribution of abdominal and visceral fat[86-88] have also been proposed as risk factors of metabolic changes predisposing for POPF formation. With the increasing use of computed tomography (CT) and magnetic resonance imaging (MRI) in the modern era, several

prediction scores have developed using radiological parameters, with the aim of facilitating preoperative risk stratification[7, 89, 90].

Commonly reported risk factors have been summarised here in categories relating to patient factors, procedure related factors, and pancreas related factors (Table 1). More complex multivariate models and prediction nomograms combining the aforementioned patient factors, intraoperative findings, and biochemical markers have been proposed[43, 91, 92]. None has been shown to be superior or widely incorporated into clinical practice.

Biomarkers and other clinical indices. There has been growing interest in the utility of clinical indicators for acute pancreatitis to predict for POPF. Urinary trypsinogen-2[73, 74], serum biomarkers[93, 94], and pancreatic enzymes in postoperative drain fluid[95, 96] have been identified as early postoperative predictors in the literature. The association with elevated amylase in postoperative drain fluid is well established and now comprise the biochemical criterion for POPF diagnosis according to ISGPS guidelines[1]. A meta-analysis by Giglio et al., including 4416 patients from 13 studies published before 2015, demonstrated the utility of drain amylase on the first postoperative day as an accurate predictor for POPF following major pancreatic resection[95]. A subsequent meta-analysis including studies published before 2018 corroborated this finding, but cautioned the inconsistency in optimal thresholds and timing between studies[97]. The use of drain lipase levels for this purpose has featured in far fewer studies and its prognostic value remains subject to debate.

Urine trypsinogen-2 and trypsinogen activation peptide concentrations have similarly been explored as potential prognostic indicators for POPF[74, 98]. The utility of a urinary dipstick test for trypsinogen-2 as a point-of-care diagnostic test for acute pancreatitis has not yet been applied in the postoperative setting but has the potential to facilitate early risk stratification for POPF. Recognising the role of the systemic inflammatory response in acute

pancreatitis, markers including CRP, procalcitonin, and WCC have also been identified as prognostic indicators for POPF[93, 94, 99].

More recently, acinar cell density of the pancreatic remnant has been established as a predictor of POPF risk. This is achieved by histological assessment of the pancreatic resection margin on frozen section to determine the relative acinar cell, collagen, and fat content. Acinar cell density has been correlated to gland texture, a well-established risk factor for POPF formation, and proposed to provide a more objective and reliable alternative to the surgeon's intraoperative assessment of a soft versus firm remnant gland[6, 8, 10].

Finally, with the growing availability of CT imaging worldwide, a number of studies have attempted to characterise the radiologic features of a "high-risk" pancreas to predict for POPF. For example, CT enhancement characteristics have been correlated to the degree of pancreatic fibrosis[100]. In another study, the radiodensity of the pancreatic tail on preoperative non-contrast CT imaging was found to correlate with the acinar cell density of the pancreatic resection margin and postoperative day 1 drain amylase levels for patients undergoing PD[7]. The recently proposed image-based preoperative fistula risk score, developed in 2022, includes CT-derived factors of normal pancreatic morphology, small pancreatic duct diameter, radiologically determined high-risk pathology, and high pancreatic remnant volume. The authors demonstrated comparable accuracy to intraoperative evaluation of established risk factors[101].

The continual development of alternative scoring systems is a testament to the ongoing challenge of POPF prediction and prognostication. The development of a reliable prediction index for CR-POPF could alter the postoperative course of patients deemed to be at-risk and reduce POPF-associated morbidity and mortality.

5. Post-pancreatectomy pancreatitis contributes to POPF

For decades, acute inflammation of the pancreatic remnant following partial pancreatic resections has been a recognized phenomenon. Yet, post-operative or post-pancreatectomy acute pancreatitis (PPAP) only gained formal recognition as a distinct complication entity by the ISGPS in 2021[102]. According to this recent consensus criteria, PPAP is diagnosed by early sustained elevation of serum amylase (exceeding the upper limit of normal for 48 hours), in the presence of radiological evidence consistent with acute pancreatitis and "clinically relevant features". Like POPF grading, the proposed stratification of PPAP severity progresses from benign postoperative hyperamylasaemia (POH), with no impact on postoperative recovery, to mild-moderate (Grade B) and severe life-threatening (Grade C) complications. This represents the first universally accepted and objective definition for PPAP, which will be essential for standardised reporting and comparison of outcomes in future studies. The current ISGPS criteria have been instrumental in establishing PPAP as a distinct phenomenon from acute pancreatitis observed in the non-surgical setting, encouraging future studies to inquire into the mechanisms of operative trauma and iatrogenic acinar cell injury. The grading schemes proposed for PPAP and POPF further reflects the contemporary understanding that postoperative hyperamylasaemia and a benign biochemical leak are frequent observations in the immediate postoperative period, which should be distinguished from their corresponding pathological or clinically relevant states.

However, as a newly defined clinical entity, PPAP is poorly characterised. Early validation studies have already reported limitations relating to non-specific radiological and clinical criteria. The present ISGPS criteria for clinically relevant PPAP lists POPF among its manifestations. Although a number of previous studies have reported CR-POPF without serum

hyperamylasemia, in the absence of technical failure PPAP appears to be the major driving mechanism for the development of a CR-POPF. Considering the emerging evidence, it is conceivable that PPAP and POPF represent manifestations of a common underling ischaemic-inflammatory process. Recent studies have demonstrated that PPAP precedes POPF, by measurement of serum amylase, serum lipase, urinary trypsinogen-2 and postoperative drain amylase levels[6, 103]. Furthermore, the severity of both processes appears to depend on the viable acinar component of the pancreatic remnant[6, 8]. Similarly, PPAP and POPF appear to be modified by common perioperative factors[104], highlighting practical implications for prevention and risk management.

CONCLUSION

Despite decades of research, the high morbidity and mortality associated with POPF continues to plague patient outcomes in pancreatic surgery. The lack of progress over the years relates to an incomplete understanding of its fundamental pathophysiology. The development of PPAP is now recognized as a critical component in the pathophysiology of POPF. Future studies should focus on characterising this relationship and their underlying pathophysiologic mechanisms, which will open new avenues for future prevention strategies.

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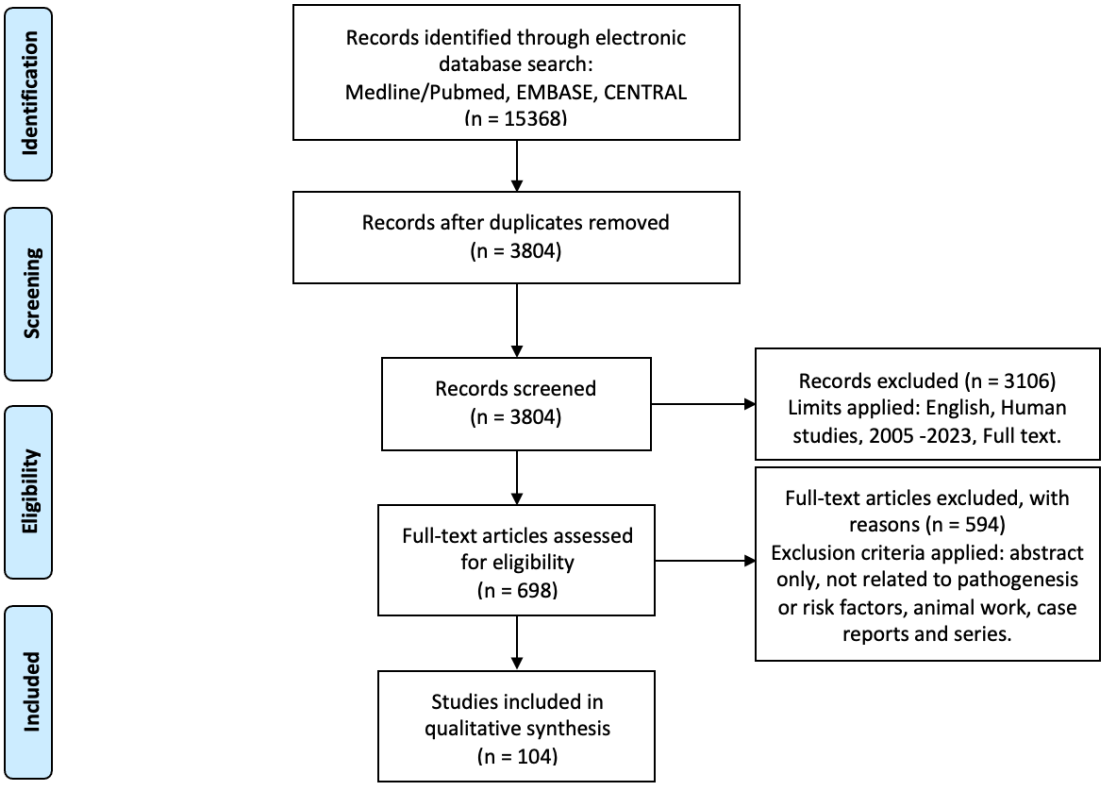

Figure 1. Flow chart of the search strategy employed.

Table 1. Risk factors for POPF used in validated risk prediction scores.

Patient factors	Intrinsic (pancreas-related) factors	Intraoperative and technical factors
▪ Male sex ▪ Old age ▪ High BMI ▪ Intra-abdominal and visceral fat distribution: difficult access, fatty infiltration of pancreas ▪ Poor nutritional status, decreased albumin level ▪ Smoking history ▪ ASA	▪ High-risk pathology: Diagnosis other than PDAC/pancreatitis ▪ Soft gland texture ▪ Small duct diameter ▪ High acinar cell density ▪ Neoadjuvant treatment	▪ Intraoperative blood loss ▪ Operation time ▪ Technical factors, relating to: transection, anastomosis, reconstruction ▪ Adjuncts, including: stents, fibrin sealants, patches, tissue wrap

Table 2. Summary of evidence on acinar cell density and postoperative pancreatitis and pancreatic fistula.

Study	Study period	Type	Resection	Cohort Size	Findings	Predictive Threshold
Partelli *et al.*, 2021[8]	January 2018 to December 2019	Single centre, retrospective cohort	PD	388	Acinar content predicted for: - CR-POPF (60-80%, OR 2.51, p = 0.008; >80%, OR 2.93, p = 0.010). - CR-PPAP (60-80%, OR 9.42, p < 0.001; >80%, OR 10.16, p < 0.001).	Third quartile cut-off: Acinar content at the pancreatic resection margin ≥60%.
Nahm *et al.,* 2021[5]	2011 to 2017	Single centre, retrospective cohort	PD, DP	294	Acinar score had significant predictive capacity for the development of POPF (AUROC 0.658, 95%CI: 0.583-0.732, p = 0.001).	Acinar score >50 was independently associated with the development of POPF (OR 6.457, P = .003).
Teranen *et al.*, 2021[10]	2006 to 2015	Single centre, retrospective cohort	PD	87	Acinar cell count method predicted for CR-POPF (p = 0.043).	Optimal cut-off for acinar cells at the transection line: Greater than 40% (sensitivity 88.9%, specificity 52.6%).
Umezaki *et al.*, 2018[9]	April 2012 to July 2016	Single centre, retrospective cohort	PD	121	Number of pancreatic acinar cells were associated with POPF (AUROC 0.83, p < 0.0001).	Optimal cut-off for number of pancreatic acinar cells: 890 cells (sensitivity 82.6%, specificity 77.6%).
Nahm *et al.*, 2018[6]	June 2016 to July 2017	Single centre, retrospective cohort	PD, DP, CP	61	Acinar cell density correlated with POPF (p = 0.003) PPAP (p < 0.001), intra-operative amylase concentration, (p < 0.001). Acinar cell score (%) predicts for POPF (AUROC 0.744, 95%CI: 0.623 – 0.866, p = 0.003).	Optimal cut-off for acinar score: 55%.
Laaninen et al., 2012[56]	2007 to 2009	Single centre, retrospective cohort	PD	40	Acinar cells covering more than 40% of the cut edge of pancreas correlated significantly with high drain amylase (r = 0.532, p = 0.001), number of positive urine trypsinogen days (r = 0.516, p = 0.001), and incidence of acute pancreatitis p = 0.03.	Cut-off for acinar cells on cut edge of pancreas, demonstrating significant difference: 40%.

PD, Pancreaticoduodenectomy; DP, Distal pancreatectomy; CP, Central pancreatectomy; CR-POPF, clinically-relevant postoperative pancreatic fistula; CR-PPAP, clinically-relevant post-pancreatectomy acute pancreatitis; OR, odds ratio; AUROC, area under the receiver operating characteristic.

CHAPTER III.

