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RADIOFREQUENCY-ASSISTED VS STAPLER PANCREATIC TRANSECTION IN DISTAL PANCREATECTOMY: A RANDOMIZED CLINICAL TRIAL (TRANSPAIRE)

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DOCTORAL THESIS

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ABBREVIATIONS

AEMPS:	Agencia Española de Medicamentos y Productos Sanitarios (Spanish Agency of Medicines and Medical Devices)
ASA:	American Society of Anesthesiologists
AUC:	Area under the curve
BMI:	Body Mass Index
ºC:	Degrees Celsius
CCI:	Comprehensive Complication Index
CI:	Confidence Interval
CEIM-PSMAR:	Clinical Research Ethics Committee of Parc de Salut Mar
CO	Colorado
CONSORT:	Consolidated Standars of Reportings Trials
CR:	Clinically Relevant
CR-POPF:	Clinically Relevant Postoperative Pancreatic Fistula
CS:	Coagulating Shears
CT:	Computed Tomography
CUSA*:*	Cavitron Ultrasonic Surgical Aspirator
DGE:	Delayed Gastric Emptyng
DM:	Diabetes mellitus
DP:	Distal Pancreatectomy
eCRF:	electronic Case Report Form
EORTC-30	European Organisation for Research and Treatment of Cancer Quality of Life Questionaire-Cancer 30
EPP:	Initials of Researcher
FBP:	Initials of Researcher
FIS:	Fondo de Investigación Sanitaria
GC-MS:	Gas Chromatography – Mass Spectrometry
GCP:	Good Clinical Practice
H/E:	Hematoxylin and Eosin
IDEAL	Idea, Development, Exploration, Long-term-study
IL-6:	Interleukin-6
ISGPF:	International Study Group on Pancreatic Fistula
ITT:	Intention To Treat
kHz:	Kilohertz
LC-MS:	Liquid Chromatography- Mass Spectrometry
LDP:	laparoscopic distal pancreatectomy

LPN:	Laparoscopic Partial Nephrectomy
Min:	Minute
mL:	Milliliter
mm:	Millimeter
MN:	Minnesota
MRI:	Magnetic Resonance Imaging
MS:	Mass Spectrometry
NaCL:	Sodium Chloride
NCT:	National Clinical Trial
NETs:	Neuroendocrine Tumors
NH:	New Hampshire
NY:	New York
OH:	Ohio
OR:	Odds Ratio
PAN-26:	Pancreatic Cancer-specific module of the EORTC
QoL	Quality of Life Questionnaire
PCA:	Principal component analysis
PD:	Pancreatic Duodenectomy
PF:	Pancreatic fistula
pNETs:	Pancreatic Neuroendocrine Tumors
PO:	Postoperative
POPF:	Postoperative pancreatic fistula
PPH:	Postpancreatectomy Hemorrhage
PS:	Pancreatic Surgery
PSM:	Propensity Score Matching
QLQ-C30:	Quality of Life Questionnaire-Core 30 (EORTC)
RAMPS:	Radical Antegrade Modular Pancreatosplenectomy
RCT:	Randomized Controlled Trial
RF:	Radiofrequency
SAP:	Statistical Analysis Plan
SD:	Standard Derivation
ST:	Stapler
USA:	United States of America

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ABSTRACT

This study comparatively assesses radiofrequency-assisted (RF) versus endostapler pancreatic transection in distal pancreatectomy (TRANSPAIRE) to mitigate postoperative pancreatic fistula (POPF) incidence.

The project acknowledges the enduring clinical significance of POPF despite advancements in technique and describes both a retrospective pilot study and a prospective, multi-center, randomized clinical trial to evaluate RF efficacy against standard endostapler transection.

A comprehensive review of distal pancreatectomy indications, surgical approaches, complications, and stump closure methods, with a focus on RF application, is included. Preliminary findings indicate that RF-assisted transection lowers the POPF rate relative to the conventional technique, based on the hypothesis that RF induces a mechanical barrier of connective tissue, thereby impeding pancreatic juice leakage.

This document further details the study's ethical and methodological considerations, including informed consent, data handling, and statistical analyses. The TRANSPAIRE trial aims to validate the efficacy of RF in POPF reduction, facilitating improved clinical outcomes and quality of life for distal pancreatectomy recipients.

RESUMEN

El siguiente trabajo evalúa comparativamente la transección pancreática asistida por radiofrecuencia (RF) frente a la endograpadora en la pancreatectomía distal (TRANSPAIRE), con el objetivo de disminuir la incidencia de fístula pancreática postoperatoria (POPF).

El proyecto se justifica por la persistente relevancia de la POPF como complicación quirúrgica, a pesar de los avances técnicos. Se describen un estudio piloto retrospectivo y un ensayo clínico multicéntrico, prospectivo y aleatorizado para evaluar la eficacia de la RF frente a la transección estándar con endograpadora.

El estudio examina exhaustivamente las indicaciones de la pancreatectomía distal, los abordajes quirúrgicos, las complicaciones y las técnicas de cierre del muñón pancreático, con énfasis en la aplicación de la RF. Los resultados preliminares sugieren que la transección asistida por RF disminuye la tasa de POPF en comparación con la técnica convencional, basándose en la hipótesis de que la RF genera una barrera mecánica de tejido conectivo que impide la fuga de jugo pancreático.

Este documento también detalla los aspectos éticos y metodológicos del estudio, incluido el consentimiento informado, la gestión de datos y los análisis estadísticos. Los resultados del ensayo TRANSPAIRE confirman la eficacia de la RF en la reducción de la POPF, contribuyendo a mejorar los resultados clínicos y la calidad de vida de los pacientes sometidos a pancreatectomía distal

INTRODUCTION

1 INTRODUCTION

1.1 PANCREATIC SURGERY: BACKGROUND AND CONTEMPORARY PRACTICE

Pancreatic surgery (PS) is the only option available as a curative treatment modality in countless neoplastic diseases such as pancreatic adenocarcinoma, or neuroendocrine tumours (pNETs), as well as in benign diseases and mucinous cystic neoplasms. PS, therefore, is a challenge for surgeons, not only because of its complexity but also because of the serious complications that can potentially occur in the postoperative period. Since the first pancreatic resection in history was described by Friedrich Trendelenburg at the end of the nineteenth century, the surgeon has always been faced with the unsolved problem of the postoperative pancreatic fistula (POPF). It is, therefore, not surprising that the efforts of pancreatic surgeons in the twentieth century were focused on trying to reduce and control the effects of this dreaded complication. Unlike other surgeries in the field of general surgery, these patients often generate prolonged hospital stays, with the need for intensive treatments in resuscitation units that require repeated invasive procedures as well as reinterventions in some cases. All this undoubtedly aggravates healthcare costs and negatively impacts patients' quality of life due to the consequent emotional and social impact. In recent times, with the centralization and sectorization of surgery and the multidisciplinary approach, a very significant decrease in postoperative (PO) mortality and morbidity related to the technique has been achieved (1)(2), however, there is a rate of pancreatic fistula, which has not yet been reduced.

Throughout the history of PS, multiple techniques have been proposed for closure of the pancreatic remnant. However, despite these efforts, the incidence of POPF has remained largely unchanged, persisting at approximately 30- 40%, although reported rates vary considerably among different patient cohorts(3)(4). Owing to the lack of consensus in the scientific literature regarding the optimal transection and closure technique, and in the absence of clear recommendations in current guidelines, most centers continue to employ the method with which they have the greatest experience. Consequently, no clear international consensus has yet been established. This unresolved and clinically relevant (CR) problem underscores the need for further systematic investigation and provides a strong rationale for dedicated academic work, including a doctoral thesis, aimed at clarifying the optimal surgical approach.

1.2 DISTAL PANCREATECTOMY

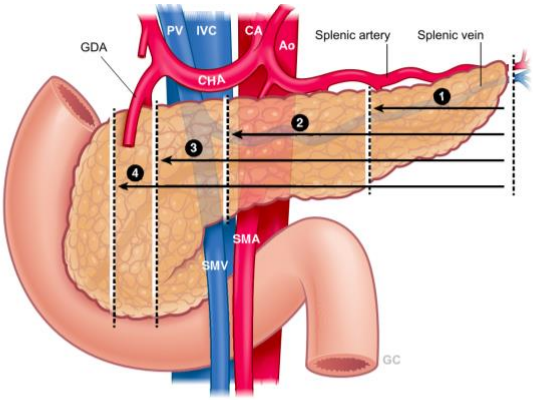
1.2.1 EVOLUTION AND SURGICAL TECHNIQUE OF DP

PS and its initial results are clearly associated with the difficulty of access to this organ, located deep in the abdominal cavity and surrounded by noble and extremely fragile structures. It is not surprising, therefore, that we must wait until the late nineteenth and early twentieth centuries to find descriptions of the first pancreatic resections. Until now, the few and not very positive experiences of surgical management of pancreatic diseases were limited almost exclusively to the drainage of cystic lesions. In the last decades of the 1800s and the first decades of the 1900s, the figures of great surgeons such as Billroth, Trendelemburg or Mayo coincided, which coincided with the beginnings of general anaesthesia techniques. The combination of these circumstances made it possible to establish the foundations of current pancreatic surgery (5).

It was in 1882 Friedrich Trendelemburg, then Professor of Surgery at the University of Bonn, who performed the resection of a tumour of unknown origin that included the excision of the spleen and the tail of the pancreas (6). The experience was described four years later by his assistant Oscar Witzel. Only two years later Theodor Billroth performed a spleno-pancreatectomy to resect a splenic sarcoma. Subsequent experiences of pancreatic resections were very sporadic (there are only 11 documented cases and no series of more than two cases) until the beginning of the twentieth century, when Finney in Baltimore and Mayo in Rochester, described the surgical technique and reported the first series of cases observing the survival of 9 patients out of 17 operated on using this technique (5)(7)(8).

The evident increase in the number of diagnostic tests in recent years has resulted in an increase in the number of incidental distal pancreatic neoplasms diagnosed, and therefore in a greater number of distal pancreatectomies performed, both open and minimally invasive (laparoscopy and robot). Although most pancreatic tumours are located at the cephalic level (74%), around 9% are in the pancreatic body and 16% at the distal level, in the tail. The diagnosis of cephalic pancreatic neoplasms is usually in earlier stages compared to those of the pancreatic head, and therefore they have less lymph node invasion and are usually better differentiated compared to those of the body and tail (9).

The term distal pancreatectomy (DP) includes surgical resections of portions of the pancreas up to the medially defined boundary by the superior mesenteric vein (10). splenopancreatectomy is defined as the surgical resection of both the body and the tail of the pancreas, achieved through glandular transection at the level of the neck, and includes lymphadenectomy of the peripancreatic and splenic hilar lymph nodes, with or without concomitant splenectomy (figure 1). Recently, this definition has been standardized by an international Delphi consensus resulting in 4 categories (11).



1	Pancreatic tail resection – to half the distance between the pancreatic tail edge and the SMV
2	Pancreatic body and tail resection – to the left border of the SMV
3	Pancreatic neck, body and tail resection – to the left border of the GDA
4	Extended pancreatic neck, body and tail resection – to the right of the GDA

Figure 1. Left pancreatectomy has been categorized at 4 different levels depending on the transection line. Source: reproduced from Van Ramshorst et al., BR J Surg, 2024.

From a technical standpoint, the initial step in performing a DP is incision of the gastrocolic ligament to provide extensive access to the omental bursa.