Prediction of the Clinically Relevant Postoperative Pancreatic Fistula

Lipase-to-Amylase Ratio for the Prediction of Clinically Relevant Postoperative Pancreatic Fistula following Pancreaticoduodenectomy

Juanita N Chui[1] MD, William A Ziaziaris[1,2] MBBS MPhil, Christopher B Nahm[2,3,4] PhD FRACS, Talia Fuchs[2,5,6] PhD FRCPA, Sumit Sahni[5] PhD, Christopher SH Lim[1] MS FRACS, Anthony J Gill[2,5,6] MD FRCPA, Jaswinder S Samra[1,2,7] DPhil (Oxon) FRACS, Anubhav Mittal[1,2,7,8] PhD FRACS

[6] Department of Upper Gastrointestinal Surgery, Royal North Shore Hospital, Sydney, Australia

[7] Faculty of Medical and Health Sciences, The University of Sydney, Sydney, Australia

[8] Department of Hepatobiliary, Pancreatic/Upper Gastrointestinal Surgery, Westmead Hospital, Sydney, Australia

[9] Surgical Innovations Unit, Westmead Hospital, Sydney NSW Australia

[10] Cancer Diagnosis and Pathology Group, Kolling Institute of Medical Research, University of Sydney, Sydney, Australia

[11] NSW Health Pathology, department of Anatomical Pathology, Royal North Shore Hospital, Sydney, Australia

[12] Australian Pancreatic Centre, Sydney, Australia

[13] School of Medicine, The University of Notre Dame, Sydney, Australia

ABSTRACT (199 words)

Aim: Postoperative pancreatic fistula (POPF) represents a leading cause of morbidity and mortality following major pancreatic resections. This study aimed to evaluate the use of post-operative drain fluid lipase-to-amylase ratio (LAR) for the prediction of clinically relevant fistulae (CR-POPF).

Methods: Consecutive patients undergoing pancreaticoduodenectomy between 2017-2021 at a tertiary centre were retrospectively reviewed. Univariate and multivariate analyses were performed to identify predictors for CR-POPF (ISGPS Grades B/C). Receiver operator characteristic (ROC) curve analyses were performed to evaluate the performance of LAR and determine optimum prediction thresholds.

Results: Of 130 patients analysed, 28 (21.5%) developed CR-POPF. Variables positively associated with CR-POPF included soft gland texture, acinar cell density, diagnosis other than PDAC or chronic pancreatitis, resection without neoadjuvant therapy, and postoperative drain fluid lipase, amylase, and LAR (all $p<0.05$). Multivariate regression analysis identified LAR as an independent predictor of CR-POPF ($p<0.05$). ROC curve analysis showed that LAR had moderate ability to predict CR-POPF on POD1 (AUC=0.64,95%CI=0.54-0.74) and excellent ability on POD3 and 5 (AUC=0.85,95%CI=0.78-0.92; AUC=0.86,95%CI=0.79-0.92). Optimum thresholds were consistent over POD1-5 (ratio >2.6) and associated with 92% sensitivity and 46–71% specificity.

Conclusion: Postoperative drain fluid LAR represents a reliable indicator for CR-POPF with the potential to facilitate early stratification of patients undergoing pancreaticoduodenectomy.

1. BACKGROUND

Postoperative pancreatic fistula (POPF) is widely recognized as the leading cause of morbidity and mortality related to partial pancreatic resection[1, 2]. It represents a challenge even in high-volume centres, with incidence rates of up to 45%, potentially lethal sequalae, and a mortality risk of up to 25% for high-grade fistulas[2-6]. Despite its clinical burden, the pathophysiology of POPF is poorly understood with ongoing research to identify a reliable prognostic indicator.

POPF is defined by the *International Study Group on Pancreatic Surger*y (ISGPS)[5] as an external pancreatic fistula with persistent abdominal drain output containing an amylase concentration greater than three times the upper reference limit of serum amylase, on or after the third postoperative day[7]. Additional clinical criteria are used to grade POPF severity, ranging from a benign "biochemical leak" (Grade A) of no clinical importance, to those requiring minimally invasive intervention (Grade B) and those resulting in organ failure, re-operation, or death (Grade C). Grades B and C are regarded as clinically relevant fistulae (CR-POPF). In recent years, there has been compelling evidence to suggest that post-pancreatectomy acute pancreatitis (PPAP) predisposes to the development of POPF[8-15]. The ISGPS regards PPAP as the acute ischaemic-inflammatory process that occurs in the remnant pancreas following partial pancreatectomy. It is thought to arise from operative trauma and iatrogenic injury to acinar cells, resulting in the release and subsequent activation of pancreatic enzymes[16, 17]. While the exact mechanisms have yet to be elucidated, the severity of this acute inflammatory response is thought to depend fundamentally on the viable acinar cell content[8, 16, 18]. Recently published research has demonstrated that PPAP and POPF are independently associated with acinar cell density at the resection margin of the remnant pancreas, and that PPAP precedes POPF[19-22]. This has led to a paradigm shift in our understanding of POPF pathogenesis, with the emphasis moving away from mechanical

determinants of anastomotic integrity to the intrinsic histologic composition of the remnant pancreas and its predisposition to PPAP.

In light of the emerging evidence, the acinar component of the remnant pancreas, responsible for the secretion of pancreatic enzymes, may represent a key determinant of PPAP and POPF risk and severity. Early experimental studies have shown that amylase and lipase may be differentially released by the exocrine pancreas[23]. Nonparallel secretion is known to occur in postprandial states and in pathological states of inflammation and neoplastic disease[24-27]. Under such circumstances, lipase secretion appears to be more susceptible to altered stimuli and appears to be released in higher concentrations than amylase. We therefore hypothesised that the relative concentration of lipase and amylase measured in the postoperative drain fluid may reflect the degree of acinar cell injury within the remnant pancreas, and thereby serve as an early indicator for PPAP and CR-POPF risk.

This study aimed to i) validate previously reported risk factors, including acinar cell density, for CR-POPF development in a cohort of patients undergoing pancreaticoduodenectomy (PD); ii) evaluate the novel use of postoperative drain fluid lipase-to-amylase ratio (LAR) in the prediction of PPAP and POPF severity; and iii) examine the correlation between LAR and the validated histopathologic acinar cell score.

2. METHODS

2.1 Patient selection and data collection

Institutional review board ethics approval was obtained for this study. All consecutive patients undergoing pancreaticoduodenectomy (PD) performed at a single public tertiary referral hospital in Sydney, NSW, Australia from January 2017 to December 2021 were identified from a prospectively maintained database. Patients proceeding to completion pancreatectomy were

excluded. A retrospective review of medical records was performed to extract demographic, clinicopathologic, intraoperative, and postoperative data. Demographics included the patient's age, sex, body mass index (BMI), comorbidities, and American Society of Anaesthesiologists (ASA) score. Histopathologic diagnoses were recorded for all patients. In those undergoing PD for malignancy, neoadjuvant treatment was recorded. Intraoperative data included established Fistula Risk Score (FRS) parameters[28] of gland texture, main pancreatic duct diameter, and estimated blood loss. Postoperative data included patient outcomes, inflammatory serum markers, and surgical drain fluid composition.

2.2 Surgical technique and post-operative care

All operations were performed by one of two consultant hepatopancreatobiliary surgeons (AM and JS) in a high-volume tertiary hospital and referral pancreatic centre, according to previously described methods[8]. In all PD procedures two large-bore surgical drains were placed; one in the subhepatic space lying posterior to the hepaticojejunostomy with the tip of the drain lying posterior to the pancreaticojejunostomy and one from the left side positioned posterior to the gastrojejunostomy with the tip lying anterior to the pancreaticojejunostomy. All PD patients at our centre are routinely admitted to Intensive Care Unit (ICU) for a minimum of 24-hours. Drain amylase and lipase levels are routinely measured on post-operative days (POD) 1, 3 and 5 and corresponding LAR were determined. Serological markers for inflammation, including white cell count (WCC), neutrophil levels, and C-reactive protein (CRP), and serum pancreatic amylase levels were additionally recorded and correlated to CR-POPF outcome.

2.3 Definition and grading of outcomes

POPF was defined as a drain fluid amylase concentration greater than three times the upper limit of normal for serum amylase (> 110 U/L) on or after the third postoperative day, in accordance with the latest ISGPS criteria[7]. The clinical impact of POPF development was used to distinguish between cases of biochemical leak or clinically relevant fistula (Grades B or C). All patients requiring persistent drainage for a duration exceeding 3 weeks, percutaneous or endoscopic drainage of intra-abdominal collections, angiographic procedures for haemorrhage secondary to POPF, or treatment for sepsis without organ failure were classified as Grade B. POPF requiring reoperation, or resulting in organ failure or death were classified as Grade C.

All partial pancreatectomy-specific complications were defined according to ISGPS definitions, including delayed gastric emptying (DGE)[29], post-pancreatectomy haemorrhage (PPH)[30], and recently recognized post-pancreatectomy acute pancreatitis (PPAP)[31]. Of note, PPAP was defined by the presence of sustained hyperamylasemia for at least 48 hours. Postoperative mortality was defined as in-hospital death or death occurring within 30-days from the initial operation. Postoperative complications were graded according to Clavien-Dindo classification. Additional outcomes of interest included return to theatre, length of postoperative ICU admission, and total length of hospital stay.

2.4 Determining the viable acinar component

Histology haematoxylin and eosin (H&E) stained sections of the paraffin-embedded pancreatic resection margin were scored for acinar cell density using previously described methods[19-22]. Briefly, all slides from the pancreatic neck resection margin were assessed and the percentage of the cross-sectional area of the pancreas occupied by acinar cells, fibrous connective tissue and fat were recorded in five percentage intervals – (Supplementary Figure

1). The acinar score was evaluated by two authors (CN or AG) who were blinded to clinical outcomes.

2.5 Statistical Analysis

Quantitative data were assessed for normal distribution using the Shapiro-Wilk test. Comparisons between CR-POPF and control groups were performed with Mann-Whitney U-test and Pearson Chi-squared or Fisher's exact tests for non-parametric data. Continuous data were presented as median with interquartile ranges (IQR) and categorical data as absolute and relative frequencies, as appropriate. Univariate analysis was performed for patient demographic and perioperative variables to predict the odds of CR-POPF development. Variables that were approaching statistical significance in univariate analysis ($p < 0.10$) were selected for multivariable logistic regression analysis. The pairwise correlation among predictor variables was computed using a bivariate correlation matrix. Multicollinearity was assessed using the Variance Inflation Factor. Continuous variables were dichotomised at the upper quartile and backward and forward stepwise regression was performed based on the Wald Statistic. Calibration of the model was calculated for using the Hosmer-Lemeshow goodness of fit test, where a *p*-value of less than 0.05 was assumed to indicate lack of fit.

Receiver operator characteristic (ROC) curves were used to evaluate the performance of LAR as a predictor for CR-POPF. An area under the ROC curve (AUC) of greater than 0.5 was regarded as a non-random result. A Youden index was calculated to determine optimum prediction thresholds. Finally, Spearman's rank correlation coefficient (r_s) was used to analyse the bivariate association between the validated histopathologic acinar cell score and LAR. The corresponding 95% confidence intervals (CI 95%) were calculated using the method of Bonnett and Wright[32]. In all analyses, a *P*-value of less than 0.05 was considered statistically

significant. Statistical analyses were performed with SPSS version 28.0 software (IBM Corp., Armonk, NY, USA).

3. RESULTS

3.1 Patient characteristics and operative outcome

A total of 130 consecutive patients undergoing PD were retrospectively analysed. The median patient age was 66 years (IQR, 57-72 years). There was a slight preponderance of males (52.6%) and patients classified as ASA III or greater (57.1%), with at least one significant comorbidity identified in 76.3% prior to surgery. The leading indications for surgery were pancreatic ductal adenocarcinoma (PDAC) (*n = 64,* 49%), pancreatic neuroendocrine tumour (PNET) (*n* = 14, 11%), and intraductal papillary mucinous neoplasm (IPMN) (*n* = 14,11%). Forty-five patients (33.4%) received neoadjuvant chemotherapy or radiotherapy, of which forty-three patients were being treated for PDAC.

Overall, 56 (43.1%) patients developed POPF. A total of 28 (21.5%) patients developed a biochemical leak, while 28 (21.5%) patients were complicated by CR-POPF (Grade B, *n = 23*; Grade C, *n = 5*). Of these, three required returns to theatre and there was one mortality. Sixty-six patients had recorded data on postoperative serum amylase, of which 36 (55%) fulfilled criteria for PPAP diagnosis. Among these, twenty (56%) also developed CR-POPF. Baseline characteristics of this study cohort and patient outcomes are summarised in Table 1.