Subsequently, division of the gastrosplenic ligament and short gastric vessels allows for the release of the greater gastric curvature and cranial displacement of the stomach and caudal colon, yielding complete exposure of the anterior aspect of the gland. Then, the splenic artery is identified, dissected, and ligated at its origin from the celiac trunk. Following dissection of the peritoneum along the superior and inferior borders of the pancreas at the level of the isthmus, the space between the posterior aspect of the pancreas and the superior mesenteric vein is dissected at this level. Two traction points are placed on each side of the section line of the pancreatic parenchyma and pancreatic transection is performed using the chosen modality. The splenic vein is dissected and ligated at its origin. Subsequently, if necessary, the spleen can be mobilized by sectioning its ligaments,

followed by the release of the entire surgical piece in a lateral-medial direction. An alternative may be to start the dissection medially, freeing the posterior aspect of the pancreas from the retroperitoneum until it reaches the spleen. In both cases, care must be taken to identify and ligate the inferior mesenteric vein at its point of entry into the splenic vein (12)(13).

Splenic-preserving DP is usually easier from right to left (clockwise) and is based on:

- Preservation of splenic vessels (Kimura technique)(14): after the section of the isthmus, the artery is carefully released and splenic vein. Careful attention must be directed toward the mobilization of the pancreatic tail, as this process can be rendered difficult by the presence of numerous collateral vessels arising from the splenic vessels. The injury of one or both vessels usually involves the ligation of both, to avoid the generation of segmental portal hypertension. Therefore, this technique is a challenge (figure 2).

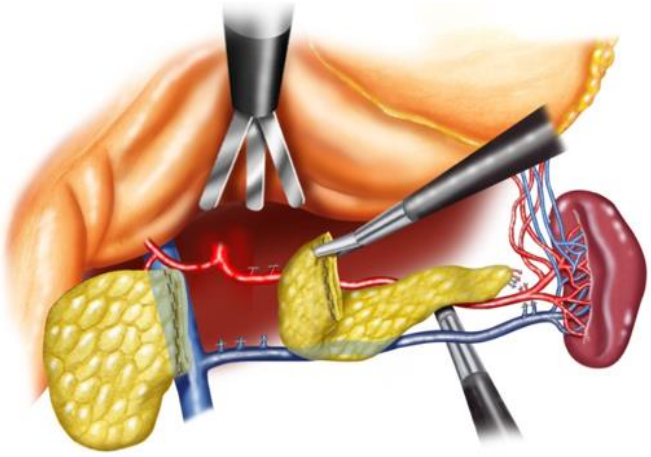


Figure 2. Kimura's technique. The sectioned isthmus can be seen with preservation of the splenic vessels. Source: reproduced from Fingerhut et al., Transl Gastroenterol Hepatol, 2016.

- Splenic vessel removal or Warshaw technique (15)(initially described by him in 1988): this approach includes preservation of the right gastroepiploic pedicle, the greater curvature arcade, and the left gastroepiploic pedicle. Subsequently, careful attention must be paid to the division of splenic vessel branches at the splenic hilum to maintain proper splenic vascularization. It is usually the technique used in inflammatory pathology (difficult to separate the splenic vessels) and especially as a rescue when technical problems arise (uncontrollable bleeding) when trying to preserve the splenic vessels (15). This technique has a lower bleeding rate,

shorter operating time, less blood loss and shorter hospital stay, but a higher rate of posterior splenectomy (2%) and splenic infarctions (22%)(16) as shown in figure 3.

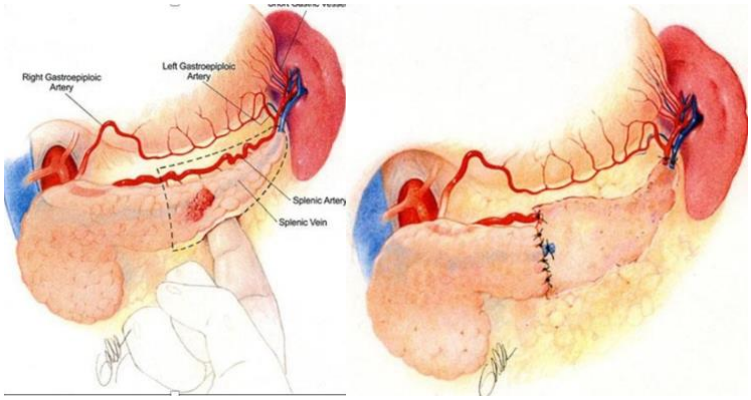


Figure 3. Distal pancreatectomy with splenic preservation and splenic vessel ligation. Source: reproduced from Fingerhut et al., Transl Gastroenterol Hepatol, 2016.

In 2003, Strasberg et al (17) Strasberg et al. described the radical antegrade modular pancreatosplenectomy (RAMPS), offering some improvements over a standard technique. First, it diminishes hemorrhagic risk due to initial vascular isolation prior to mobilization of the specimen. Second, it improves oncologic outcomes through a more posterior dissection of the retroperitoneal plane and more extensive lymphadenectomy at the expense of performing a lymphadenectomy of the portal nodes, celiac trunk to the proper hepatic artery, and para-aortic ganglia to the origin of the superior mesenteric artery. Once access to the lesser sac has been achieved, the pancreatic isthmus is dissected along the mesentericoportal axis, followed by transection. This allows for addressing the splenic artery and splenic vein at their origin, where they can be ligated and divided. Subsequently, the short gastric vessels are divided. The pancreato-splenic block is then mobilized from right to left following one of the two posterior planes, depending on local infiltration, and the specimen is extracted after the spleen has been freed. Retroperitoneal dissection can be performed anterior to the left adrenal gland (anterior dissection), or posterior to it, including it in the surgical specimen (posterior dissection). Thus, the posterior plane of dissection is delimited by the renal and suprarenal veins in the anterior dissection (figure 4) or by the renal artery and diaphragmatic crura in the posterior dissection.The safety of this technique has been validated by different prospective studies(18), but its advantage in terms of disease-free survival or overall survival over

routine resection techniques has not been demonstrated by randomized controlled trials (RCT).

It is a technique that can be applied on all occasions, but it is especially useful in:

– Bulky hypervascular tumour (e.g. in NETs)
– Splenic vein thrombosis and/or segmental portal hypertension
– Block excision of neighbouring organs due to invasion or marked adhesions

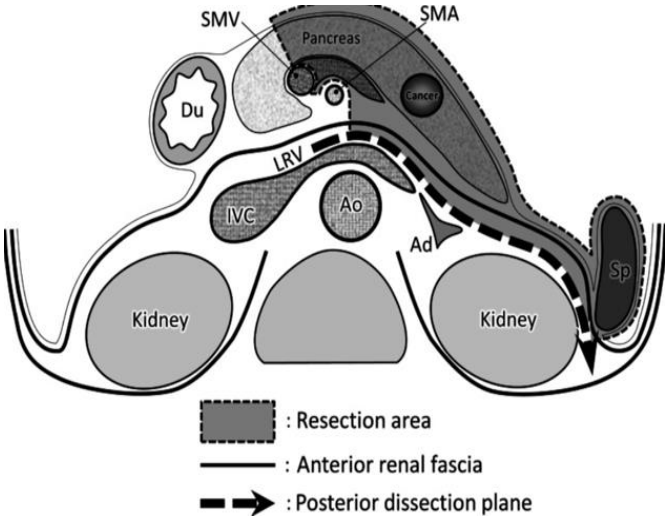


Figure 4. RAMPS. Anatomy and posterior resection plane showing the location of the pancreas, spleen and adjacent structures allowing the surgical plane to be co-ignited in RAMPS. Source: Adapted from Strasberg et al., Gastrointestinal Surg, 2003.

For tumors involving the pancreatic body or isthmus that extend to the splenomesenteric confluence or celiac trunk, a left splenopancreatectomy including vascular resection, a modified Appleby procedure, may be necessary (19). These are generally indicated in cases of NETs or locally advanced pancreatic adenocarcinomas deemed "controlled" with neoadjuvant therapy. To ensure adequate hepatic and gastric perfusion, preservation of the gastroduodenal artery is essential.

1.2.2 OPEN AND MINIMALLY INVASIVE DP

The first experiences in minimally invasive surgery of the pancreas were carried out in the 90s (20). Initially, this type of surgical access was used as a diagnostic tool to evidence the presence of tumour invasion at a distance and save the patient from a useless laparotomy (21). In the years prior to the development of modern abdominal imaging techniques, this type of examination allowed for a change in the number of in the therapeutic decision in

up to 30% of patients with carcinoma of the pancreas (22)(23).

However, the first distal pancreatectomies assisted by laparoscopy were performed by Sussman (24) and Gagner (25) in 1996. Since then, laparoscopic distal pancreatectomy (LDP) has experienced a very rapid diffusion and is currently the procedure minimally invasive most often performed on the pancreas (26)(27). Despite the difficulties due to the complex access to the retroperitoneal region, the proximity to larger vascular structures and the potential severity of postoperative complications, LDP has the important advantage of not requiring pancreato-intestinal anastomosis, so its diffusion has been much greater than that of minimally invasive PD (9)(26)(27).

With the emergence and development of minimally invasive surgery, there has been a concern in the surgical scientific community to compare both approaches to improve the results of the high incidence of POPF associated with distal pancreatectomy (28). In this scenario, several studies have appeared, including the one known by the acronym LEOPARD, which shows that in patients with pancreatic tumours on the left side confined to the pancreas, minimally invasive DP reduces functional recovery time compared to the open approach, although it is true that the complication rate was not reduced. Nevertheless, minimally invasive approach was associated with less delay in gastric emptying (DGE) and improved quality of life without increased costs (3).

Another multicentric study that provided evidence on the non-inferiority of the minimally invasive approach compared to the open one was the one known as DIPLOMA (22). Current findings support the applicability of minimally invasive surgery in patients with resectable left-sided pancreatic cancer. However, with the advent and increasing adoption of robotic surgery in recent years, studies comparing robotic DP to both laparoscopic and open approaches have emerged. These papers suggest that robotic DP is associated with a lower conversion rate, reduced need for blood transfusion, fewer postoperative complications, and improved splenic preservation compared to laparoscopic DP, particularly in obese patients (Body Mass Index, BMI <30) (29), albeit at the expense of longer operative times and higher costs (30) (31). Nevertheless, although robotic distal pancreatectomy has been suggested to offer advantages over laparoscopic DP, clear consensus and data on specific subgroups and the impact of surgical approach on morbidity remain limited.

1.3 POSTOPERATIVE PANCREATIC FISTULA (POPF)

As previously mentioned, the most feared, frequent, and potentially serious complication that can occur after pancreatic resection is POPF, and it can produce severe PO bleeding leading to the patient's death in up to 5% of cases.

During the last decade, great efforts have been made in the search for a common and clinically reproducible definition of POPF. In 2005, the International Study Group on Pancreatic Fistula (ISGPF), composed of 37 surgeons specializing in PS, created a simple and easy-to-apply definition for pancreatic fistula (PF) (32). This definition, universally accepted, was subsequently validated in prospective studies on many patients (33)(34)(35). In 2016, given that POPF remained the most feared and clinically significant complication associated with this type of surgery, this international group developed a new report aimed to verify the validity of the definition established in 2005 and updating that classification based on the most current evidence available at the time. Ultimately, the ISGPF refined the definition of CR-POPF as the drainage of any measurable volume of fluid with an amylase level more than three times the upper limit of normal serum amylase, associated with a CR condition directly related to the POPF.