3.2 Identifying risk factors

Baseline demographic data were similar across CR-POPF and control groups (Table 2). There was no significant difference in age, sex, BMI, ASA grade, selected comorbidities, main

pancreatic duct diameter, or estimated intraoperative blood loss between patients who developed CR-POPF in this cohort and those who did not (all $p > 0.05$). By contrast, there was evidence to suggest a negative association between neoadjuvant treatment and CR-POPF ($p < .001$). The incidence of CR-POPF was significantly lower among those who received neoadjuvant chemotherapy or radiotherapy (11%) than those who proceeded to upfront resection or resection for benign disease (37%). Similarly, a histopathological diagnosis of PDAC or chronic pancreatitis was associated with a reduced incidence of CR-POPF ($p < .001$), representing a significantly smaller proportion of patients who developed CR-POPF (25%) compared to patients who did not (62%). Pancreatic gland texture also demonstrated a significant difference when comparing between control and CR-POPF groups ($p < .001$). Soft gland texture was reported in a larger proportion of those who developed CR-POPF (89%) than those who did not (46%). Additionally, there was evidence of positive association between acinar cell scores and CR-POPF ($p < .001$); a median score of 80% (IQR 65 - 90%) was observed for the CR-POPF group, compared to 40% (IQR, 10 - 70%) in the control.

On evaluation of postoperative serum biomarkers, WCC and neutrophil count demonstrated no significant association with CR-POPF incidence, while CRP was found to be significant from POD 3 onwards. Higher median CRP levels were observed on POD 3 and 5 for those who subsequently developed CR-POPF, than those who did not (both $p < .001$, Mann-Whitney U test). With regards to drain fluid composition, absolute and relative drain fluid levels for amylase and lipase were significantly associated with CR-POPF on POD 1, 3, and 5; on average, patients who developed CR-POPF were found to have had higher postoperative drain fluid lipase and amylase levels ($p < .001$ for POD 1-5), and higher corresponding LAR ($p = 0.03$ for POD1, $p < .001$ for POD 3-5). compared to the control group.

3.3 Identifying independent predictors

Univariate and multivariate logistic regression analyses were performed to define predictive factors for CR-POPF (Table 3). On univariate analysis, soft gland texture (OR = 0.10, 95%CI 0.02 – 0.50, $p = 0.005$) and acinar score (OR = 1.05, 95%CI 1.02 – 1.07, $p < .001$) were found to be significantly associated with risk of CR-POPF development. Regarding postoperative variables, CRP and drain fluid lipase concentration, amylase concentration, and LAR were similarly found to be positively associated with CR-POPF for POD 1, 3 and 5 ($p < .001$). Meanwhile, neoadjuvant therapy (OR = 4.95, 95%CI 1.40 – 17.50, $p = 0.013$) and a diagnosis of PDAC or chronic pancreatitis (OR = 4.65, 95%CI 1.82 – 11.94, $p < .001$) were negatively associated with CR-POPF. Patient factors of age, sex, BMI, and ASA score did not achieve statistical significance ($p > 0.05$). Similarly, there was no evidence of an association with main pancreatic duct diameter, estimated intraoperative blood loss, or postoperative WCC.

On multivariable analysis, LAR, acinar cell density, and CRP retained significant association with CR-POPF ($p < 0.05$). Histopathologic diagnosis of PDAC or chronic pancreatitis and WCC were included in the final model but did not demonstrate statistical significance. Homer-Lemeshow test yielded a *p*-value of 0.99, consistent with a well calibrated model, and the final model accounted for 87.3% of variance in CR-POPF incidence.

3.4 Correlation between the validated acinar cell score and LAR

Spearman Rank Correlation test demonstrated a positive correlation LAR and corresponding histopathologic acinar cell scores on POD 1 ($r_s = 0.47$, 95%CI 0.32 – 0.61, $p = <.001$), POD 3 ($r_s = 0.59$, 95%CI 0.44 – 0.70, $p = <.001$) and POD 5 ($r_s = 0.48$, 95%CI 0.31 – 0.62, $p = <.001$) (Figure 1.)

3.5 LAR as a clinical predictor for CR-POPF

ROC curve analyses and calculation of optimum prediction thresholds were used to evaluate the performance of postoperative drain fluid LAR as an early predictor for CR-POPF. Results were compared to that of drain fluid amylase and lipase as a standard of reference (Table 4; Figure 2).

ROC analyses for LAR indicated acceptable predictive performance on POD1 (AUC = 0.64, 95%CI = 0.54 – 0.74) and excellent performance on POD 3 and 5 (AUC = 0.85, 95%CI = 0.78 - 0.92 and AUC = 0.86, 95%CI = 0.79 – 0.92, respectively) (Figure 2a). Optimum prediction values for LAR over POD 1 to 5 were 2.6 – 3. 0, with a sensitivity of 92% and specificity of 46 – 71%.

Absolute drain fluid levels for amylase and lipase were similarly associated with CR-POPF (p <.001) on univariate analysis in the early postoperative period but demonstrated significant multicollinearity with LAR and were therefore excluded from multivariable analysis. Independently, amylase and lipase drain levels demonstrated excellent prediction for CR-POPF across POD 1, 3 and 5. In contrast to LAR, the excellent prediction performance was observed on POD 1 for both amylase and lipase (AUC = 0.90, 95%CI = 0.85 - 0.95 and AUC = 0.90, 95%CI = 0.84 - 0.95, respectively). Prediction performance was comparable to LAR on POD 3 and 5 for amylase (AUC = 0.86, 95%CI 0.79 – 0.93 and AUC = 0.83, 95%CI 0.74 – 0.92) and lipase (AUC = 0.87, 95%CI 0.81 – 0.94 and AUC = 0.89, 95%CI 0.83 – 0.96). However, cut-off values for amylase and lipase drain fluid levels displayed marked variation, ranging between 160 - 1747 U/L (sensitivity 79 - 89% and specificity 72 - 83%) and 669 - 4063 U/L respectively (sensitivity 93 - 100% and specificity 73 - 84%) across POD 1 to 5 (Figure 2b and 2c).

4. DISCUSSION

4.1 Lipase-to-Amylase ratio as a prognostic indicator

This study has demonstrated the utility of drain fluid LAR as an independent predictor of CR-POPF development following PD and its correlation to the intrinsic acinar cell content of the pancreas. The results demonstrate that drain fluid levels for lipase and amylase predict for CR-POPF with excellent sensitivity and specificity; however, a wide range was observed in optimal prediction thresholds on postoperative days 1, 3, and 5, highlighting a potential limitation in their use as a clinical indicator (Table 4). This finding is consistent with previous studies exploring the utility of postoperative drain fluid amylase and lipase as predictors for POPF[32, 33]. By contrast, LAR optimal thresholds for the prediction of CR-POPF were consistent (> 2.6) over the early postoperative period, making it a more clinically applicable and reliable predictor. As an early indicator for CR-POPF risk, LAR can be readily incorporated into routine postoperative care in most centres and achieved in a timely manner, compared to the histologic determination of acinar cell content at the pancreatic resection margin, which may not be in the workflow of the pathologist. Furthermore, there is evidence to suggest that the biochemical events preceding POPF formation begins intraoperatively and drain fluid levels are likely to be sensitive to these early changes[8, 11, 16, 35]

4.2 Insight into POPF pathophysiology

The findings of this study lend further evidence to the role of the exocrine pancreas in the development of POPF. It has previously been demonstrated that a higher acinar cell content at the pancreatic resection margin is strongly associated with CR-POPF in patients following pancreatic resection[19-22]. The role of the acinar cell component, may also explain the reduced incidence of POPF in states of exocrine dysfunction, as observed in chronic pancreatitis, pancreatic cancer, and neoadjuvant chemotherapy[19, 21, 28]. The results of this

study support these findings, and further suggest that LAR may be used as a biochemical correlate of the histologic acinar cell score.

The differential secretion of pancreatic enzymes has been previously been described in both in homeostatic and pathologic states[23-25]. In normal physiology, nonparallel secretion has been described in the postprandial state, in response to dietary changes, and in nocturnal phases of the circadian cycle. For example, reduced pancreatic amylase production has been associated with high protein diet and poor diabetic control, resulting in reduced intra-acinar cell glucose[23, 36]. Pancreatic lipase production appears to be more sensitive to stimuli, and outputs have been reported to exceed that of amylase up to six-fold in the postprandial state[24]. Nonparallel secretion of amylase and lipase has also been observed in pathologic states, including chronic pancreatitis and pancreatic cancer[26, 27]. In the postoperative setting, iatrogenic acinar cell injury, systemic inflammatory response mechanisms, and reduced urinary excretion have been proposed to explain elevated pancreatic enzyme concentrations in peripheral blood[37, 38]. These factors may similarly be responsible for the raised enzyme levels observed in postoperative drain fluid in this study. The precise mechanisms underlying the differential release of pancreatic amylase and lipase have yet to be characterised.

4.3 Strengths and limitations

This study has evaluated the use of serum inflammatory markers, drain fluid enzyme levels, and routine histology for the prediction of CR-POPF, and identified LAR as a novel predictor. However, several limitations should be acknowledged. Firstly, our results represent a relatively small cohort derived from a single centre, and the retrospective design of this study predisposes to selection bias. Furthermore, this study only includes patients undergoing PD and does not consider other partial pancreatectomies. Future studies using larger patient cohorts and in the context of other pancreatic resections are required. Finally, this study has only evaluated

clinical predictors encountered in routine practice. Other potential indicators of interest include pancreas-specific amylase and lipase, phospholipase A, and urinary trypsinogen activation peptide[39-41]. Furthermore, metabolomic biomarkers represent an area for future research. While beyond the scope of this study, there is growing evidence to support their diagnostic and prognostic utility in acute pancreatitis[42]. Their use, either independently or combined with parameters evaluated in this study, may improve prediction of CR-POPF and warrant future investigation.

CONCLUSION

In conclusion, LAR represents a promising clinical predictor for the development of CR-POPF. The identification of a clinically validated predictor has significant implications, with the potential for early risk stratification of patients for the development of CR-POPF and related complications. This study further validates previously reported risk factors, including the histopathologic acinar score, to suggest that PPAP and POPF severity critically depends on the acinar cell content in the remnant gland. Future studies are warranted to further our understanding of POPF pathophysiology and to inform the optimization of perioperative management for patients undergoing pancreaticoduodenectomy.

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Table 1. Summary of Demographics.

Patient demographics	**Number of cases (%) *n = 130***
Age (y) Median (IQR)	66 (57 - 72)
Sex (% Male)	71 (52.6%)
BMI (kg/m^2) Median (IQR)	24 (22.0 - 27.7)
Risk profile	
ASA status (%)	
Not reported	5 (3.7)
II	53 (39.3)
III	73 (54.1)
IV	4 (3.0)
Comorbidities (%)	
None	32 (23.7)
Cardiovascular	12 (8.9)
Renal	7 (5.2)
Diabetes	24 (17.8)
Hypertension	46 (34.1)
Current smoker	21 (15.6)
Former smoker	54 (40.0)
Immunosuppression	6 (4.4)
Histopathological diagnosis	
PDAC	64 (49)
PNET	14 (11)
IPMN/ MCN	14 (11)
Cholangiocarcinoma	11 (8)
Ampullary adenocarcinoma	7 (5)
GIST	4 (3)
Chronic pancreatitis	4 (3)
Other	12 (9)
Neoadjuvant therapy	
Neoadjuvant Chemotherapy	41 (30.4)
Neoadjuvant Radiotherapy	4 (3.0)
Transection method	
Diathermy	86
Surgical stapler	38
Energy device*	6
Pancreaticoenteric anastomosis	
PJ	126
PG	4
Multi-visceral resection	
Vascular resection and reconstruction	8 (6)
	39 (30)
Modifying therapies	
Pancreatic duct stent	47
Internal	35
External	12
Pharmacological prophylaxis	
Octreotide	31
Indomethacin	3
Postoperative Outcome	
Postoperative pancreatitis	
Postoperative pancreatic fistula	36 (26.7)
Biochemical Leak	56 (43.1)
Clinically relevant POPF	28 (21.5)
ISGPS Grade B (% of CR-POPF)	
ISGPS Grade C (% of CR-POPF)	23 (17.7)
Other non-POPF complications	5 (3.8)
Return to theatre	76 (58.5)
30-day Mortality	5 (3.8)
	1 (0.7)

BMI, Body mass index; ASA, American society of anaesthesiologists; PDAC, Pancreatic ductal adenocarcinoma; PNET, pancreatic neuroendocrine tumour; IPMN, intra-ductal papillary mucinous neoplasm; MCN, Mucinous cystic neoplasm; GIST, gastrointestinal stromal tumour; ISGPS, International Study Group of Pancreatic Surgery; POPF, postoperative pancreatic fistula.

Table 2. Demographics, clinicopathologic, intraoperative, and early postoperative variables.

Variable	CR-POPF *n = 28 (%)*	No POPF *n = 102 (%)*	*p*
Perioperative features			
Male sex	19 (68)	52 (51)	0.112
Age (years)	68 (61 – 73)	66 (56 – 72)	0.212
BMI (kg/m2)	25.8 (23.1 – 28.9)	23.4 (21.9 – 26.9)	0.089
ASA > 3	12 (43)	57 (56)	0.116
Smoking history	10 (36)	45 (44)	0.403
Diabetes	6 (21)	18 (18)	0.664
Hypertension	11 (39)	35 (34)	0.651
Cardiovascular disease	2 (7)	10 (10)	0.100
Renal insufficiency	1 (4)	6 (6)	0.100
Immunosuppression	3 (11)	3 (3)	0.116
Neoadjuvant treatment (%)	3 (11)	38 (37)	<.001*
PDAC or pancreatitis	7 (25)	62 (61)	<.001*
Soft gland texture	17 (89)	20 (46)	<.001*
Not reported	9 (32)	59 (58)	
MPD diameter >3mm	6 (35)	19 (48)	~~0~~.396
Not reported	11 (39)	62 (61)	
EBL >500mL	7 (41)	18 (35)	0.625
Not reported	11 (39)	50 (49)	
Postoperative features			
Acinar score (%)	80 (65 - 90)	40 (10 - 70)	<.001*
WCC (x10^9/L)			
POD1	11.4 (9.9 – 13.9)	13.0 (11.1 – 16.0)	0.050
POD3	9.2 (7.4 – 12.5)	10.8 (8.4 – 13.5)	0.356
POD5	8.9 (7.6 – 11.7)	9.1 (7.1 – 12.3)	0.968
CRP (mg/L)			
POD1	139.5 (83.7 – 301.7)	108.0 (77.3 – 206.8)	0.188
POD3	307.0 (266.0 – 380.0)	190.5 (116.3 – 256.3)	<.001*
POD5	233.5 (183.3 – 302.8)	115.0 (66 – 192)	<.001*
Neutrophil (x10^9/L)			
POD1	9.6 (8.6 – 12.3)	10.9 (9.1 – 14.0)	0.126
POD3	8.7 (5.8 – 11.1)	8.7 (6.6 – 11.3)	0.919
POD5	7.2 (5.5 – 9.6)	7.1 (5.2 – 10.2)	0.817
Amylase (U/L)			
POD1	4787 (2584.8 – 10367.5)	144 (29 – 1179)	<.001*
POD3	1962 (807 – 5220)	71 (16.3 – 300.5)	<.001*
POD5	860 (175 – 2008.8)	25 (11 – 80)	<.001*
Lipase (U/L)			
POD1	23770 (10892 – 56375.5)	371 (42 – 4816.5)	< .001*
POD3	7978 (4445.8 – 26849.3)	120 (8 – 905)	<.001*
POD5	3235 (1042.8 – 16072.8)	21 (4 – 255.5)	<.001*
LAR			
POD1	4 (3.2 – 4.5)	3 (1.2 – 4.6)	0.030*
POD3	5 (3.9 – 7.2)	2 (0.7 – 3.2)	<.001*
POD5	5 (3.1 – 9.0)	1 (0.6 – 2.8)	<.001*

* $P < 0.05$. Data are represented as median and interquartile ranges.
Chi-squared or Fisher's exact tests were applied for categorical data and Mann-Whitney U test for continuous data.
BMI, Body mass index; ASA, American society of anaesthesiologists; MPD, main pancreatic duct; WCC, white cell count; CRP, C-reactive protein, LAR, lipase-to-amylase ratio; POD, postoperative day; 95% CI, 95% Confidence Interval; U/L, units per litre.

Table 3. Univariate and multivariate analyses.

Variable	Univariate		Multivariate	
	p	OR (95% CI)	*p*	OR (95% CI)
Perioperative features				
Male sex	0.116	2.03 (0.84 – 4.91)		
Age (years)	0.165	1.03 (0.99 – 1.07)		
BMI (kg/m2)	0.141	1.07 (0.98 – 1.16)		
ASA ≥ 3	0.120	0.50 (0.21 – 1.20)		
Neoadjuvant treatment	0.013*	4.95 (1.40 – 17.50)		
PDAC or pancreatitis	<.001*	4.60 (1.81 – 11.94)	0.063#	0.26 (0.07 – 1.08)
Soft gland texture	0.005*	0.10 (0.02 – 0.50)		
MPD diameter >3mm	0.396	1.66 (0.51 – 5.36)		
EBL >500mL	0.626	0.76 (0.25 – 2.32)		
Postoperative features				
Acinar score (%)	<.001*	1.05 (1.02 – 1.07)	0.007#	7.19 (1.71 – 30.25)
WCC (x10⁹/L)				
POD1	0.072*	0.91 (0.83 – 1.01)	0.059	0.20 (0.04 – 1.06)
POD3	0.586	0.97 (0.88 – 1.07)		
POD5	0.719	0.98 (0.90 – 1.08)		
CRP (mg/L)				
POD1	0.083*	1.00 (1.00 – 1.01)		
POD3	<.001*	1.02 (1.01 – 1.02)		
POD5	<.001*	1.01 (1.01 – 1.02)	<.001#	26.9 (5.54 – 130.28)
Neutrophil (x10⁹/L)				
POD1	0.114	0.913 (0.82 – 1.02)		
POD3	0.753	1.02 (0.91 – 1.13)		
POD5	0.869	0.99 (0.90 – 1.10)		
Amylase (U/L)				
POD1	0.003*	1.00 (1.00 – 1.00)		
POD3	0.096*	1.00 (1.00 – 1.00)		
POD5	0.010*	1.00 (1.00 – 1.00)		
Lipase (U/L)				
POD1	< .001*	1.00 (1.00 – 1.00)		
POD3	0.012*	1.00 (1.00 – 1.00)		
POD5	0.001*	1.00 (1.00 – 1.00)		
LAR				
POD1	0.284	1.04 (0.97 – 1.10)		
POD3	0.034*	1.09 (1.00 – 1.17)		
POD5	<.001*	1.31 (1.15 – 1.50)	0.008#	7.40 (1.70 – 32.12)

* These variables approached statistical significance ($p < 0.1$) and were selected for multivariate regression analyses.
# These variables retained significance ($p < 0.05$) in the final multivariable model.
BMI, Body mass index; ASA, American society of anaesthesiologists; MPD, main pancreatic duct; WCC, white cell count; CRP, C-reactive protein, LAR, lipase-to-amylase ratio; POD, postoperative day; 95% CI, 95% Confidence Interval; U/L, units per litre.

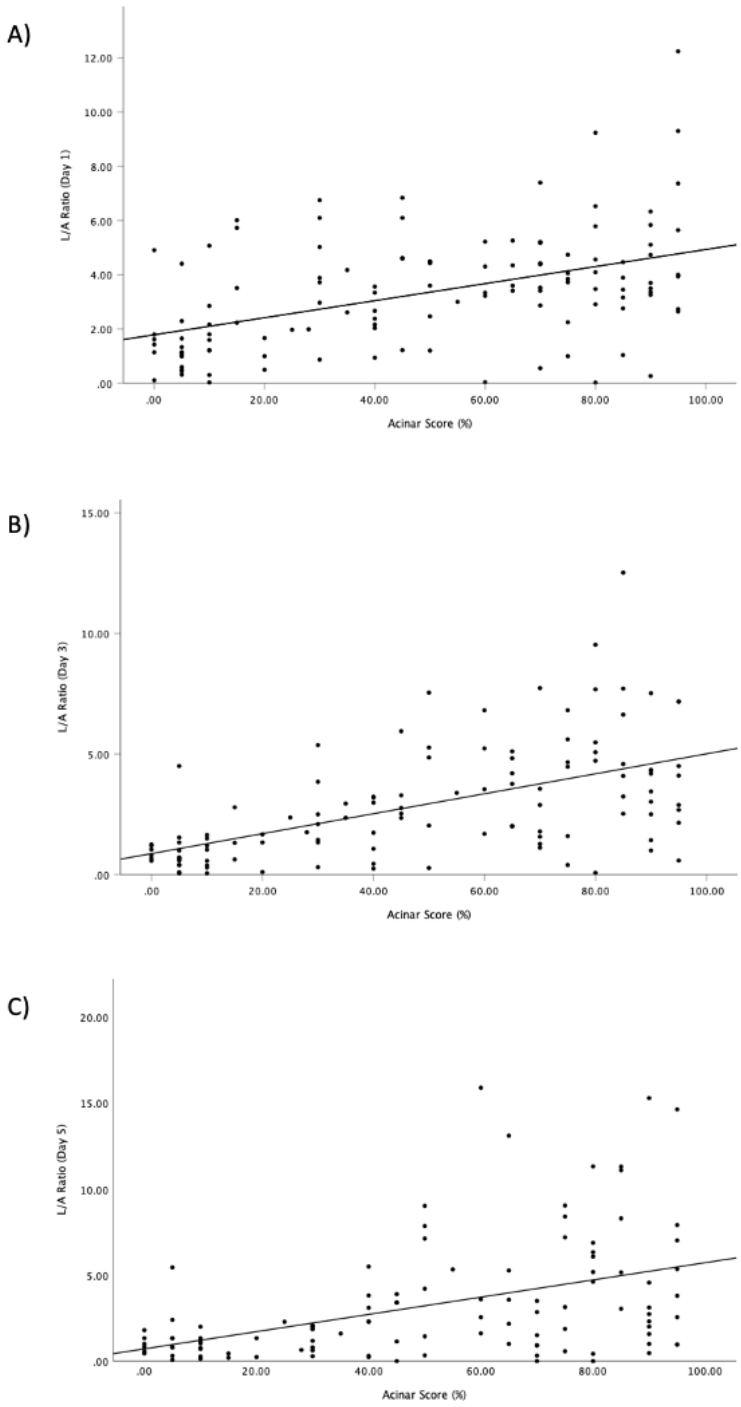


Figure 1. Correlation of acinar score and lipase-to-amylase ratio (LAR) on postoperative **A)** Day 1 (r_s = 0.47, p <.001), **B)** Day 3 (r_s = 0.59, p = <.001), and **C)** Day 5 (r_s = 0.48, p <.001).

Table 4. Area under the Receiver Operating Characteristic (ROC) curve analysis. Confidence interval, optimum prediction thresholds, sensitivity and specificity, and predictive values of drain fluid predictors for clinically relevant POPF.

	Area under the ROC curve	*P* value	95% CI	Optimum cut-off	Sensitivity (%)	Specificity (%)
LAR						
POD1	0.64	0.029	0.54 – 0.74	2.7	0.93	0.46
POD3	0.85	<.001	0.78 - 0.92	3.0	0.89	0.71
POD5	0.86	<.001	0.79 - 0.92	2.6	0.89	0.73
Amylase (U/L)						
POD1	0.90	<.001	0.85 - 0.95	1747.0	0.89	0.80
POD3	0.86	<.001	0.79 - 0.93	266.5	0.93	0.75
POD5	0.83	<.001	0.74 - 0.92	160.0	0.93	0.84
Lipase (U/L)						
POD1	0.90	<.001	0.84 - 0.95	4062.5	1.00	0.73
POD3	0.87	<.001	0.81 - 0.94	1119.0	0.93	0.75
POD5	0.89	<.001	0.82 - 0.96	669.0	0.93	0.84

LAR, Lipase-to-amylase ratio; 95% CI, 95% Confidence Interval; U/L, units per litre.
Optimum thresholds calculated by Youden Index (sensitivity + specificity - 1).