Consequently, the former POPF type A was redefined as a *biochemical leak*, since it lacks significant clinical repercussions and, therefore, is no longer considered a true POPF. In addition, types B and C were refined to include more stringent criteria. Specifically, type B refers to fistulas that require a change in PO management, in which drains are maintained for more than three weeks or need to be repositioned through endoscopic or percutaneous procedures. Finally, type C encompasses those POPFs that necessitate surgical reintervention or are associated with single or multiple organ failure and/or patient mortality directly attributable to the fistula (36).

Other classifications worthy of mention for their high diffusion and rigor are the classification made by the Universities of Heildelberg and John Hopkins (37) (38) and the adaptation of the Dindo-Clavien classification published in 2007 by Strasberg (39). The latter combines Clavien's classification of PO complications into five grades according to their clinical impact, with the definition of pancreatic fistula proposed by Yeo et al., which is characterized by the presence of more than 50 milliliters (ml) per day of fluid in the

drains with an amylase concentration greater than three times the upper normal value of serum amylase, occurring on or after the tenth PO day (37).

Several authors have attempted to identify perioperative risk factors associated with an increased likelihood of developing a PF (40). Although most studies have focused on factors related to the technique used for closure of the pancreatic remnant, some groups have investigated patient-related factors as well as other technical aspects of the surgical procedure. The vast majority of POPFs are usually managed and resolved conservatively. This high rate of successful non-surgical management has become possible in recent years due to major advances in antibiotic therapy, nutritional support, and, above all, interventional radiology techniques.

1.4 METHODS OF PARENCHYMAL TRANSECTION AND CLOSURE OF THE PANCREATIC STUMP

Throughout the history of PS different techniques have been used for closing the remnant pancreas stump (manual suture, autologous patches, heterologous patches, glues, endostapler... etc), without any consensus on the superiority of one technique over another and therefore, without achieving to date that the percentage of POPF decreases from around 30- 40%, as mentioned before (3)(4).

A. Manual and mechanical suturing

Traditionally, closure of the pancreatic remnant was performed by continuous manual suturing with or without selective ligation of the Wirsung's duct (41) (42). The birth and great ergonomic and functional development of mechanical sutures have conditioned their growing use in PS since the seventies (43) as illustrated in figure 5. In the lasts decades we have witnessed a greater diffusion of this method of transection, probably in relation to the spread of minimally invasive pancreatic resection techniques and due to the difficulty of performing a manual suture using this type of approach (26).

To highlight the heterogeneity in the management of pancreatic stump closure in DP, multiple studies have been undertaken. This included DISPACT, a prospective, European, multicenter study evaluating 336 randomized patients across more than 20 high-volume centers for PS. In this trial, no significant differences were observed in PF

incidence or other complications within 30 days of surgery when comparing mechanical stapling to manual suturing (4).

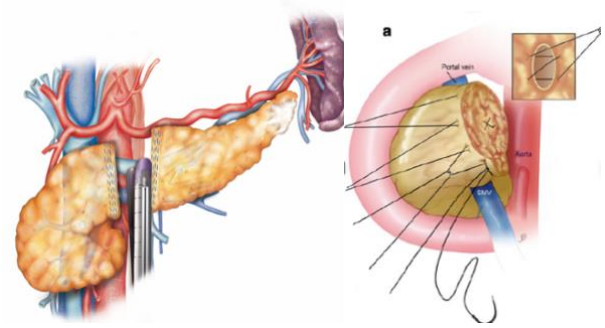


Figure 5. Line of staples following pancreatic parenchyma transection using a conventional stapler (left). Closure of the pancreatic stump via manual suturing (right). Source: Adapted from Diener et al., Ann Surg, 2011.

B. Selective closure of Wirsung duct

Despite discordant results, great interest has been placed on the need to selectively locate and ligate the pancreatic duct prior to closure of the remnant, especially if the latter is performed by manual suture (44). In 2003, Bilimoria (41) published a retrospective study which was conducted on 126 patients that showed a decrease in the rate of PF after selective ligation of the Wirsung (9.6% vs 34%). These results, although endorsed by other similar authors, they have been refuted by some authors (45)(46) but have not been confirmed by prospective RCT. Nathan et al. in 2009, presented a retrospective analysis of the most extensive single-center series of DPs performed to that point (n=704 patients), reporting that POPFs rates did not significantly differ based on selective ligation of the Wirsung duct or the specific closure technique employed.

Whipple's intervention was originally described in 1935 as a pancreatoduodenectomy associating the occlusion of the main pancreatic duct (PD) or Wirsung's duct of the pancreatic remnant (47). In 1971 Goldsmith et al. (48) published a study comparing the efficiency of anastomosis and after performing a PD compared with ligation of the duct with similar POPF indices for both procedures.

Between the 1970s and 1980s, multiple cases of the use of surgical occlusion of the PD to avoid pain in patients in cases of chronic pancreatitis, without the appearance of POPF or subsequent diabetes mellitus (DM) in patients, are described (49–51).

Gall described the use of glue in 328 patients, with rates of 1.2% mortality and 12.7% morbidity (52). Occlusion of PD with glues led to a decrease in the mortality rate and

associated morbidity. However, as suggested by two more recent RCT (53,54), the complications are still very high and some studies warn of the risk of haemorrhagic pancreatitis if the injection of the glue is done at high pressures (55) or with an insufficient seal, which would increase the secretion of pancreatic juice by secondary canaliculi (56).

Other subsequent studies, such as a single center, prospective investigation by Mazzaferro et al., have assessed the safety and efficacy of PD occlusion with neoprene adhesive in select patients undergoing pancreaticoduodenectomy at high risk for postoperative pancreatic fistula. The authors concluded that duct occlusion with neoprene-based adhesive is a safe technique. However, they observed a threefold increase in the risk of diabetes at 1 and 3 years (57).

C. Barrier methods: Glues and Patches

To achieve a barrier or containment effect in the pancreatic transection plane and thus control the discharge of pancreatic fluid into the abdominal cavity giving rise to POPF, various materials such as glues and biological sealing agents have also been tried (figure 6).

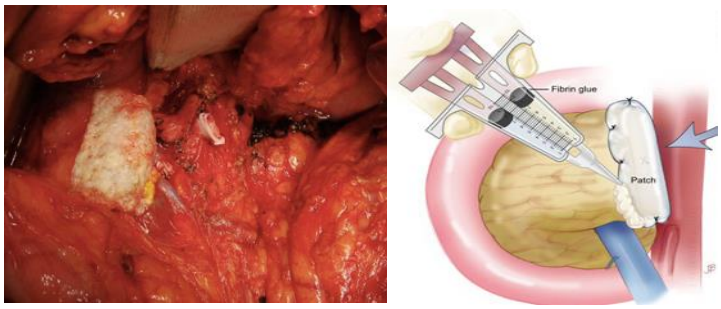


Figure 6. Left: Surgical image with haemostatic material placed in the pancreatic stump after pancreatic transection. Right: Schematic drawing illustrating pancreatic stump fixed with fibrine glue. Source: adapted from Montorsi et al., Ann Surg, 2012.

The use of fibrin sealants to coat the surface of the pancreatic remnant has been proposed by Ohwada et al in 1998 (56) and by Suzuki et al in 1995, in non-randomised studies, with favourable results, despite both studies presented an imprecise definition of POPF and a high risk of selection bias. One RCT conducted in France in 2003 (53) published the postoperative results of different pancreatic surgical techniques with and without the use of glues were found, without finding significant differences between groups. It should be noted that in these historical studies the definition of POPF did not follow the

standardised definition of vocational training as we consider it today (as defined by the ISGPF and the "updated"), therefore, is not comparable.

In the study published by Montosori et al, in 2012, where the fibrin sealant known as Tachosil® was used, one of the highest rates of POPF was reported, around 60% after the use of this sealant on the surface of the pancreatic section (58). The possibility of adding a sickle cell ligament patch and fibrin glue after closure of the standard remnant with mechanical ST or sutures has also been explored. Similarly, this method has not been able to demonstrate a decrease in the rate or severity of POPF in patients undergoing DP (59). In fact, based on the current available evidence, fibrin seals may have little or no effect on postoperative pancreatic fistula in patients undergoing DP (60)(61).

D. Stapler line reinforcement

The reinforcement of the staple line with absorbable suture has been favorably evaluated in several non-randomized studies (62)(63)(64). This type of reinforcement does not require additional maneuvers that increase the complexity of the surgical technique and can be easily applied and reproduced in minimally invasive procedures. However, it should be noted that this reinforcing suture may cause minor parenchymal lacerations due to needle passage, particularly in cases involving a soft pancreas, which could be associated with a higher incidence of fistula formation. In 2013, Jensen et al (63) published a meta-analysis that evaluated the results of 5 prospective and 5 retrospective case series conducted between 2007 and 2009, finding no significant differences in the prevalence of PO complications between patients in whom staple line reinforcement was performed with resorbable material (n=234) or not (n=249). However, none of the studies included in the meta-analysis were RCT.

In 2012, Professor Strasberg's group from St. Louis (62), published a prospective, RCT comparing closure of the pancreatic remnant by mechanical suturing with or without reinforcement. In this study, no significant differences were observed between groups in the overall incidence of POPF. However, the subgroup analysis of patients who developed a CR-POPF (grade B or C according to the international classification) showed no significant differences favoring the reinforced closure (32). It should be highlighted, as a possible bias of the study, the presence of a significant difference in the preoperative BMI between groups (higher in the control group), a well-known risk factor for the development of POPF (65)(66) and the peculiarity that almost half of the patients in both

groups were discharged from hospital before the surgical abdominal drainage had been removed.

Recent meta-analyses have highlighted that the incidence of CR-POPF (types B and C) is significantly lower following DP performed with reinforced STs compared to those using bare STs. Nevertheless, this reinforcement strategy failed to prevent the development of biochemical (type A) fistula (67)(68).

E. Pancreatic duct drainage methods

Some authors have proposed drainage of the pancreatic stump through an anastomosis with a Roux-en-Y jejunal loop, reporting favorable outcomes in retrospective studies (69)(70)(71). Of course, this is an option that considerably increases the technical difficulty of the intervention, especially through a minimally invasive approach. It is also necessary to consider the greater risk involved in a POPF where there is contact between intestinal and pancreatic fluid, due to the activation of the enzymes contained in the latter (71).

F. Heat sealing

Since the late 1990s, there has been a rapid increase in reports and clinical series describing the use of various energy sources for tumor ablation in solid organs (72)(73). Subsequently, several devices were developed that used such sources of thermal energy (mainly radiofrequency, RF and ultrasound) for surgical haemostasis and parenchymal transection of solid organs, particularly the liver (74)(75). The positive results of these experiments and the wide diffusion of these coagulation devices have awakened the curiosity of different groups at an international level about the possibility of its use in PS. The rationale for the use of RF-assisted devices or other hyperthermia-based energy sources to seal the pancreatic remnant in DP lies in their ability to achieve sufficiently high temperatures within the glandular parenchyma to induce coagulative necrosis, collagen fiber retraction, and subsequent sealing of vessels and ducts. (76)(77)(78)(79). In addition, some of these devices could allow both parenchymal cutting and sealing to be performed, without the need for additional gestures, greatly facilitating the performance of pancreatic transection, especially by minimally approach.

In summary, transection instruments are divided into two types: those whose primary function is dissection (e.g., haemostatic forceps or ultrasonic dissector) and those whose most notable function is haemostasis-coagulation (e.g., sutures, endostaplers, sealants, etc.). Within the second group, those who use RF as an energy source (e.g. Habib® or

TissueLink®) have emerged in recent years.