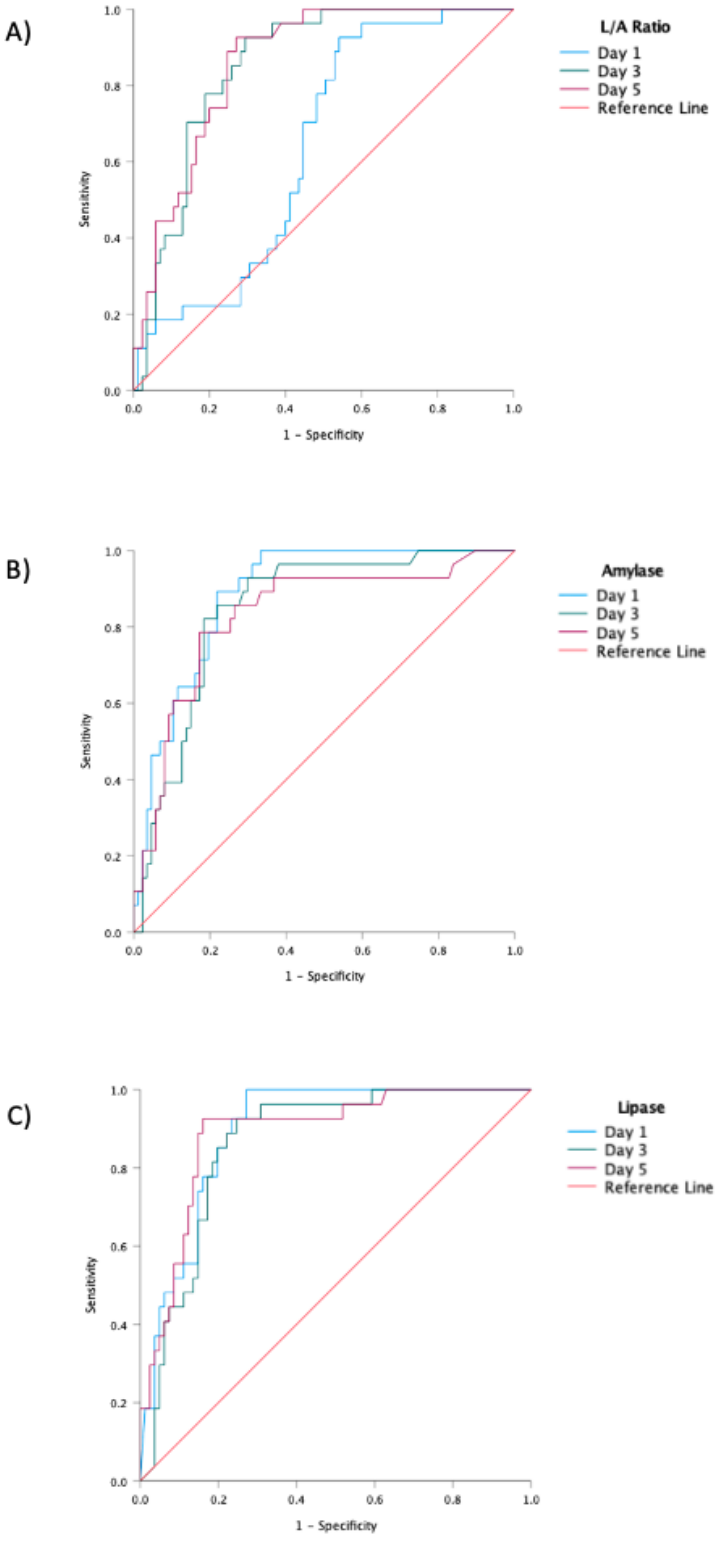
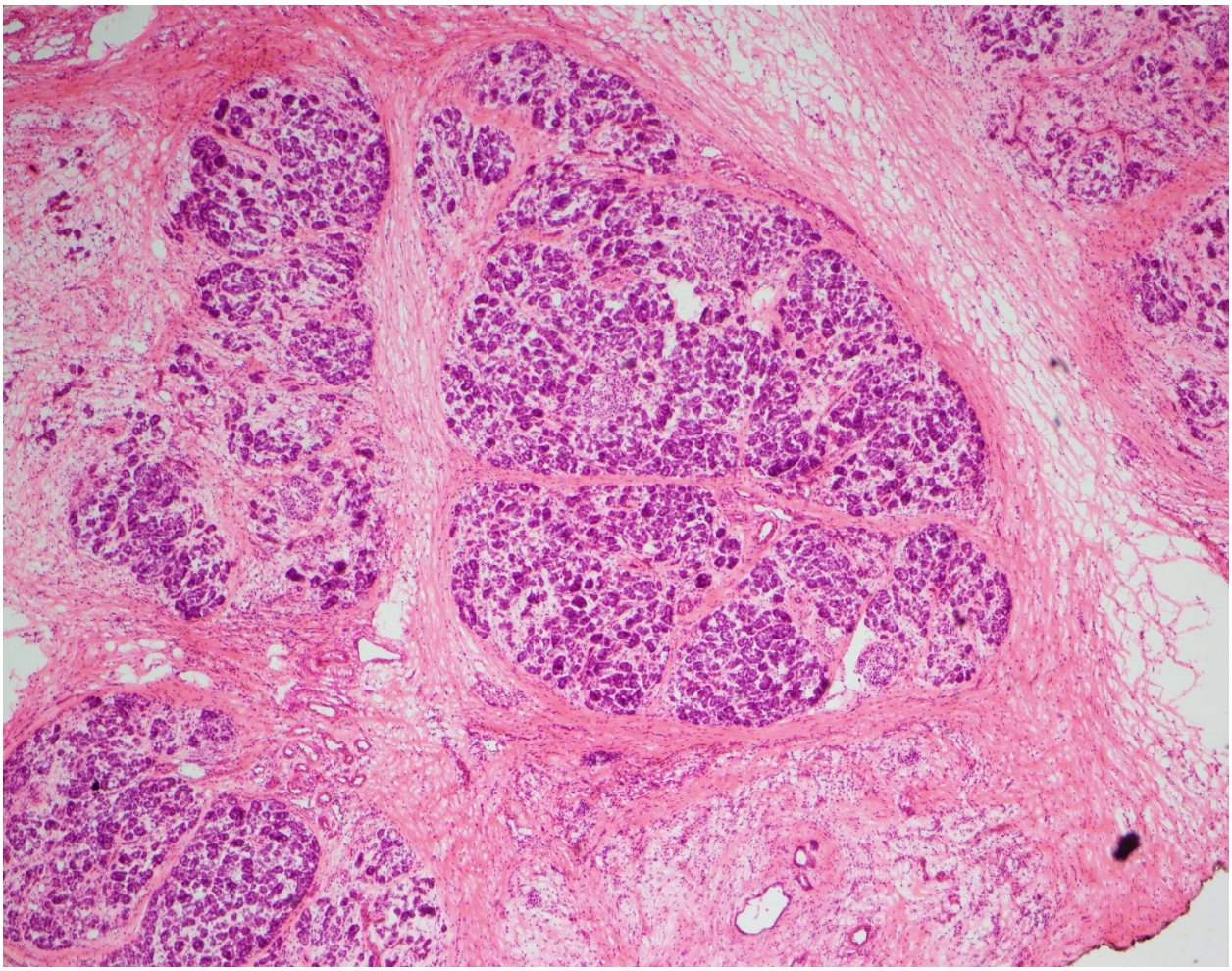


Figure 2. Receiver Operator Characteristic (ROC) curves for drain fluid LAR, lipase level, and amylase level for postoperative days 1, 3, and 5 (A-C respectively).



Supplementary – Figure 1. Transverse section of pancreas at the neck resection margin (Original magnification 100x). In this case, approximately 40% of the cross-sectional area of the pancreas is occupied by acinar tissue, 55% by collagenous fibrous tissue and 5% by fat; therefore, the acinar score is 40%.

CHAPTER IV.

Post-pancreatectomy Acute Pancreatitis as a Distinct Complication Entity

Clinical Validation of the International Study Group of Pancreatic Surgery (ISGPS) Definition for Post-Pancreatectomy Acute Pancreatitis

Juanita Noeline Chui[1,2] MD, Ai Jen Yang[1] Christopher B Nahm[2,3,4] PhD FRACS, Saxon Connor MBChB[5] FRACS, Anthony J Gill [2,6,7] FRCPA, Jaswinder S Samra[1,2,8] DPhil (Oxon) FRACS, Anubhav Mittal[1,2,8,9], PhD FRACS

[1] Department of Gastrointestinal Surgery, Royal North Shore Hospital, Sydney, Australia

[2] Faculty of Medical and Health Sciences, The University of Sydney, Sydney, Australia

[3] Department of Hepatobiliary, Pancreatic/Upper Gastrointestinal Surgery, Westmead Hospital, Sydney, Australia

[4] Surgical Innovations Unit, Westmead Hospital, Sydney NSW Australia

[5] Department of Surgery, Christchurch Hospital, Christchurch, New Zealand

[6] Kolling Institute of Medical Research, University of Sydney, Sydney, Australia

[7] NSW Health Pathology, Department of Anatomical Pathology, Royal North Shore Hospital, Sydney, Australia

[8] Australian Pancreatic Centre, Sydney, Australia

[9] School of Medicine, The University of Notre Dame, Sydney, Australia

ABSTRACT (200 words)

Background: The diagnosis of postoperative or post-pancreatectomy acute pancreatitis (PPAP) is controversial. In 2021, the *International Study Group of Pancreatic Surgery* (ISGPS) published the first unifying definition and grading system for PPAP. This study sought to validate recent consensus criteria, using a cohort of patients undergoing pancreaticoduodenectomy (PD) in a high-volume pancreaticobiliary specialty unit.

Methods: All consecutive patients undergoing PD at a tertiary referral centre between January 2016 and December 2021 were retrospectively reviewed. Patients with serum amylase recorded within 48 hours from surgery were included for analysis. Postoperative data were extracted and evaluated against the ISGPS criteria, including the presence of postoperative hyperamylasaemia, radiologic features consistent with acute pancreatitis, and clinical deterioration.

Results: A total of 82 patients were evaluated. The overall incidence of PPAP was 32% (26/82) in this cohort, of which 3/26 demonstrated postoperative hyperamylasaemia and 23/26 had clinically relevant PPAP (Grade B or C) when correlated radiologic and clinical criteria.

Conclusions: This study is among the first to apply the recently published consensus criteria for PPAP diagnosis and grading to clinical data. While the results support their utility in establishing PPAP as a distinct post-pancreatectomy complication, there remains a need for future large scale validation studies.

1. BACKGROUND

Postoperative or post-pancreatectomy acute pancreatitis (PPAP) has recently gained formal recognition by the *International Study Group of Pancreatic Surgery* (ISGPS) as a distinct complication entity following partial pancreatic resection[1]. The ISGPS consensus criteria, published in 2021, represent the first unifying definition and grading system for PPAP, which have yet to be validated and applied to patient data.

According to the ISGPS, PPAP is defined as the acute inflammatory condition of the pancreatic remnant following partial pancreatic resections, occurring in the early postoperative period (within the first three days from surgery)[1]. Based on biochemical, radiologic and clinical criteria, PPAP is diagnosed by the presence of: i) early sustained elevation of serum amylase (above the upper limit of normal for at least 48 hours) postoperatively; ii) radiological evidence consistent with acute pancreatitis and iii) clinically relevant features. The proposed grading scheme stratifies its severity, ranging from benign postoperative hyperamylasaemia, without no appreciable impact on postoperative recovery, to mild-moderate (Grade B) and severe life-threatening complications (Grade C) (Table 1).

Prior to the ISGPS consensus statement, there was no universally accepted and objective definition for PPAP to permit standardised reporting and comparison of outcomes. As such, there has been significant controversy surrounding its incidence, clinical significance, pathophysiology, and management[2]. In a recent review of 39 studies published before 2019 (comprising data from 9,220 patients), Bannone *et al.* reported an incidence of 64% for postoperative hyperamylasaemia but there was insufficient evidence to determine the incidence for postoperative pancreatitis and approximately 40% of the studies in this review lacked a definition for PPAP.

Previously, most studies have adapted the revised Atlanta Classification for acute pancreatitis (serum amylase above three times the upper limit of normal with associated

morbidity) to define acute pancreatitis in the postoperative setting[3]. However, it has become apparent in recent years that acute pancreatitis following partial pancreatectomy is a distinct phenomenon to that occurring in the non-surgical setting. In an attempt to define postoperative acute pancreatitis, Connor proposed a definition based on the presence of any serum pancreatic enzyme level exceeding the upper limit of normal[4]. This resulted in exceptionally high rates with subsequent studies questioning the validity of the proposed diagnostic threshold[5]. Furthermore, these definitions do not distinguish between postoperative hyperamylasaemia of no clinical importance and clinically relevant PPAP. In contrast, the ISGPS recognizes that early elevation of pancreatic serum enzymes is frequent following major pancreatic resections and does not reflect or predict PPAP. The inclusion of radiologic and clinical criteria permits correlation with clinical outcomes. The proposed grading system formally recognizes, for the first time, the spectrum of severity of PPAP and distinguishes clinically relevant acute pancreatitis, from postoperative hyperamylasemia.

This study sought to evaluate the utility of the recent ISGPS criteria, using a cohort of patients undergoing pancreaticoduodenectomy (PD) in a high-volume pancreaticobiliary surgical specialty unit, and to summarise the current evidence on the elusive entity of PPAP.

Table 1. Summary of ISGPS criteria for PPAP diagnosis and Grading[1].

PPAP is diagnosed by the presence of:
i) Early sustained elevation of serum amylase (above the upper limit of normal for at least 48 hours) postoperatively
ii) Radiological evidence consistent with acute pancreatitis
iii) Clinically relevant features
The proposed grading scheme stratifies its severity from benign postoperative hyperamylasaemia, without no appreciable impact on postoperative recovery, to mild-moderate (Grade B) and severe life-threatening complications (Grade C).

2. MATERIALS AND METHODS

2.1 Patient selection and data collection

Institutional review board ethics approval was obtained for this study. All consecutive patients undergoing pancreaticoduodenectomy (PD) at a single public tertiary referral hospital in Sydney, NSW, Australia, between January 2016 and December 2021 were identified from a prospectively maintained database. Only patients who had postoperative serum amylase recorded within 48 hours from surgery were included to permit evaluation of the ISGPS criteria. All operations were performed by one of two consultant hepatopancreatobiliary surgeons (AM and JS), according to previously described methods[10].

A retrospective review of records was performed to extract demographic, clinicopathologic, intraoperative and postoperative data. Demographic clinical data included the patient's age, sex, body mass index (BMI), comorbidities, and American Society of Anaesthesiologists (ASA) score. Histopathological diagnosis was recorded for all patients. Where PD was indicated for malignant lesions, this included tumour staging related data and the use of neoadjuvant treatment. Postoperative serum amylase levels were recorded up until postoperative day three. Clinical outcomes of interest included length of hospital stay, length of admission to Intensive Care Unit (ICU), postoperative complications, reoperation, and mortality (defined as within 30 days from surgery).

2.2 Definition and Classification of PPAP

Three grades of severity were classified according to the ISGPS criteria, incorporating postoperative serum amylase levels, radiological findings, and patient outcomes. Patients demonstrating sustained elevations to serum amylase (above the upper limit of normal for 48

hours) were identified as positive for PPAP. Clinical outcomes were used to distinguish clinically relevant cases (ISGPS Grades B and C) from benign postoperative hyperamylasemia (POH) (ISGPS Grade A). All pancreatectomy-specific complications were similarly defined according to ISGPS definitions, including delayed gastric emptying (DGE)[6], postpancreatectomy haemorrhage (PPH)[7], and postoperative pancreatic fistula (POPF)[8]. Other major complications of interest included intraabdominal abscess formation and organ space infection, sepsis, and relaparotomy. Postoperative complications were graded according to Clavien-Dindo classification and mortality was recorded where death occurred within 30 days from surgery.

As the ISGPS criteria does not specify timing of relevant radiological findings and related complications, data within a single admission were included for analysis and used to clinically correlate biochemical evidence of PPAP. With the lack of consensus on criteria for radiologic features, CT reports were reviewed for radiologic abnormalities consistent with PPAP independently by two authors (JC and AJY) and a consensus was reached. Where there were positive findings for PPAP on postoperative imaging, time from surgery was additionally recorded.

2.3 Determining the viable acinar component

For all cases, haematoxylin, and eosin (H&E) stained sections of the paraffin-embedded pancreatic resection margin were examined for acinar cell density, using previously described methods[9-11]. All slides from the pancreatic neck resection margin were examined under light microscopy and the percentage of the cross-sectional area occupied by acinar cells, fibrous connective tissue, and fat were recorded. The acinar score was evaluated by two authors (CN and AG) who were blinded to clinical outcomes.

2.4 Statistical analysis

Patients developing POH were included within the control group and compared with those who developed clinically relevant PPAP. Quantitative data were assessed for normal distribution using the Shapiro-Wilk test. Continuous data were presented as median with interquartile ranges (IQR) and categorical data as absolute and relative frequencies unless indicated otherwise. Factors associated with clinically relevant PPAP severity were calculated based on univariate analyses. Nonparametric statistical methods were used including Mann-Whitney U, Chi-square, or Fisher's exact tests as appropriate. A p-value of <0.05 represented the threshold of statistical significance. All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 28.0 software (IBM Corp., Armonk, NY, USA).

3. RESULTS

3.1 Patient characteristics and outcomes

A total of 82 patients who underwent PD were evaluated against the ISGPS consensus criteria. Baseline characteristics of this study cohort and patient outcomes are summarised in Tables 2 and 3. The incidence of PPAP was 26 cases (32%) in this cohort, of which 3 cases (4%) demonstrated POH and 23 cases (28%) clinically relevant PPAP (Grade B and C) when correlated to patient outcomes. Overall, 37 (45%) suffered pancreatectomy-specific complications, with 23 (28%) developing clinically relevant POPF, 9 (11%) DGE, and 7 (9%) PPH. Additionally, 18 (22%) were complicated by organ space infection and 11 (13%) by intraabdominal sepsis. Non-POPF complications were reported in 39 (48%) of patients. Seven (9%) cases were returned to theatre, of which 5 required relaparotomy for intra-abdominal

bleeding and 2 underwent drainage of superficial wound collections. The median length of hospital stay was 21 days (IQR, 14-32), and median duration of ICU admission was 3 days (IQR, 2-6). There was one 30-day mortality.

Among the control group, 44/59 (75%) patients underwent postoperative CT imaging, including all patients who developed POH (3/3). Eighteen patients demonstrated radiological features consistent with PPAP as defined by the ISGPS criteria, of which peripancreatic collections was the most common positive finding (16/18).

3.2 Risk factors for PPAP as defined by the ISGPS criteria

A comparison of characteristics for clinically relevant PPAP and non-PPAP groups is presented in Table 4. There was evidence to suggest a positive association between clinically relevant PPAP and acinar cell score (p<.001), soft gland texture (p=0.003), and BMI (p=0.034). Meanwhile, diagnosis of PDAC or chronic pancreatitis (p<.001) and neoadjuvant treatment were associated with reduced incidence of clinically relevant PPAP. There was no significant association with clinically relevant PPAP incidence and patient age, sex, and comorbidities.

Using the established Fistula Risk Score (FRS)[12], patients who were identified as immediate to high risk (score of greater than or equal to 3) for the development of POPF also had an increased risk of developing clinically relevant PPAP, compared to those with a low FRS score (less than 3). Interestingly, the use of pancreatic stents or pharmacological agents, such as prophylactic octreotide, indomethacin, or dexamethasone demonstrated no association with clinically relevant PPAP in this cohort (p>0.05).

Postoperatively, there was a significant difference in morbidity between patients who fulfilled the ISGPS criteria for PPAP and those who did not (p<.001). Among the PPAP group, a larger proportion of patients (24/26, 92%) developed pancreas-specific or major complications (Clavien-Dindo score >3), compared to the non-PPAP group (13/56, 23%). No

difference was observed in relation to length of stay, length of ICU admission postoperatively, or thirty-day mortality.

3.3 Correlation between acinar cell scores and postoperative serum amylase

Spearman Rank Correlation test demonstrated a positive correlation between acinar score and POD 1 serum amylase (r_s=0.57, 95%CI=0.36-0.73, p=<.001).

Table 2. Study characteristics.

Patient demographics	**Number of cases (%) *n = 82***
Age (y) Median (IQR)	66 (57.5 – 72.5)
Sex (% Male)	45 (55)
BMI Median (IQR)	23 (20.9 – 27.3)
ASA status (%)	
II	34 (41)
III	44 (54)
IV	4 (5)
Histopathological diagnosis	
PDAC	43 (52)
Cystic neoplasm	9 (11)
Cholangiocarcinoma	8 (9)
Ampullary adenocarcinoma	8 (9)
PNET	7 (9)
Other	7 (9)
Neoadjuvant treatment	26 (32)
Postoperative course	
Length of stay (day) Median (IQR)	21(14 – 32)
Length of ICU stay (day) Median (IQR)	3 (2 – 6)
Major complication	
PPAP-related complication	37 (45)
Other complication	39 (48)
Postoperative mortality	1 (1)

BMI, Body mass index; ASA, American society of anaesthesiologists; PDAC, Pancreatic ductal adenocarcinoma; PNET, pancreatic neuroendocrine tumour; PPAP, Post-pancreatectomy Acute Pancreatitis; IQR, Interquartile range

Table 3. ISGPS criteria for PPAP diagnosis and grading.

ISGPS Diagnostic Criteria	
Biochemical Evidence (Sustained hyperamylasemia)	**Number of cases (%) *n* = *82***
ISGPS Criteria (>ULN for at least 48h)	26 (32)
Radiological Evidence	
No post-operative imaging	16 (20)
Consistent with PPAP	36 (44)
Peripancreatic fluid	35
Parenchymal oedema	22
Parenchymal necrosis	1
Positive findings on POD > 3	32 (89)
Time from surgery (days) Median (IQR)	6 (4 – 8)
Clinical Evidence	
Clinically Relevant Pancreatic fistula (ISGPS Grades B/ C)	23 (28)
Organ space infection / Intraabdominal abscess	18 (22)
Percutaneous or endoscopic drainage	13
Sepsis	11 (13)
Delayed gastric emptying	9 (11)
Post-pancreatectomy haemorrhage	7 (9)
Return to theatre	7 (9)
Relaparotomy	5
Wound washout	2
ISGPS Grading	**Number of cases (%) *n* = 26**
Postoperative hyperamylasaemia	3 (12)
Grade B PPAP	16 (62)
Grade C PPAP	7 (27)

ISGPS, International Study Group of Pancreatic Surgery; PPAP, Post-pancreatectomy Acute Pancreatitis; ULN, upper limit of normal; POD, postoperative day; IQR, Interquartile range; IR, Interventional Radiology

Table 4. Comparison of PPAP and non-PPAP groups.

Variable	CR-PPAP *n = 23 (%)*	Control *n = 59 (%)*	*p*
Perioperative features			
Male sex	14 (61)	31 (53)	0.497
Age (years)	66 (57 – 73)	66 (57 – 72)	0.617
BMI (kg/m2)	23.6 (22.2 – 29.0)	22.9 (20.5 – 26.3)	0.046*
ASA ≥ 3	11 (48)	37 (63)	0.222
Neoadjuvant treatment (%)	3 (13)	23 (39)	0.043
Histopathologic features			
Acinar cell score (%)[1]	80.0 (65.0 – 86.8)	40.0 (10.0 – 73.8)	0.002*
Soft gland texture[2]	18 (95)	16 (50)	0.008*
PDAC or chronic pancreatitis	6 (26)	37 (63)	0.004*
Other	19 (82)	20 (34)	
Cystic neoplasm	5 (22)	4 (7)	
PNET	3 (13)	4 (7)	
Cholangiocarcinoma	4 (17)	4 (7)	
Ampullary adenocarcinoma	1 (4)	7 (12)	
Benign pathology	2 (9)	0 (0)	
Fistula Risk Score[3]			
≥ 3 (Intermediate – High)	17 (94)	22 (63)	0.034*
< 3 (Negligible – Low)	1 (6)	13 (37)	
Reconstruction with external stent	7 (30)	25 (42)	0.322
Pharmacological treatment (Octreotide, Indomethacin, Dexamethasone)	13 (57)	30 (51)	0.644
Postoperative course			
Length of stay (days), Median (IQR)	30.0 (21.3 – 38.9)	17.0 (13.0 – 26.2)	0.029*
Length of ICU stay (days), Median (IQR)	3.0 (1.5 – 7.0)	3.0 (2.0 – 5.0)	0.296
Other post-pancreatectomy complication[4]	21 (91)	18 (31)	<.001*
In-hospital mortality	0 (0)	1 (2)	

* *P* < 0.05. Chi-square or Fisher's exact tests were applied for categorical data and Mann-Whitney U test for continuous data.

[1] Missing acinar scores for PPAP group (n = 4) and no PPAP group (n = 14)

[2] Missing gland texture for PPAP group (n = 10) and no PPAP group (n = 33)

[3] Postoperative pancreatic fistula, delayed gastric emptying, post-pancreatectomy haemorrhage, Intra-abdominal abscess or collections, sepsis

PPAP, Post-pancreatectomy Acute Pancreatitis; 95% CI, 95% Confidence Interval; IQR, Interquartile range; BMI, Body mass index; ASA, American society of anaesthesiologists; PDAC, Pancreatic ductal adenocarcinoma; PNET, pancreatic neuroendocrine tumour; ICU, Intensive Care Unit.

4. DISCUSSION

4.1 Evaluation of the ISGPS criteria

In the absence of a unifying definition for PPAP until the recent ISGPS criteria, reports on its incidence have varied widely within the literature, with rates as high as 53-64%[4, 13, 14]. In this cohort of patients undergoing PD, there was an incidence of 28% (23/82) for clinically relevant acute pancreatitis as defined by the ISGPS (PPAP Grades B or C). Of note, the proposed threshold for sustained hyperamylasemia (at least 48 hours) correlated well with postoperative morbidity. While the present study lacked the power to detect a difference in mortality, the incidence of pancreas-specific and major complications was significantly higher for patients who met this criterion compared to those who did not. The results of this study therefore support the ISGPS criterion on biochemical evidence of sustained hyperamylasemia for identifying patients with poorer clinical outcomes.

In contrast, there are potential limitations to be acknowledged in relation to the application of ISGPS clinical and radiological criteria. The clinical features of PPAP as outlined in the consensus statement are non-specific. In a large proportion of patients in this study, this depended on the presence of other pancreas-specific complications including, POPF, DGE, and PPH. Meanwhile, radiological features of PPAP and the appropriate timing of postoperative imaging are poorly defined – early postoperative inflammatory changes may misinform grade and late changes do not facilitate prognosis. Among the cases that demonstrated positive radiological findings in this study, 89% (32/36) were observed to occur after the third postoperative day – exceeding the proposed timeframe for PPAP diagnosis based on biochemical evidence. As such, while they may be useful in the evaluation of equivocal cases, the diagnostic utility of radiologic and clinical criteria in the absence of biochemical evidence remains in question.

In the only other study to evaluate the new ISGPS consensus definition to date, Ikenaga *et al*.[15] reported a low incidence of clinically relevant PPAP (24%) within their cohort, similar to that observed in the present study. However, the authors cautioned for under detection of PPAP using the proposed criteria for sustained hyperamylasaemia, as they observed that patients who only demonstrated elevated serum amylase on POD1 also experienced poorer clinical outcomes. The same study further reported that for patients with CT-determined acute pancreatitis, positive radiologic findings were detected late in their postoperative course (median of POD10, range POD4-24) in agreement with the findings of this study. In relation to clinical and radiological criteria further appraisal at other institutions may therefore be necessary.