Ultrasound

The possibility of using transection and sealing instruments that use the energy generated by ultrasound to seal the pancreatic remnant has been evaluated in experimental work on animal models (80)(81) and in humans (77)(82)(83). In 1999, Suzuki et al (77) evaluated, in a prospective RCT, the use of an ultrasound-assisted dissection tool (CUSA system; Cooper Medical Devices, Mountainview, California, United States of America, USA) with very favourable results. Although this study represents one of the few RCT addressing pancreatic transection in DP, it presents several limitations. Firstly, the device employed was suitable only for parenchymal dissection; therefore, the authors systematically ligated the main PD and secondary ducts during transection. In addition, the study employed a less stringent definition of POPF than that later proposed by the ISGPF, and most patients underwent surgery for gastric neoplasia, a characteristic common to most Japanese DP series. Therefore, despite being, to date, the only RCT evaluating a pancreatic parenchymal resection method with clearly positive results, this trial has not had a substantial impact on the subsequent adoption or dissemination of the technique. In 2001 Sugo et al. (83) in a retrospective study on a small number of patients, the use of a transection and sealing device (Harmonic scalpel; Johnson & Johnson Medical, Ethicon, Tokyo, Japan). In this study, the authors did not identify or ligate the main PD during ultrasound-assisted transection and achieved a significant reduction in the rate of POPF. Matsumoto (264) evaluated the results obtained by performing parenchymal transection using another device (Coagulating shears, CS, Ethicon Endo-Surgery, Cincinnati, OH, USA) in 5 patients undergoing PD, with 1 POPF.

Bipolar devices

The usefulness of the use of bipolar devices associated with electrothermal energy has recently been evaluated in several studies on animal models (84)(85)(86), in isolated clinical cases (87) or in reduced case series (88)(89). Although the evidence provided by these studies remains limited, these devices appear to offer a high degree of safety, combined with the ease of use required for minimally invasive surgery. In an experimental study on the pig model carried out by the group at the University of Heidelberg, the safety and efficacy of one of these devices (LigaSure Impact device®; Covidien®, Boulder®, CO, USA) with promising results confirming the need for further clinical studies.

Radiofrequency-assisted devices:

In 2007, Truty et al (79) conducted a RCT on a porcine model comparing the closure of the pancreatic remnant after DP by manual suture or with a device (Tissuelink®; Salient Surgical Technologies, Portsmouth, NH, USA) that uses RF for thermocoagulation, associated with dripping saline solution to lower the temperature of the interface between the metal head of the instrument and the tissue. The authors demonstrated a significant reduction in the rate of PF in the group of animals treated by RF.

Hanly et al. (80) also employed a porcine model to compare the efficacy of different methods of transection and parenchymal closure during LDP: conventional endostapler closure, ultrasound-assisted device closure, and a prototype stapler (ST) connected to a radiofrequency (RF) source. No POPF was observed in the ultrasound-assisted closure groups, whereas both the conventional and RF-assisted stapling groups presented one case of POPF each (33% incidence in both groups).

In 2010, Fronza et al. (90) described his experience, not entirely positive, in the use of another RF-associated device (Habib 4x, Angiodynamics, Queensbury, New York (NY), USA) for the sealing of the pancreatic remnant after LDP in 14 patients. Of these, 4 patients had a clinically silent fistula (28%) and 2 had a grade B or C fistula as defined by the ISGPF (14%).

Subsequently, Blansfield's group (91) conducted a retrospective study involving 29 patients who underwent distal pancreatectomy in which parenchymal transection was performed exclusively using the Tissuelink® RF-assisted device. The authors compared these results with those obtained in a historical control group of 33 patients. Not only was a reduction in the POPF rate observed (36% vs 10%), but there was also a decrease in operative time and intraoperative blood loss in the study group. In this series, 19 of the 29 patients underwent LDP.

Rostas et al. (92), retrospectively evaluated the results obtained in 33 patients using a variation of the RF-assisted device associated with saline drip (Tissuelink® EndoSH 2.0™; Salient Surgical Technologies, Portsmouth, NH, USA) during hand-assisted LDP. The authors observed only 3 POPFs, two grade A and one grade B.

Our group has one of these RF ablation devices (Coolingbis®), which is approved for this indication and is currently used as standard of care at the Hospital del Mar in Barcelona in the hepatobiliary-pancreatic surgery unit and in other centres (93).

1.5 RADIOFREQUENCY

1.5.1 THEORETICAL BACKGROUND

RF is a type of energy that is part of the "electromagnetic wave spectrum." An intrinsic characteristic of electromagnetic waves is their ability to move at the speed of light in empty space, without the need for a propagation medium. The electromagnetic spectrum encompasses multiple types of energy, from gamma rays to RF through the spectrum of visible light, microwaves, lasers, etc. These types of energy are distributed within the electromagnetic spectrum according to the frequency and wavelength and are measured in cycles per second or Hertz (Hz).

Electromagnetic waves can be classified into ionizing and non-ionizing. Ionizing radiation (X-rays and gamma rays) are waves of very high frequency and energy, capable of producing ionization, which is, breaking molecular bonds and atomic structures. The effects caused by the absorption of this energy by biological materials are cumulative and irreversible, profoundly altering their biology.

Non-ionizing radiation, on the other hand, is part of the spectrum of low-energy photons (low frequency and high wavelength), incapable of breaking the atomic or molecular structure. Ultraviolet radiation, visible light, infrared, RF and microwaves, even with the highest intensity, are incapable of ionizing biological media, but they can cause other effects such as the generation of an electric current that passes through the medium where they act and causes it to heat up.

Cellular homeostatic mechanisms can withstand temperatures of until 42 degrees Celsius (ºC), depending on also of the time during which one are subjected to such temperatures (table 1). Between 42 and 45ºC the cells are more susceptible to the damage caused by external mechanisms (e.g. Radiation or chemotherapy), while to split of the 45ºC herself begin to produce irreversible structural damage. These damages are due to the denaturation of proteins in the cell membrane, cytoplasm, of enzymes mitochondrial and of acids aucleic (94).

The damage tissular caused depends not only on the temperature achieved in the tissues but also of the exposure time: the higher the is the temperature, the shorter the time needed to produce cell death and coagulative necrosis (95)(96)(97).

Table 1. Tissue changes according to temperature

Temperature	*Microscopic changes*	*Biological Changes*
37º to 60ºC	Minimal changes	Beginning of tissue destruction
69º to 65ºC	Color changes (Whitish)	Coagulation, collagen fusion, tissue necrosis
65º to 90ºC	Extensive color changes (bright)	Desiccation, denaturation of proteins
90º to 100ºC	Steam formation Shrinkage	Vaporization, drying
120ºC	Plumes of smoke appear	Carbonization

One of the first effects suffered by tissues exposed to a progressive rise in temperature is the retraction of type 1 collagen fibres, which are mainly found in the walls of blood vessels, bile canaliculi and small airways. The retraction of collagen fibres results in the permanent collapse of arteries, veins, bile ducts and small airways. This mechanism therefore represents the basis of the haemostasis and sealing capacity of RF-assisted devices. During RF application, the tissue is gradually heated, exceeding 60ºC and causes denaturation of cell membrane proteins, cytoplasm, mitochondrial enzymes and nucleic acids, known as coagulative necrosis (98) (99), and therefore the latter could prevent the appearance of POPF by causing fibrosis and collagen contraction (100) (101).

1.5.2 PRINCIPLES OF RADIOFREQUENCY SURGICAL DEVICES

RF-assisted electrocautery devices operate by converting electrical current into heat, producing specific effects on biological tissues. Understanding the structure of conventional electrosurgical units is therefore essential to comprehend the functioning of modern RF generators.

Electrosurgical units convert standard electrical current, which operates at a frequency of 60 Hz, into a higher frequency current of approximately 500 kilohertz (kHz), capable of producing thermal effects on treated tissues. Electrocoagulation can be applied in either monopolar or bipolar modes. The current is concentrated on a very small surface area at the active electrode (scalpel tip), producing sufficient heat to generate tissue effects. The return electrode is a large plate in contact with a wide skin area, allowing electrical energy to dissipate safely without causing thermal injury. In bipolar mode, the current flows between the two poles, typically the tips of forceps, so that only the tissue grasped between them is affected.

Modern RF devices consist of three main components: the RF source or generator (which modulates the electric current), the application electrodes (complex modifications of the simple electrode used in traditional electrosurgery), and the control and monitoring systems (for impedance, power output, temperature, etc.).

RF generators convert commercial electrical energy (50- 60 Hz) into RF energy (450- 600 kHz). The parameters of RF sources can be manually or automatically adjusted to maintain constant temperature, impedance, current intensity, and a defined application time during the procedure.

During RF application, it is not the electrode that heats up but rather the surrounding tissue, which resists the passage of current. Thus, the temperature measured corresponds to that of the tissue around the electrode. By monitoring tissue impedance and temperature (or the energy delivered), the diameter of the tissue volume exposed to cytotoxic heat can be estimated.

Currently, numerous electrode types are commercially available for the delivery of electrosurgical and RF energy. Variations in size, number, shape, and cooling mechanisms of these instruments aim to achieve a controlled increase in the depth of the coagulative necrosis produced in the target tissue. Among the most recent advances in this field, the perfusion of saline into tissue surrounding the electrode or its cooling through a closed-circuit system has allowed greater energy deposition and more effective tissue ablation.

The most researched and documented effect of RF on biological tissues is the transformation of the electrical energy that penetrates them into an increase in the kinetic energy of the molecules, which produces a heating of the medium in which they settle. The higher the intensity of the current applied to the tissue, the more vigorous the movement of the molecules and the higher the temperature will be reached.

Regarding the application of RF in hepatic transection, multiple studies have demonstrated that intraoperative bleeding occurring during hepatic parenchymal transection is one of the main complications of liver resection. It contributes to increased perioperative morbidity and mortality, a higher rate of local tumor recurrence, and decreased long-term survival (74)(102). The empirical observation that RF-assisted devices used during tumor ablation sessions can coagulate vessels up to 3- 5 millimeter

(mm) in diameter served as the basis for the first experiences of hepatic parenchymal transection using these instruments (74)(103)(104).

In 2002, Weber et al. (158) reported their experience with liver transection using an innovative RF-assisted device in 15 patients who underwent segmental or atypical resections. The authors observed a decrease in intraoperative bleeding and absence of PO morbidity and mortality. Ten years later, the same group presented a series that include 604 hepatectomies, performed among 2001 and 2010, using a modified version of the same device, and confirmed the reduction of intraoperative blood loss, as well as low rates of PO morbidity and mortality (105).

1.5.3 PRECLINICAL TRIALS

Given the lack of clear evidence and the persistence of a frequent and clinically relevant complication, our group initiated a series of preclinical studies in 2010 to assess the safety of pancreatic transection using RF in an experimental model. In this context, Dorcaratto et al. (2012) (106) demonstrated in a porcine model (n = 10) that RF-assisted pancreatic transection is a safe and reproducible technique, not associated with intraoperative or immediate postoperative complications. In this initial pilot study, not CR- POPF was observed, as shown in table 2 below.

Table 2. Preoperative and postoperative (4 hours, 4 days, and 4 weeks postoperatively) serum amylase and glucose levels.

		Preoperative	*4 hours PO*	*4 days PO*	*4 weeks PO*	p value
Serum amylase (IU/L), (mean, SD)	**RF** group	1.408 (1.072 - 3.003)	2.010 (1.013 - 3.603)	1.327 (1.015 - 4.056)	1.458 (1.048 - 2.816)	>0.05
	ST group	1.764 (898 - 2.810)	1.904 (1.058 - 3.456)	1.415 (969 - 2.528)	1.797 (1.314 - 2.940)	
Serum glucose (mg/dl), (mean, SD)	**RF** group	110 (63 - 211)	126 (85 - 2 30)	119 (93 - 263)	99 (62 - 207)	>0.05
	ST group	110 (73 - 154)	115 (94 -203)	124 (94 -213)	116 (64 -161)	

Once the clinical safety of the technique had been demonstrated, we proceeded to perform a randomized experimental efficacy study comparing RF transection with Coolingbis against ST closure of the pancreatic stump (107). This study again showed a higher incidence of POPF in the ST group, confirming both the reproducibility and potential advantages of the RF approach. Importantly, it also represented the first RCT specifically designed to evaluate the efficacy of this method.

Beyond clinical safety, experimental data from our group provided relevant mechanistic insights. RF-assisted transection was associated with a marked early inflammatory response, reflected by increased levels of acute-phase reactants such as interleukin-6 (IL-6), followed by normalization at one month postoperatively (figure 7). This temporal profile suggests that RF induces a controlled and transient inflammatory response rather than a sustained proinflammatory state.

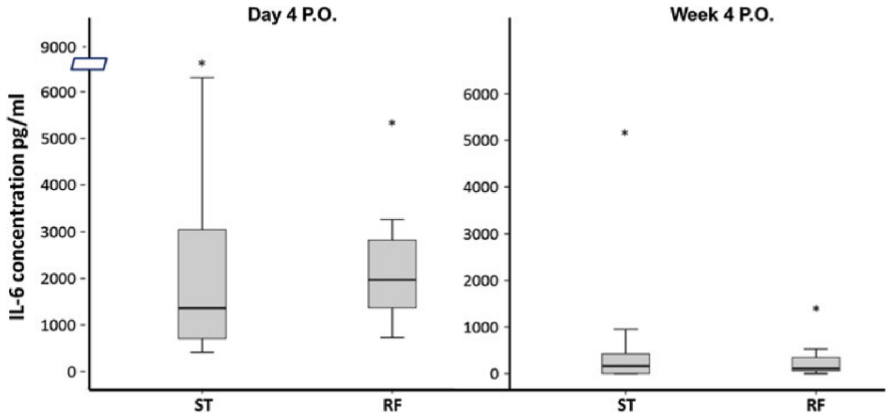


Figure 7. Temporal evolution of interleukin-6 (IL-6) levels following pancreatic transection in a porcine experimental model. An early postoperative increase is observed, followed by normalization at one month, indicating a transient inflammatory response. Source: Dorcaratto et al., Surg Endosc, 2013.

Following confirmation of safety, a RCT efficacy study comparing RF transection with ST closure was conducted (107). This study demonstrated a significantly higher incidence of POPF in the ST group, representing the first RCT specifically designed to evaluate the efficacy of RF-assisted pancreatic transection. The experimental model was performed using the same RF device as that applied in the present study.

Histological analysis further elucidated the underlying mechanisms. Specimens obtained after RF transection demonstrated a central area of coagulative necrosis involving the

islets of Langerhans, surrounded by a dense fibrotic capsule approximately 1.8 mm thick, forming a mechanical barrier at the transection margin (figure 8). In contrast, specimens from the ST group exhibited a markedly weaker inflammatory and fibrotic response (figure 9).

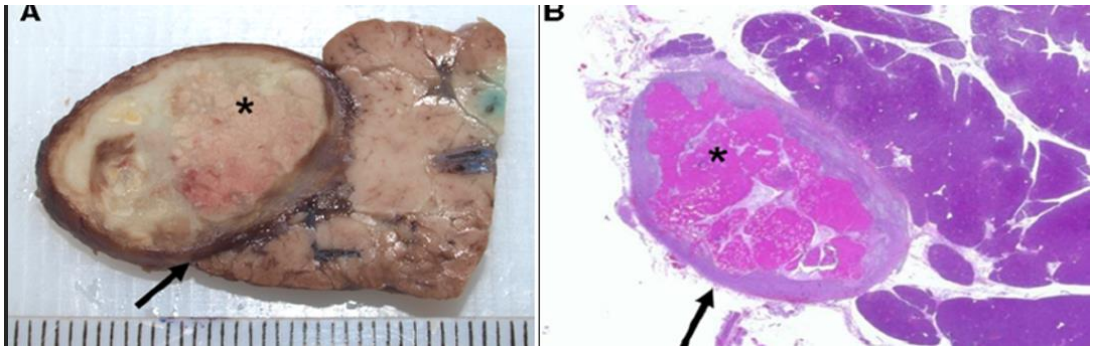


Figure 8. (A) Macroscopic section showing the area of coagulative necrosis (asterisk) surrounded by fibrosis (arrow). (B) Complete histological section (H/E) showing the area of coagulative necrosis (asterisk) surrounded by fibrosis (arrow) Source: Reproduced from the doctoral thesis of Dorcaratto (2013).

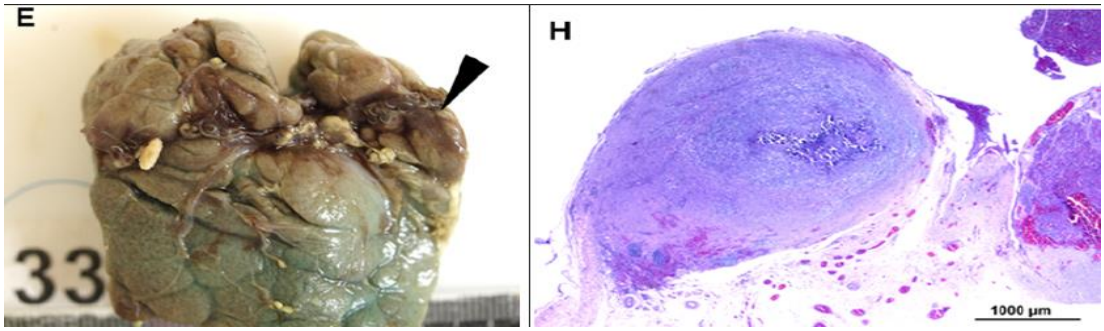


Figure 9. (E) Macroscopic image showing the staple line on the transection surface (arrowhead). (F) Weak fibrotic reaction (arrowhead) at the transection margin after staple removal (H/E). Source: Reproduced from the doctoral thesis of Dorcaratto (2013).

These findings suggest that RF-induced fibrosis and connective tissue encapsulation may play a key role in preventing leakage of pancreatic secretions into the abdominal cavity, thereby reducing the incidence of CR- POPF.

Building upon these findings, Quesada et al. (108) from our group employed a murine model to specifically assess whether RF pancreatic transection could trigger necrotizing pancreatitis, which represents often a major concern. In a cohort of 32 rats undergoing transection at the pancreatic neck, no cases of necrotizing pancreatitis were observed. These results demonstrate that RF-assisted transection does not induce pancreatic necrosis or inflammatory destruction, thereby reinforcing the safety profile of this technique. To reinforce this fact, a more recent study published by Burdío et al. (109) also

compared the RF-assisted device with ST closure in a porcine model, performing pancreatic transection at the level of the portal vein. This model was even more challenging than the one previously described, as the transection was carried out precisely at the pancreatic neck, with exclusion of the transected pancreatic stump. The results suggested that RF was more effective in controlling POPF than ST closure, with POPF rates of 14% versus 75%, respectively.

These experimental animal studies, together with the subsequent pilot study in humans, demonstrated a significant reduction in the incidence of this complication without introducing additional risk to patients. As a result, this intervention has evolved from being a procedure associated with potentially life-threatening risks to one that is safe, rapid, and achieves oncological outcomes comparable to those obtained with conventional ST transection.

1.5.4 DEVELOPMENT AND APPLICATION OF THE COOLINGBIS DEVICE

The device initially used at our institution, Hospital del Mar (Coolingbis®, Apeiron Medical, Valencia, Spain), was originally developed and approved for use as a device for hepatic parenchymal transection (103)(110).

This instrument consists of an electrode connected to an RF generator and internally cooled by the circulation of saline in a closed circuit (figure 10).

The electrode used in our study is the version developed for the application of the device in minimally invasive surgery (figure 11). It is an electrode with a total length of 445 mm, coated with insulating material, which includes the following systems:

- Coagulation system: at its distal end the electrode has an angle with an inclination of approximately 135º and an active distal portion, without insulation, approximately 20 mm long and 3 mm in diameter, which distributes energy in the tissue, generating heat.
- Cutting system: The electrode has a 2.5 mm high scalpel blade at the end of the distal end that allows the section of the previously coagulated parenchyma by means of a retrograde movement.
- Internal cooling system: The electrode has 2 internal ducts communicating with each other that constitute a cold serum inlet and hot serum outlet circuit. The circulation of cold serum allows the heat at the tip of the electrode to be reduced,

with a phenomenon like cooling by blood flow in the tissues. The main advantage of electrode cooling is that it helps prevent tissue charring/carbonization at the electrode–tissue interface. By limiting carbonization, cooling reduces impedance and allows a more efficient energy delivery and dissipation within the tissue, thereby improving the effectiveness of RF application.

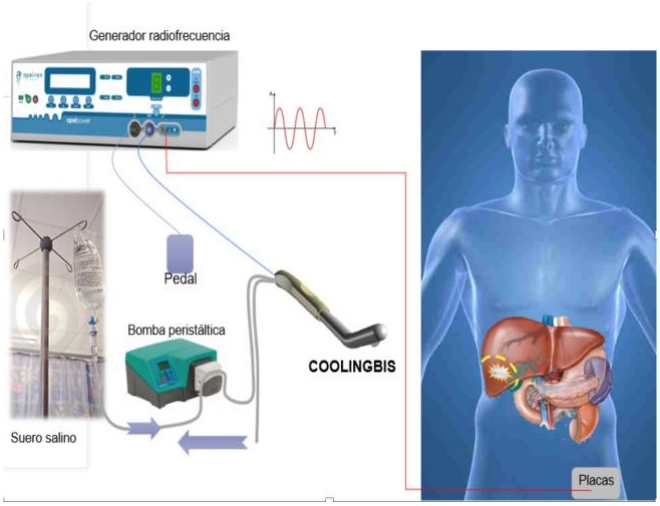
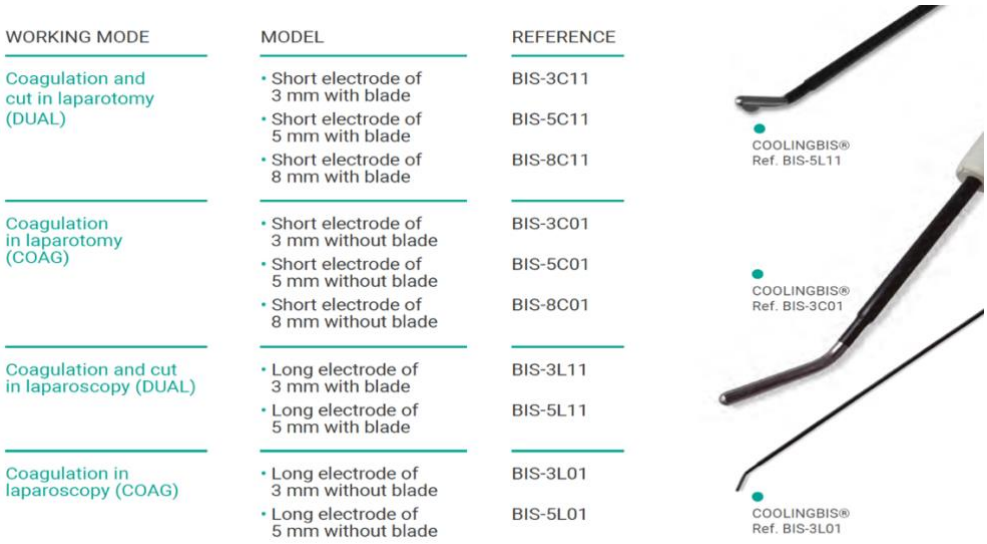


Figure 10. Schematic summary of the connection circuit between the generator, the subject undergoing treatment and the peristaltic pump, as well as the fundamental components of the electrode in contact with the tissue (in this case hepatic parenchyma). Source: technical datasheet of the Coolingbis® RF device.