4.2 Insights into PPAP pathophysiology

At present, PPAP is a well-known but poorly studied complication. Overall, the ISGPS consensus statement has provided standardization of criteria for evaluating PPAP as a newly recognized post-pancreatectomy complication, which will be essential in permitting future studies into its pathophysiology and management strategies. The proposed nomenclature accompanying the new criteria distinguishes acute pancreatitis occurring after partial pancreatectomy from other aetiologies and from late-onset obstructive pancreatitis. Additionally, the proposed grading scheme recognizes the spectrum of clinical severity, which has not previously been defined. Notably, the ISGPS classification scheme distinguishes benign POH from clinically relevant PPAP (ISGPS Grades B and C). It suggests that hyperamylasemia, frequently observed following major pancreatic resection, should be regarded as a separate entity from the pathological process of PPAP with its adverse implications for patient outcomes. Furthermore, the notion of clinical relevance and the recognition of its various grades of severity holds significant implications for risk stratification

of patients undergoing partial pancreatectomy, conferring the potential to reduce postoperative morbidity.

As understanding of PPAP continues to evolve, ongoing revisions to current diagnostic and grading criteria will be necessary. While the pathophysiology of PPAP has yet to be elucidated, there is a growing body of evidence to suggest that the ischaemic-inflammatory response that occurs within the pancreatic remnant following major resection depends on its viable acinar cell component[10, 16, 17]. Similar to acute pancreatitis in the non-surgical setting, clinical manifestations of postoperative pancreatitis are postulated to occur on a spectrum of severity from inflammation to necrosis[2, 5]. In the present study, a positive association was observed between acinar scores and clinically relevant acute pancreatitis, lending evidence to the notion that the grade of PPAP severity depends on acinar cell density. Furthermore, there is a growing body of evidence to support a pathophysiologic relationship between PPAP and POPF[4, 14]. In this study cohort, 17 out of 23 of patients who developed clinically relevant POPF demonstrated sustained hyperamylasaemia, fulfilling the proposed biochemical criteria for PPAP. This study further lends evidence to the association between clinically relevant PPAP and increased acinar cell density at the resection margin, soft gland texture, neoadjuvant treatment, and histopathologic diagnoses other than PDAC or chronic pancreatitis, which are known risk factors for POPF[10, 12, 16]. The relatively low incidence of PPAP in this cohort may be attributed to the significant proportion of cases performed for PDAC (52%) and following neoadjuvant therapy (31%). Evidently, the standardisation of PPAP definition will be important in furthering our understanding of PPAP pathophysiology and its related complications.

4.3 Limitations

Major limitations of the present study are attributed to its retrospective design. Firstly, there was a relatively small patient cohort, due to the lack of data for postoperative serum amylase. Over the study period, approximately one third of patients who underwent PD at our centre were excluded, representing a potential source of selection bias. Similarly, not all patients were evaluated with postoperative CT imaging. Furthermore, this study analyses PPAP within the context of PD only and has not controlled for variation in surgical approach and anastomotic techniques. Future studies using data from larger patient cohorts and in the context of other pancreatic resections will be of value in further characterising the clinical entity of PPAP.

CONCLUSION

This study is among the first to apply the recently published international consensus criteria for the diagnosis and grading of PPAP to clinical data. These early results support its utility in recognizing PPAP as a distinct post-pancreatectomy complication and its spectrum of severity but highlight the need for future larger scale validation studies.

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CHAPTER V.

Concluding Remands and Future Directions

1. Summary of Findings

Postoperative pancreatic fistula (POPF) is a universally dreaded complication following pancreatic resection, representing a leading cause of morbidity and mortality[1-3]. In recent years, there has been a paradigm shift in our understanding of its pathogenesis[4, 5]. Chapter 1 summarises the contemporary literature, which has challenged the traditional concept of POPF as a mere anastomotic leak. It is now apparent that post-pancreatectomy acute pancreatitis (PPAP) plays a critical role in the development of POPF, with major implications for future risk assessment and mitigation strategies.

Chapter 2 is concerned with the prediction of the clinically relevant fistula, defined by the *International Study Group of Pancreatic Surgery* (ISGPS) as those of Grade B or C severity[6]. Here, we validated established risk factors, including components of the Fistula Risk Score[7], acinar cell density, and explored the use of surgical drain fluid and serological biomarkers for the prediction of clinically relevant POPF. The results support acinar cell density as a strong predictor for POPF. In keeping with this, a reduced POPF risk was observed in patients who had a history of neoadjuvant therapy, diagnosis of chronic pancreatitis or pancreatic ductal adenocarcinoma, or intraoperative finding of firm gland texture. These factors are known to be associated with reduced acinar cell density and are identified as protective factors for the development of POPF in this study.

With regards to identifying a prognostic indicator, it was shown that postoperative drain fluid amylase and lipase concentrations predicted for clinically relevant POPF with excellent sensitivity and specificity. The lipase-to-amylase ratio (LAR) was proposed as a novel predictor with the advantage of consistent prediction thresholds in the early postoperative period. Furthermore, LAR was significantly correlated with acinar cell density at the pancreatic resection margin examined on frozen sections. While further research will be required to

validate our findings, there is potential for the development of an updated prognostic score or nomogram using the risk factors identified in our study.

Finally, Chapter 3 validates the current consensus criteria for the diagnosis and grading of PPAP. With the growing interest in PPAP as a mechanism for the development of POPF it gained international recognition as a distinct complication entity by the ISGPS in 2021[8]. Formerly regarded as postoperative pancreatitis, there has been no universally accepted definition to permit standardised reporting and comparison of outcomes in the literature until now. Our results demonstrate that the understanding of PPAP as a clinical entity remains incomplete. Larger-scale validation studies are required to better characterise its clinical and radiologic correlates, and further research into its fundamental pathophysiology is warranted.

2. Discussion

The evidence-base for postoperative pancreatitis and pancreatic fistulae is still evolving. While perioperative risk assessment in relation to POPF has conventionally been based on identifying the structural characteristics of a "high-risk gland"[7, 9], there is now a growing emphasis on the histologic composition of the remnant pancreas. In particular, acinar cell density has been recognized as an intrinsic determinant of POPF risk[10-16]. With this knowledge, new strategies for risk mitigation are emerging and mechanisms of iatrogenic acinar cell injury are increasingly being recognised as targets for prevention. The minimisation of intraoperative blood loss and traumatic tissue handling, coupled with the use of pharmacologic adjuvants, including anti-inflammatory agents and pancreatic enzyme inhibitors, have shown promise in modulating the ischemic-inflammatory response that may occur in the pancreatic remnant[17-21]. Furthermore, with the growing appreciation for PPAP in the development of POPF, pancreatic enzyme concentrations in surgical drain fluid[22-24] and serological markers of systemic inflammation[25, 26] have gained interest as prognostic factors, with the potential to

facilitate early risk stratification and timely intervention for at-risk patients. Insights from early research, such as those presented in this thesis, have yet to translate into clinical practice.

3. Future Directions

The work presented in this thesis has provided valuable insights into areas for future research with respect to the pathogenesis, prognostication, and risk mitigation of PPAP and POPF. Firstly, the precise mechanisms of acinar cell injury proposed to underly the development of postoperative pancreatitis and pancreatic fistula have yet to be characterised. Similarly, the biochemical and physiologic changes that occur in the remnant pancreas are not fully understood. Dedicated research into the pathophysiology of PPAP and POPF is warranted and will be essential for the development of future prevention and treatment strategies.

Secondly, ongoing research is required to identify new diagnostic biomarkers for clinically relevant PPAP and POPF. In particular, products of acinar cell injury, such as pancreas-specific amylase and lipase in serum and drain fluid, serum phospholipase A, and urinary trypsinogen activation peptide, may be of interest to future investigators. Metabolomic profiling represents another potential avenue for future research.

Finally, this thesis has predominantly evaluated PPAP and POPF in the context of pancreaticoduodenectomy and further studies are required to substantiate our findings in relation to other partial pancreatic resections. It is well-known that distal pancreatectomy, central pancreatectomy, and pancreatic enucleations, differ in their associated incidence of POPF. Furthermore, with the growing role of minimally invasive surgery, risk factors for acinar cell injury and mitigation strategies for PPAP and POPF should also be explored in the context of laparoscopic and robotic-assisted pancreatectomy.

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APPENDIX I.

Additional Publications Relevant to this Thesis

Construction of a Pancreatojejunostomy with an External Stent: A Technical Description

Christopher Seng Hong Lim[1] MS FRACS, Juanita Noeline Chui[1] MD, Varsha Sharma[1] MSurg FRACS, Jaswinder S Samra[1,2,3] DPhil (Oxon) FRACS, Anubhav Mittal[1,2,3], PhD FRACS

[1] Department of Upper Gastrointestinal Surgery, Royal North Shore Hospital, Sydney, New South Wales, Australia

[2] Northern Clinical School, The University of Sydney, Sydney, New South Wales, Australia

[3] Australian Pancreatic Centre, Sydney, Australia

[4] Notre Dame University of Australia

INTRODUCTION

Post-operative pancreatic fistula (POPF) is perhaps the most dreaded complication of a pancreatoduodenectomy, with a reported incidence of 10-30% in the literature.[147] Various approaches to mitigate this risk have been explored, with conflicting results in the literature.[148] These include the use of pharmacological agents, various pancreatic anastomotic techniques, placement of pancreatic stents, glues and placement of drains.

There has been no consensus amongst the surgical community on the comparative superiority of different pancreatic anastomotic techniques. The RECOPANC randomised controlled trial (RCT) comparing pancreatogastrostomy (PG) versus pancreatojejunostomy (PJ) anastomoses showed no difference in rates of POPF.[149] A recent meta-analysis came to the same conclusion.[150] Variations on the PJ technique (duct-to-mucosa and invagination being the two most common) have been studied, again with no difference in rates of POPF.[151] In light of this, most pancreatic surgeons perform the anastomosis that they are most comfortable with. In terms of pancreatic stents, the use of internal stents have not been found to be associated with lower rates or lower severity of POPF.[152] The use of externalised pancreatic stents on the other hand has shown promise, with RCTs and a recent meta-analysis showing lower rates of POPF.[35, 39, 153-155]

At the Royal North Shore Hospital in Sydney, we have adopted the use of external pancreatic stents for high-risk pancreatic anastomoses, based on a high Fistula Risk Score or a high acinar score on frozen section, both of which have been correlated with higher risk of POPF.[6, 156] Here, we describe our technique using a fine bore infant feeding tube and a closed suction drainage system.

TECHNIQUE

First, the infant feeding tube is prepared. The proportion of the tube that will sit within the pancreatic duct is measured by inserting the tube into the pancreatic duct. Multiple side holes are made in this segment without kinking the tube. This can be done by holding the tube with a gentle bend in one hand and cutting small side holes using a pair of curved Mayo scissors. Once the tube is prepared, a silk stitch is tied to its tip, and it is delivered through the abdomen using the Endo Close (Figure 1a).

In our experience, this method is compatible with a Cattell Warren or a Modified Blumgart anastomosis. The posterior wall of the PJ is fashioned first, then an enterotomy is created for the planned duct-to-mucosa part of the anastomosis. Next, a Medtronic Endo Close™ Trocar Site Closure Device is introduced through the enterotomy and passed distally into the jejunum, exiting through its wall at least 10 cm distal to the planned hepaticojejunostomy. Alternatively, a paediatric Yankauer suction device may be used. The suture attached to the prepared infant feeding tube is grasped by the Endo Close and pulled into the jejunum, exiting the enterotomy (Figure 1b). The posterior wall of the duct-to-mucosa anastomosis is created with a 6/0 PDS interrupted suture. The suture at the tip of the tube is removed, and the tip is introduced into the pancreatic duct (Figure 2a). A 5/0 PDS suture is used to secure the infant feeding tube to the jejunal mucosa at the enterotomy site.

The anterior wall of the duct-to-mucosa anastomosis is completed, followed by the anterior wall of the pancreatic capsule to the jejunal serosal 5/0 PDS sutures (Figure 2b). Finally, a

jejunal Witzel tunnel is created around the tube for a length of 5 cm (Figure 2b). At this point, the tube is secured to the skin with a stitch and marked 2-5 cm from the skin to indicate the drain position. As an added measure, the tube is coiled and a Tegaderm is placed over it. It is important to ensure redundancy of the infant feeding tube at the entry point in the jejunum (again marked 4-5cm from the jejunum) at each stage of creation of the anastomosis, and that it is not inadvertently pulled out. If inadvertently displaced, the situation can be salvaged by palpation of the infant feeding tube within the jejunum and gently advancing it further into the pancreatic duct. Intraoperative ultrasound can be used to confirm its position within the pancreas at the completion of the PJ. The bowel is not secured to the abdominal wall as one would a feeding jejunostomy, as it risks internal herniation and tension on the hepaticojejunostomy. This is followed by completion of the rest of the operation.