WORKING MODE	MODEL	REFERENCE
Coagulation and cut in laparotomy (DUAL)	• Short electrode of 3 mm with blade	BIS-3C11
	• Short electrode of 5 mm with blade	BIS-5C11
	• Short electrode of 8 mm with blade	BIS-8C11
Coagulation in laparotomy (COAG)	• Short electrode of 3 mm without blade	BIS-3C01
	• Short electrode of 5 mm without blade	BIS-5C01
	• Short electrode of 8 mm without blade	BIS-8C01
Coagulation and cut in laparoscopy (DUAL)	• Long electrode of 3 mm with blade	BIS-3L11
	• Long electrode of 5 mm with blade	BIS-5L11
Coagulation in laparoscopy (COAG)	• Long electrode of 3 mm without blade	BIS-3L01
	• Long electrode of 5 mm without blade	BIS-5L01

Figure 11. Laparoscopic version of the Coolingbis® device electrode. Source: technical datasheet of the Coolingbis® RF device.

Using a peristaltic pump (Radionics, Burlington, USA) (figure 10), ice-cooled saline (0.9% sodium chloride, NaCl solution at an approximate temperature of 0 °C) is circulated into the interior of the electrode at a rate of 130 (mL/min). The efferent warm saline, after

cooling the non-insulated and active portion of the electrode, allowing continuous internal cooling, and is subsequently recirculated through a closed-loop system, maintaining a stable temperature throughout the RF application. The electrode is connected to a conventional 485 KHz RF generator and generating a power of 0 to 150 W (Radionics CC-1, Burlington, Massachusetts), used in manual mode (figure 11).

The operation of the device is based primarily on two actions:

1. **Simultaneous cutting and coagulation of tissue** (figure 12): When the device is applied and moved retrogradely over the parenchymal surface, the blunt portion of the instrument coagulates the tissue, while the sharp edge simultaneously transects the coagulated area.
2. **Coagulation of the tissue surface without cutting**: The surgeon uses only the blunt tip of the electrode to coagulate a tissue surface without performing any transection.

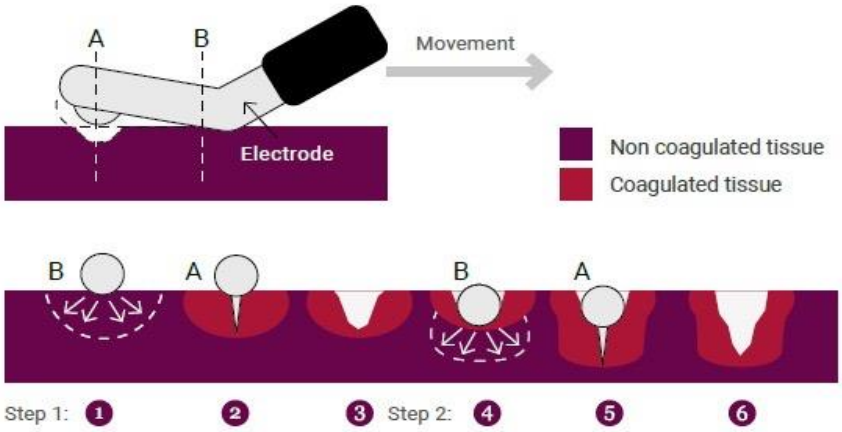


Figure12. Main operation of the Coolingbis® device. Above: lateral section of the active part of the device, showing the distal part (D) with the scalpel blade, the proximal part (P) connected to the non-active part (in green) and the direction of movement of the device. electrode. Bottom: cross-section of the action of the electrode on the tissue by two consecutive applications. Each application consists of two parts: first the coagulation of the tissue with the P part of the electrode applying RF (arrows) as in steps 1 and 4; then the section of the coagulated tissue using the scalpel blade (D) as in steps 2 and 5. Technical datasheet of the Coolingbis® radiofrequency device.

After the computational modeling phase, the device was studied in an ex vivo and in vivo porcine liver model, comparing it with another RF-assisted device (Tissuelink®). which is habitually employed in liver transection (111). The first result of this study was to confirm the feasibility of the application of the device Coolingbis® for hepatic

parenchymal transection in an animal model. The comparison with Tissuelink® in vivo showed a decrease in intraoperative blood loss and liver transection time in animals treated by the Coolingbis® device. These differences were explained by a greater depth of tissue coagulation on the surface of the hepatic transection, achieved by Coolingbis® The depth of tissue coagulation was 6 mm in the Coolingbis® group and 3 mm in the Tissuelink® group. This marked difference may be partially explained by the non-uniform distribution of saline solution over the hepatic surface when using the Tissuelink® device. In contrast, the closed-circuit internal cooling system of the Coolingbis® device always ensures consistent and uniform cooling of the active portion of the electrode.

In the same year 2008, the applicability of the device in hepatectomy was tested in a laparoscopic pig model (110). The results of the study confirmed the feasibility of application of the laparoscopic version of the device and positive results in terms of reduced blood losses and transection times, as well as the ability to seal bile canaliculi.

The positive results obtained in the animal model enabled the initiation of a clinical "safety" study, which received prior approval from the Clinical Research Ethics Committee of Hospital del Mar in Barcelona and from the Spanish Agency of Medicines and Medical Devices (AEMPS). The results of this study, in which 11 hepatectomies were performed in 8 patients with hepatic metastases from colorectal carcinoma, were published in 2010 (103). All hepatectomies were completed using the device alone, without the need for other coagulation or dissection methods, and in the absence of temporary vascular occlusion strategies (Pringle maneuver). The mean resection time per patient was 51 minutes (range 38- 87), and the mean hepatic transection speed was 1.28 cm^2/min. The mean intraoperative blood loss related to hepatic transection was 42.5 mL (range 5–420). None of the eight patients required blood product transfusion during surgery or in the postoperative period. There were no postoperative bile leaks or mortality.

In parallel with the different phases of the study on the application of the device in hepatic surgery, its use was also initiated in laparoscopic partial nephrectomy (LPN). The first comparative study conducted in a porcine model provided initial positive data regarding the hemostatic capacity of the device in the kidney, although the rate of urinary leakage remained like that of the control group.

As with other RF-assisted devices initially used successfully in hepatic surgery, their application was later extended to pancreatic surgery. The study of the feasibility, safety, and efficacy of using the Coolingbis® device in performing PD appears to be a natural

and logical next step, considering the experimental and clinical results obtained to date (110)(112)(113).

Some experimental studies have shown that performing the pancreatic transection with RF improves the sealing efficiency of the main and secondary pancreatic ducts and reduces the incidence of POPF. Although the literature on the subject is limited, (114) recent studies indicate marked reductions in the rate of POPF when an RF device is used in the parenchymal cutting plane (115)(116). An important clinical attempt was an inconclusive RCT conducted at the Mayo Clinic in Rochester, MN, (national clinical trail (NCT)01051856) comparing an RF device (Tissuelink Medical, Dover, NH, USA) with stapling. The trial was terminated prematurely in 2014 due to poor patient recruitment. However, it was eventually published in 2016 as a multicenter randomized clinical trial evaluating the leak rate after distal pancreatectomy, comparing stapler closure with SEAMGUARD versus the TissueLink® device. Although a difference in leak rates was observed, it did not reach statistical significance; therefore, the study did not demonstrate the superiority of one technique over the other, concluding that the choice should depend on the surgeon's preference, experience, and the characteristics of the pancreas (117).

1.6 METABOLOMIC ANALYSIS

While metabolomic analysis has traditionally been applied to oncological diagnosis and biomarker discovery, its inclusion in the present study is justified by its capacity to provide a functional and integrative readout of tissue-level biological responses to surgical injury (118). Pancreatic transection represents not only a mechanical act but also a profound biological insult, triggering local and systemic metabolic changes related to inflammation, tissue necrosis, healing, and pancreatic exocrine activity. These processes are directly involved in the pathogenesis of POPF.

Importantly, different transection techniques may induce distinct metabolic responses, reflecting variations in tissue damage, inflammatory activation, ischemia, and reparative mechanisms at the pancreatic remnant. RF-assisted transection, by inducing controlled coagulative necrosis and fibrosis, is expected to generate a biological environment that differs fundamentally from that produced by mechanical stapler transection. Metabolomic profiling offers a unique opportunity to capture these differences at a molecular level,

beyond what can be detected through conventional clinical, biochemical, or radiological parameters.

The metabolome is defined as the set of small-molecule chemical entities involved in cellular metabolism. Metabolomic analysis examines the concentrations of these low–molecular-weight metabolites, allowing for a comprehensive characterization of the functional state of a cell or organism. Traditionally, its study has focused on the identification of biomarkers for disease diagnosis and prediction(118). However, in recent years, the value of metabolomic analysis has been redefined, evolving from a simple tool for biomarker detection into a technology capable of identifying active drivers of biological processes (119).

Future applications are expected to extend beyond early cancer detection to include therapeutic stratification (for example, identifying patients who are more likely to respond to chemotherapy or immunotherapy) and the discovery of new metabolic targets for cancer treatment.

1.7 JUSTIFICATION OF THE STUDY

POPF remains the most frequent and CR complication following DP, representing the main determinant of postoperative morbidity despite significant advances in surgical technique and perioperative care. Importantly, the incidence of CR-POPF has remained consistently high, at approximately 30- 40%, highlighting an unresolved problem in PS.

Currently, mechanical ST transection represents the most widely used and accepted technique for pancreatic transection, particularly in minimally invasive surgery. However, staplers were not specifically designed for pancreatic tissue, a solid and biologically active gland characterized by friability, heterogeneous texture, and a complex ductal system. Their widespread use is largely driven by technical convenience rather than by pancreas-specific biological rationale. This limitation may partially explain why mechanical transection has failed to significantly reduce POPF rates.

In contrast, RF-assisted pancreatic transection represents a true conceptual shift. Rather than relying on mechanical compression alone, radiofrequency induces controlled thermal coagulation, collagen contraction, and fibrosis at the transection margin, promoting biological sealing of pancreatic ducts and parenchyma. This mechanism directly targets

the pathophysiology of POPF and provides a sound biological rationale for expecting a reduction in fistula incidence.

The development of this technique has followed a structured and progressive translational pathway consistent with the Idea, Development, Exploration, Long-term-study (IDEAL) framework for surgical innovation. Initial preclinical validation was performed in animal models, including the pivotal experimental study by Dorcaratto et al., which demonstrated the safety, feasibility, and mechanistic basis of radiofrequency-assisted pancreatic transection. This was followed by retrospective clinical analyses from our institution, which provided preliminary evidence of reduced POPF rates compared with stapler transection. These sequential stages justified progression to the current prospective, multicenter RCT, designed to definitively evaluate efficacy and clinical impact.

Only a well-designed randomized trial can provide the level of evidence required to challenge current standards and potentially modify clinical practice. The TRANSPAIRE study therefore represents the natural and necessary culmination of a rigorous, stepwise research process aimed at addressing a long-standing unmet need in pancreatic surgery. If successful, this study has the potential to redefine the standard approach to pancreatic transection and significantly improve postoperative outcomes after distal pancreatectomy

Within the context of the TRANSPAIRE study, metabolomic analysis is used as a translational exploratory tool to link the type of pancreatic transection with systemic metabolic responses associated with postoperative outcomes. By identifying metabolic patterns related to inflammation, tissue injury, and healing, this approach may provide mechanistic insight into the clinical effects of radiofrequency-assisted transection and help identify early metabolic signals associated with the risk of POPF.

HYPOTHESIS

2 HYPOTHESIS

RF-assisted transection of pancreatic tissue in distal pancreatectomy significantly reduces the incidence of clinically relevant postoperative pancreatic fistula by approximately 22% compared with conventional transection methods using a stapling device.

Assumptions were made considering a POPF rate of 32% (120) for ST and 10% (121) for RF, respectively, so that there was a clinically relevant difference of 22%.

For sample size estimation, a two-sided alpha error of 5% and a beta error of 20% (corresponding to a statistical power of 80%) were assumed.

OBJECTIVES

3 OBJECTIVES

3.1 MAIN OBJECTIVE

The primary objective of this project is to evaluate the efficacy of radiofrequency-assisted pancreatic parenchymal transection in achieving effective sealing of the pancreatic duct, compared with the conventional stapler-based method, to significantly reduce the incidence of CR-POPF following DP.

3.2 SECONDARY OBJECTIVES

- To assess postoperative morbidity during the first year of follow-up, including late complications and the development of endocrine and/or exocrine pancreatic insufficiency.
- To evaluate in-hospital mortality, as well as mortality at 90 days and at one year postoperatively.
- To evaluate postoperative changes in pancreatic morphology through imaging studies performed at one month, six months and one year after surgery.
- Analyze the metabolomics profile of the peritoneal liquid from the drain

COMPENDIUM OF PUBLICATIONS

4 COMPENDIUM OF PUBLICATIONS

4.1 FIRST ARTICLE

Pueyo-Pérez E, Téllez-Marquès C, Radosevic A, Morató O, Visa L, Ilzarbe L, et al.

Radiofrequency-assisted transection of the pancreas vs stapler in distal pancreatectomy: a propensity scores matched cohort analysis. **Scientific Reports.** 2022; 12(1):7486. doi:10.1038/s41598-022-11583-0.

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Radiofrequency-assisted transection of the pancreas vs stapler in distal pancreatectomy: a propensity score matched cohort analysis

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To demonstrate the efficacy of radiofrequency for pancreatic stump closure in reducing the incidence of postoperative pancreatic fistula (POPF) in distal pancreatectomy (DP) compared with mechanical transection methods. Despite all the different techniques of pancreatic stump closure proposed for DP, best practice for avoiding POPF remains an unresolved issue, with an incidence of up to 30% regardless of center volume or surgical expertise. DP was performed in a cohort of patients by applying radiofrequency to stump closure (RF Group) and compared with mechanical closure (Control Group). A propensity score (PS) matched cohort study was carried out to minimize bias from nonrandomized treatment assignment. Cohorts were matched by PS accounting for factors significantly associated with either undergoing RF transection or mechanical closure through logistic regression analysis. The primary end-point was the incidence of clinically relevant POPF (CR-POPF). Of 89 patients included in the whole cohort, 13 case patients from the RF-Group were 1:1 matched to 13 control patients. In both the first independent analysis of unmatched data and subsequent adjustment to the overall propensity score-matched cohort, a higher rate of CR-POPF in the Control Group compared with the RF-Group was detected (25.4% vs 5.3%, p = 0.049 and 53.8% vs 0%; p = 0.016 respectively). The RF Group showed better outcomes in terms of readmission rate (46.2% vs 0%, p = 0.031). No significant differences were observed in terms of mortality, major complications (30.8% vs 0%, p = 0.063) or length of hospital stay (5.7 vs 5.2 days, p = 0.89). Findings suggest that the RF-assisted technique is more efficacious in reducing CR-POPF than mechanical pancreatic stump closure.

Distal pancreatectomy (DP) is the gold standard surgical procedure for most benign or malignant lesions in the body or tail of the pancreas, which is defined as any resection of the pancreas beyond the left aspect of the superior mesenteric vein/portal vein trunk[1–3]. Although this surgery is performed less often than pancreaticoduodenectomy, improvements in diagnostic imaging techniques have resulted in an expansion of DP indications. In recent years, the mortality rate following DP has decreased dramatically to between 0 and 5%[2,3] in high-volume centers due to advances in surgical techniques and especially to improvements in perioperative care[3]. Despite this reduction in the mortality rate, morbidity remains high mostly as a result of complications related to the pancreatic transection and the development of postoperative pancreatic fistula (POPF), which is associated with intra-abdominal abscesses, sepsis, respiratory complications and hemorrhage among others[1], and which prolong

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hospital stays and raise costs for specialized treatment, including revision surgery and drainage[4]. The fundamental shortcoming in outcome assessment after DP is the lack of genuine outcome data, therefore, since relevant studies report CR-POPF ranging between 10 and 40%[1,2], a fistula rate of around 30% is generally accepted.

Despite efforts to reduce the incidence of POPF through the use of many different stump closure methods such as staplers, hand-sewn closure[5], biological sealants[6,7] or even patches of fatty tissue applied to the pancreatic stump, the incidence of POPF remains a clinically relevant issue and has been relatively stable over recent decades, even with the introduction of minimally invasive surgery[2,8], with no technique demonstrating better results than others[3,5–7]. In fact, there is considerable variability in clinical practice worldwide reflecting the lack of solid evidence on the benefit of any given strategy, indicating that there are still underlying areas for improvement and that new techniques are needed in order to improve results after DP[9]. One of these emerging techniques is based on using radiofrequency (RF) energy[10,11]. During the application of RF, tissue heats up gradually, reaching above 60 °C and causes denaturation of cell membrane proteins, cytoplasm, mitochondrial enzymes, and nucleic acids, known as coagulative necrosis[12,13], and thus this latter one could prevent POPF occurrence by eliciting fibrosis and collagen shrinkage[14–16]. Some experimental studies have demonstrated that RF-based DP improves the sealing efficiency of the main and secondary pancreatic ducts and reduces the incidence of POPF. Although literature on the issue is limited[17–19], recent studies indicate notable reductions in the POPF rate when a RF device is used at the parenchyma transection plane[15–17]. One important clinical attempt was an unfinished RCT performed at the Mayo Clinic in Rochester, MN (NCT01051856) in which a RF device (TissueLink Medical, Dover, NH, USA) was compared with stapling. However, the trial was terminated early due to poor recruitment in 2014.

In absence of RCTs in this area, propensity score matching analyses provide evidence that minimizes bias from non-randomized treatment assignment and allows comparison of otherwise non-comparable cohorts. Applying this methodology to our study, we evaluate an innovative concept for pancreatic parenchymal transection, which has proved reliable and which background research[20,21] indicates to be a better method of closing the pancreas remnant to reduce POPF than other standard techniques.

Materials and methods

We performed a retrospective propensity scored matched cohort study comparing DP with RF closure of pancreas stump (RF Group) to standard DP conducted with other transection methods (Control Group). Ethical approval was obtained from the Institutional Review Board at Hospital Universitario del Mar (No. 2020/9390/I). All patients singed an informed consent to participate in the study. The study was performed in accordance with relevant named guidelines and regulations.

Patient eligibility and data collection

All consecutive patients undergoing elective distal pancreatectomy in our institution between 2006 and 2019 were retrospectively included in the study. All data proceed from a prospective electronic database audited and checked for completeness by 2 investigators (EPP and CTM). Inclusion criteria were DP conducted in adults for any solid or cystic lesion. Stapler or other mechanical transection methods such as LigaSure were employed in the control Group, while in the RF Group, a radiofrequency device was exclusively used. Exclusion criteria were patients with insufficient baseline data or missing primary outcome data.

Included cases in the RF Group were all patients undergoing elective DP and performed by three surgeons who had already reached their learning curve. The technique was conducted in a standardized fashion with either open or laparoscopic approaches. After examination of the abdominal cavity, the gastrocolic ligament was divided to allow correct visualization of the upper border of the pancreatic gland and the course of the splenic vessels. Routine dissection of splenic vessels and encircling the splenic artery was prophylactically conducted in case a hemorrhage occurred. The position of the pancreas division line was selected in the proximal normal pancreas depending on the placement of the lesion and intraoperatively guided by ultrasonography to ensure correct margins. In all cases, pancreatic transection was performed in the RF group with a 10-mm diameter version of the Coolingbis device (Apeiron Medical, Valencia, Spain). By applying the device and moving it back over the surface of the parenchyma, the blunt part of the device coagulates the tissue and the blade part cuts through the portion of tissue coagulated[17,20]. Splenic preservation was conducted in patients with benign lesions or borderline tumors (e.g., cystic neoplasm), but not for pancreatic adenocarcinoma. In those specific cases, a Radical Antegrade Modular Pancreatosplenectomy (RAMPS) was performed.

Included patients in the Control Group were all consecutive patients undergoing standard DP. The surgical procedure was performed in essentially the same way as stated previously in the RF Group except for the pancreatic transection, which was carried out mostly with a stapler. Differences between these techniques are outlined in Fig. 1.

Primary and secondary end-points

The primary end-point was comparison of the clinically relevant postoperative pancreatic fistula (CR-POPF) between the two study groups, graded using the updated International Study Group on Pancreatic Fistula classification[8]. Secondary endpoints included 90-day postoperative mortality and complications recorded and monitored until 90 days after the operation. All surgical complications were graded using the Clavien–Dindo classification[22], which categorizes the complication according to received treatment, and the CCI, a value which measures overall cumulative morbidity on a scale from 0 (no complications) to 100 (death), which was applied to cover the total number of complications by severity for each patient[23]. Complications were reported as major complications, i.e., Clavien–Dindo ≥ IIIa or minor complications, corresponding to Clavien–Dindo ≤ 2.

Within postoperative outcomes, special attention was paid to radiological evaluation of the transection zone after surgery. Computer tomography at 1-month and 1-year postoperative was assessed to evaluate the remnant

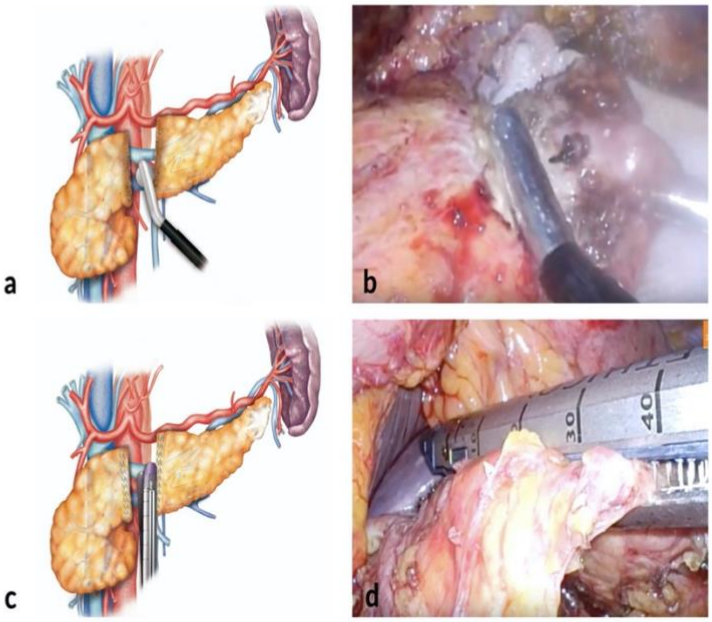

Figure 1. Pancreatic transection with the two different scenarios that have been described in this study. The upper figures (**a**,**b**) show representative (left) and original (right) methods of pancreatic transection mediated by RF. In contrast, the lower figures display the closure with a linear stapling device.

necrosis at the transection zone, measured as the larger of the two orthogonal diameters, to compare between-group differences.