To secure the tube post-abdominal closure, the tube is coiled twice, and this is covered with a fresh clear Tegaderm dressing. A highly visible sign cautioning against the removal of the tube is placed. The connection of the infant feeding tube is cut, placed into a Romovac Minivac and left on suction. Extra effort is made to communicate the importance of the tube to all staff.
The feeding tube is removed on post-operative day 14 if recovery is uneventful. This can be done in the rooms as an outpatient.

DISCUSSION

Rationale behind technique

POPF is a dreaded post-pancreatoduodenectomy complication. The widely utilised Fistula Risk Score (FRS) describes the factors that influence the risk of clinically relevant (CR) POPF.[60, 157] Additionally, our unit also describes the acinar score as a means to predict a patient's risk

of CR POPF.[156] A number of mitigation strategies have been reviewed by Ecker et al., of which only external pancreatic stenting showed significant efficacy (rate of POPF in external vs. internal vs. no stent; 15.2% vs. 43.8% vs. 33.8% respectively).[50] An RCT by Adrianello et al. also found that for patients at high risk of POPF, a pancreaticojejunostomy with an externalised stent with the omission of octreotide resulted in the lowest rate of POPF. [158] In a multicentre RCT, the use of externalised pancreatic stents showed significant benefit in patients with a high FRS Score (7-10).[159]

The insertion of a pancreatic stent serves to: (i) divert pancreatic secretions away from the anastomosis; (ii) maintain patency of the main pancreatic duct in the remnant and in the anastomosis itself; (iii) act as a probe for secure suturing of the pancreatic duct (it is a landmark for the orifice of the anastomosis and prevents inadvertent occlusion by suturing of anterior and posterior walls.) [160, 161]

We therefore adopted this technique for patients at a high risk of CR POPF. This technique solves several practical problems. First, commercial external pancreatic stent kits with a closed suction drainage system are not widely available. We use basic, accessible components in its place – an infant feeding tube, a Romovac drainage system (usually used for neck or groin dissections) and an Endo Close device. Second, the use of an Endo Close device offers an easier way to guide the stent through the jejunum for a longer distance (through the small enterotomy of the duct-to-mucosa, before exiting distal to the hepaticojejunostomy with a blunt tip). This is safer for the operator and reduces at least the theoretical the risk of button-holing the jejunum with the use of a needle.

There is limited evidence suggesting the benefit of negative pressure on external drainage of the pancreatic duct.[162-165] While some units may choose to leave their drain on free drainage, the use of suction at our unit reflects a preference as well as a judgment call based on available evidence.

Preliminary results from our unit

We recently performed an internal audit on 26 consecutive pancreatoduodenectomy patients from June to November 2021. We compared the rate of CR POPF in patients who received an external pancreatic stent as a mitigation strategy vs. patients who received an internal stent. The technique described above was employed in 13 patients (50%) judged to be at high risk of CR POPF based on their FRS (we looked at their acinar score retrospectively, which added no additional patients to the series). Despite a higher FRS and acinar score, rates of CR POPF were lower in the external stent group, at 7.7% (1/13) compared to the internal stent group, at 30.8% (4/13). These are preliminary results; however, they are promising and so far corroborates Ecker's, McMillian's and Adrianello's findings.[50, 158, 159]

Overview of existing techniques

In the literature, there a number of technical descriptions on the placement of an external pancreatic stent in a PJ anastomosis.[35, 37, 39, 153, 162, 166-177] These are detailed in Table 1 and discussed briefly below. To our knowledge, there are no systematic comparisons of the outcomes of these different methodologies.

We looked at 15 articles. There were too many gaps in the descriptions to enable a full analysis, however, there were several worthwhile observations. The majority (8 of 15) used a pancreatic stent kit and 2 of 15 used an infant feeding tube; the rest were unclear. The stent sizes ranged

from 3 to 10 French. Where described, most authors (10/12) used a 2-layered closure for the PJ outer layer and the rest (2/10) used invaginated end-to-end. Where described, the most common method of stent introduction was via enterotomy (4/8). Of the 10 authors who used 2-layered closures, 8 used interrupted duct-to-mucosa anastomosis. Eleven papers described the site of stent fixation: 4 to the jejunal mucosa, 4 to the pancreatic duct and 3 to the pancreatic parenchyma. The most common site of exteriorisation was distal to the HJ (5/12), followed by proximal to PJ (4/12). The stent was removed anywhere from 2 to 8 weeks post operatively. Two authors described their experience with complications related to external pancreatic stents. These included self-limiting peritonitis, stent blockage, post-removal pancreatitis and dislodgment of the pancreatic stent leading to intra-abdominal abscess. [37, 167]

Evidence for external vs. internal pancreatic stents

The evidence base for external vs. internal pancreatic stents in reducing POPF is largely based on small RCTs (largest n=328, many n≤100 and there seems to be no clear consensus on their relative superiority. [35, 37, 167, 168, 178] A 2016 Cochrane systematic review that compared the outcomes of stents vs. no stents showed uncertain benefits in the rate of POPF due to what the authors considered low quality of evidence (RR 0.67, 95% CI 0.39 to 1.14; 605 participants; 4 RCTs).[179] However, on subgroup analysis, they found limited evidence that the use of external stents was associated with a lower risk of POPF (RR 0.55, 95% CI 0.38-0.79). A more recent 2021 meta-analysis comparing external vs. internal stents showed that the use of external stents was associated with a lower risk of Grade C POPF (OR 0.58, p=0.03).[155] This was based on a different set of more recent RCTs and non-randomised studies.

This likely reflects the multi-factorial nature of POPF and hence the difficulty of isolating the contribution of a single intervention without large-scale RCTs. On balance, however, there appears to be a favourable tendency towards external pancreatic stents.

CONCLUSION

Herein, we describe our technique for the formation of a PJ anastomosis with an external pancreatic stent using basic equipment when specialised external pancreatic stents are not available and present an overview of the literature.

FIGURES

Figures 1a. Endo Close device delivering the infant feeding tube through the abdominal wall

Figure 1b. Endo Close device delivering the infant feeding tube through the jejunum

Figure 2a. Formation of the PJ and commencement of the Witzel tunnel

Figure 2b. Completed PJ with external pancreatic stent and Witzel tunnel

TABLES

Table 1. Summary of techniques described in the literature

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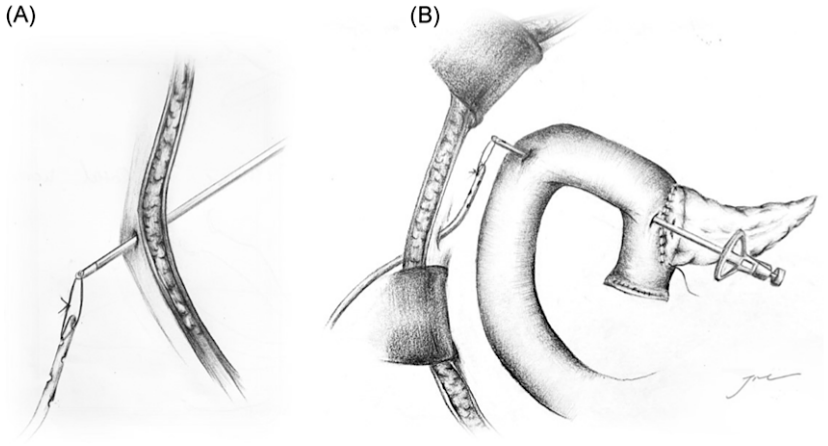

FIGURE 1 (A) Endo Close device delivering the infant feeding tube through the abdominal wall. (B) Endo Close device delivering the infant feeding tube through the jejunum

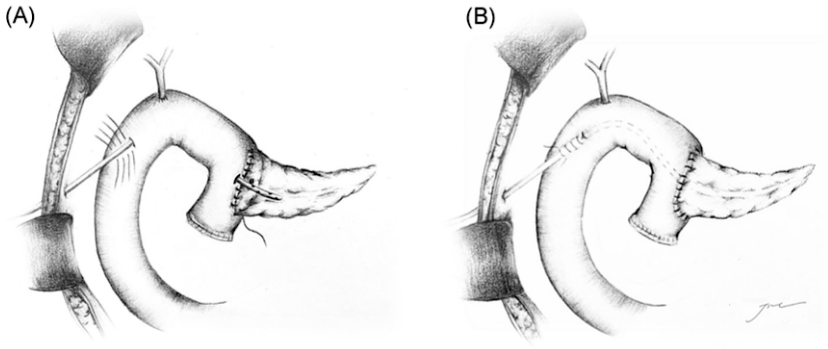

FIGURE 2 (A) Formation of the pancreatojejunostomy (PJ) and commencement of the Witzel tunnel. (B) Completed PJ with external pancreatic stent and Witzel tunnel

TABLE 1 Summary of techniques described in the literature

Author	Year	PJ outer layer	Duct-to-mucosa	Stent	Method of stent introduction	Anchoring stitch	Exit site	Enterotomy stitch	Wall fixation	Drain	Stent removal
Gu[16]	2020	Not described	Not described	6–9 Fr Silastic catheter	Not described	Not described	Proximal to PJ	Not described	Not described	Not described	4–6 weeks
Jang[17]	2016	Two-layered 4/0 Prolene	Interrupted 5/0 PDS	4–10 Fr Silastic PE catheter [a]	Enterotomy	Jejunal mucosa	10–15 cm below the anastomosis	Purse string	Not described	Jackson Pratt suction	6 weeks
Yokoyama[18]	2014	Two-layered 4/0 nonabsorbable monofilament	5/0 absorbable monofilament	4–7.5 Fr PE knotted pancreatic duct drainage tube [b]	Not described	Not described	Not described	Not described	Not described	Not described	Not described
Meng[19]	2014	Invaginated end-to-end	NA	Silicone catheter with multiple side-holes	Not described	Jejunal mucosa	Likely distal to HJ	Not described	Not described	Not described	2–3 weeks
Hakamada[20]	2014	Two-layered 4/0 PDS	2 purse-strings tied to one another; 4/0 Vicryl Rapide	5–7.5 Fr notched catheter [b]	Extracorporeally; transhepatic or transintestinal	Pancreatic duct	Not described	Not described	Not described	Not described	2 weeks
Motoi[7]	2012	Two-layered 5/0 PRONOVA	8×6/0 PDS	5 Fr × 65 cm PVC catheter [b]	Enterotomy	Jejunal mucosa	Proximal to PJ	Not described	Not described	Drainage bag	2 weeks
Pessaux[8]	2011	Not described	Not described	3–6 Fr PVC catheter	Enterotomy	Pancreatic parenchyma	Not described	Purse string	Yes	Urine bag	6–8 weeks
Azumi[21,22]	2010	Two-layered 5/0 polypropylene	Interrupted 6/0 PDS	4–6 Fr [b]	Metal needle	Pancreatic parenchyma	Proximal to PJ	Witzel tunnel	Not described	J-VAC drainage system	6 weeks
Tani[23]	2010	Two-layered 4/0 Vascufil	8 × 5/0 PDS	5 F knobbed PE pancreatic catheter [c]	Not described	Site not specified	Distal to HJ	Not described	Not described	Not described	3 weeeks
Prenzel[24]	2010	Two-layered 4/0 PDS	12 × 5/0 PDS	5–9 Fr PVC catheter [d]	Guided aborally	Pancreatic duct	Distal to HJ	Purse string and Witzel tunnel	Yes	Not described	5–6 weeks
Lee[25]	2009	Two-layered 5/0 polypropylene	Interrupted 5/0 PDS	5–8 Fr Silastic PE paediatric feeding tube [e]	Not described	Inner layer posterior stitch	Distal to HJ	Purse string	Yes	Jackson Pratt suction	4 weeks
Sriussadaporn[26,27]	2008	Invaginated end-to-end	NA	3–8 F paediatric PVC feeding tube	Not described	Pancreatic parenchyma	Distal to HJ	Not described	Not described	Urine bag	3–4 weeks
Poon[9]	2007	Two-layered fine Prolene	Interrupted suture	3–8 Fr PVC catheter	Enterotomy	Jejunal mucosa	Proximal to PJ	Purse string	Yes	Drainage bag	6 weeks
Ohwada[28]	2002	Two-layered; suture unspecified	Continuous suture	4 Fr PVC catheter [b]	Not described	Pancreatic duct	Via HJ and liver parenchyma	Not described	Not described	Not described	8 weeks
Roder[29]	1999	Not described	Not described	5–7.5 Fr cuffed PVC catheter	Guided externally	Not described	Between HJ and PJ	Likely Witzel Tunnel	Yes	Closed drainage system	8 weeks

Abbreviations: HJ, hepaticojejunostomy; NA, not applicable; PE, polyethylene; PJ, pancreaticojejunostomy; PVC, polyvinyl chloride.

[a]Dow Corning.

[b]Sumitomo Bakelite Co.

[c]Akita Sumitomo Bake, Co.

[d]Peter Pflugbeil GmbH.

[e]NIPRO O disposable feeding tube; Nipro.