Statistical analyses. Propensity score matching

With the aim of minimizing the differences and avoiding potential confounders between groups, propensity score (PS) matching was applied according to Lonjon et al.[24] recommendations. The PS is defined for each participant as the probability of receiving the treatment, given baseline covariates. With the assumption of no unmeasured confounders, a treated and an untreated patient with the same PS can be considered as though they had been randomly assigned to each group.

Accordingly, an individual's PS was calculated through logistic regression modeling based on the following covariates: age, American Society of Anesthesiologists (ASA) classification, body mass index (BMI) and type of pancreas resection (either distal pancreatectomy or RAMPS). Those covariates were selected as well-known factors that might influence the occurrence of CR-POPF and, most importantly, the surgeon's tendency to choose one technique or another mainly based on the thickness of the pancreas or its proximity to the intrapancreatic common bile duct.

The surgeon tends to avoid the use of staplers in thick pancreas, which are more likely to crush when the stapler is closing or in the tail of the pancreas where it is bulkier and offers difficulties to apply a stapler. As such, these baseline characteristics could affect surgical outcomes. As there are different modalities in calculating a PS, we chose to do PS matching, i.e., matching participants with identical or similar PS with a 1:1 ratio without replacement and a standard caliper width of 0.3. The area under the curve (AUC) of the model was 0.847, which indicated a good goodness of fit.

To assess the validity of our PS matching, data analysis was subsequently divided into two phases. First, we analyzed the row data without matching PS and applying pairwise comparisons of the subsequent endpoint variables with Fisher's exact test for categorical variables, and the Mann–Whitney *U* test for continuous variables. Following 1:1 matching of the cases (RF-Group) with the controls (Control-Group), comparability was assessed between propensity score-matched cohorts using McNemar's test for paired categorical variables, while continuous outcomes used the paired *t* test or Wilcoxon rank-sum test. Categorical data were reported as proportions and continuous data as mean and standard deviation (SD). Tests were considered statistically significant at a 2-sided $p < 0.05$. All confidence intervals (CI) were 95%. All data were handled and analyzed using IBM SPSS Statistics for Windows version 25.0 (IBM, Armonk, NY, USA).

Results

Baseline characteristics

A total of 89 patients underwent DP in our institution between 2006 and 2019. Seven patients were excluded owing to inaccurate or missing data, or due to the use of other techniques. The remaining 82 patients underwent PS matching; 19 in the RF Group and 63 in the Control Group. Of all included patients, 13 patients in RF Group

were 1:1 matched to 13 patients in Control Group using a caliper width of 0.3 of the logit of the propensity score (Fig. 2).

Almost all baseline variables were more balanced after PS matching (see Table 1). Differences between groups were notable regarding the location of the tumor; those in the Control Group had more tumors located on the neck/body with respect to the RF Group (61.9% vs 10.5%, p = 0.001) and the proportion of patients with malignancies in the histology was also higher in the Control Group before matching (see Table 1). Concerning the stump closure only 3 patients out of the initial 82 in control group had a closure different to stapler and within the matched cohort all patients from the control group were performed with stapler. Most of the patient's demographic characteristics were comparable between groups.

Assessment of primary and secondary outcomes

Concerning the primary end-point, the first independent analysis of unmatched data revealed a higher rate of CR-POPF in the Control Group compared with the RF Group (25.4% vs 5.3%, p = 0.049). In fact, after conducting the adjustment in the overall PS-matched cohort, this difference became even more striking as 53.8% of patients in Control Group developed CR-POPF vs none in the RF Group (53.8% vs 0%; p = 0.016) (Table 2).

With regard to secondary end-points, no significant differences were observed in terms of in-hospital mortality, as none of the groups presented mortality in the matched cohort. RF Group showed fewer minor and major complications compared to the Control Group, although no statistical significance was achieved. The rates of grade B/C hemorrhage were comparable in both groups. Length of hospital stay was significantly increased in the Control Group in the individual analyses, but were similar after PS matching (5.7 vs 5.2 days, p = 0.89). The results also revealed better outcomes concerning readmission rate in the RF Group (46.2% vs 0%, p = 0.031). Laparoscopic approach was significantly higher in the control group before the PS adjustment, although no between-group differences were observed by matched cohort.

At 1-month postoperative follow-up, no significant differences were found in the maximum diameter of the residual necrotic zone at the transection zone (24.6 ± 23.0 mm in the RF-Group vs 31.3 ± 17.4 mm in the Control Group, p = 0.646). Nor were changes in maximum diameter observed at 1-year follow-up. (17.6 mm vs 24.2 mm, p = 0.398).

Discussion

To our knowledge, this is the first study which compares, through exhaustive PS matching methodology, the efficacy of RF-assisted vs stapler pancreatectomy in terms of precluding the appearance of CR-POPF. Our analysis shows that this index complication after pancreatic surgery is significantly lower in cases of RF-assisted pancreas

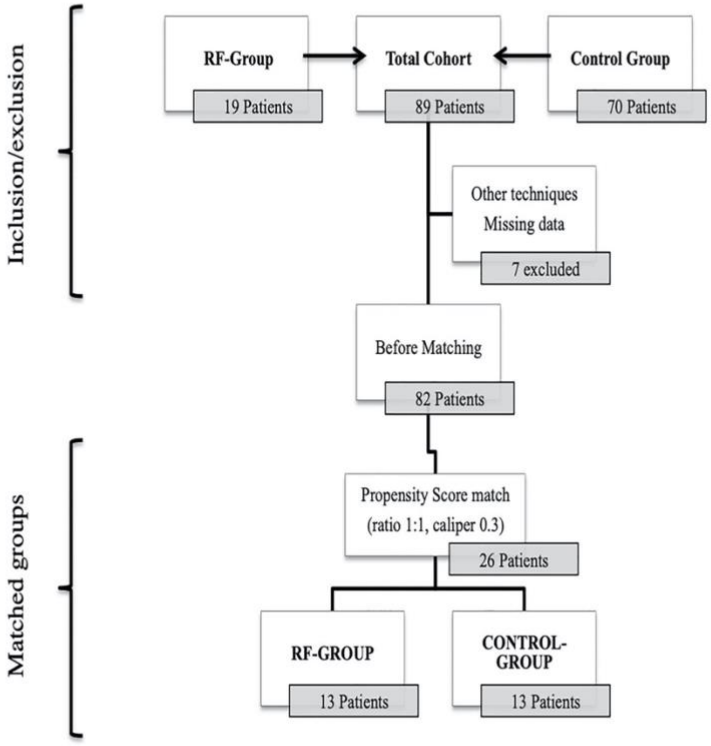

Figure 2. Flow chart of the study.

	With propensity score-matching			Without propensity score-matching		
	Control-Group	RF-Group		Control-Group	RF-Group	
	(n: 63)	(n: 19)	p	(n: 13)	(n: 13)	p
Sex, female, n (%)	34 (54)	7 (36.8)	0.191	4 (30.8)	6 (46.2)	0.420
Age, mean (SD)	64.8 (14.9)	66.8 (14.9)	0.919	53.2 (18.9)	68.3 (14.1)	0.428
BMI, mean (SD)	25.5 (5.2)	27.4 (4.6)	0.755	25.2 (3.8)	27.4 (4.9)	
ASA classification, n (%)			0.303			0.239
I–II	35 (55.6)	8 (42.1)		8 (61.5)	5 (38.5)	
III–IV	28 (44.4)	11 (57.9)		5 (38.5)	8 (61.5)	
Malignancy, n (%)	45 (71.4)	10 (52.6)	0.126	5 (38.5)	7 (53.8)	0.431
Localization, n (%)			**<0.05***			1.0
Body-neck	39 (61.9)	2 (10.5)		4 (30.8)	4 (30.8)	
Tail	24 (38.1)	17 (89.5)		9 (69.2)	9 (69.2)	
Histology, n (%)			**0.022***			0.082
Adenocarcinoma	32 (50.8)	6 (31.6)		2 (15.4)	5 (38.5)	
NET	9 (14.3)	4 (21.1)		2 (15.4)	2 (15.4)	
IPMN	10 (15.9)	9 (47.4)		3 (23.1)	6 (46.2)	
Chronic Pancreatitis	5 (7.9)	–		3 (23.1)	0	
Others	7 (11.1)	–		3 (23.1)	0	

Table 1. Comparison of baseline characteristics between RF-Group and Control Group before and after Propensity Score-Matching. *p-value for the difference in the independent analyses (row data). Significant values are in bold.

	Control-Group Pre-match	RF-Group Pre-match		Control-Group Post-match	RF-Group Post-match	
	(n: 63)	(n: 19)	p	(n: 13)	(n:13)	p
Perioperative						
Operative time, median (SD)	276 (71.4)	284 (70.9)	0.635	224 (60.8)	280 (109.5)	0.065[b]
Type of resection, n (%)			**<0.05**			1.0
RAMPS	54 (85.7)	4 (21.1)		4 (30.8)	4 (30.8)	
Distal pancreatectomy	9 (14.3)	15 (78.9)		9 (69.2)	9 (69.2)	
Laparoscopic approach (%)	45 (71.4)	19 (100)	**0.031**	92.3	100	1.0
Hard/firm pancreas (%)	27.0	15.8	**0.021**	23.1	23.1	1.0
Postoperative (90 days)						
Morbidity, n (%)						
None or minor complications CD 0–II	51 (81.0)	18 (94.7)	0.173	9 (69.2)	13 (100)	0.063[a]
Major complications CD ≥ IIIA	12 (19.0)	1 (5.3)		4 (30.8)	0	
In-hospital mortality (%)	1.6	0	0.768	0	0	1.0
Pancreatic fistula grade B/C n, (%)	16 (25.4)	1 (5.3)	**0.049**	7 (53.8)	0	**0.016[a]**
Hemorrhage grade B/C, n (%)	1.6	0	0.768	0	0	1.0[a]
CCI (median)	0	0	1.0	20.9	0	0.026
Reoperation (%)	6.3	0	0.341	0	0	1.0[a]
Length of hospital stay, mean (SD)	8.9	4.7	**0.02**	5.7 (3.3)	5.2 (1.6)	0.89[b]
Readmission rate (%)	12 (19)	1 (5.3)	0.137	6 (46.2)	0	**0.031[a]**
Transfusion rate (%)	7 (11.1)	0	0.146	0	0	1.0[a]

Table 2. Postoperative outcomes for Clinically Relevant Pancreatic Fistula (CR-POPF) occurrence and secondary end-points comparing Propensity Score-Matched Cohorts RF-Group vs Control Group. [a]McNemar's test, [b]Wilcoxon test. Significant values are in bold.

transection compared to the classical stapled pancreatectomy and reveals encouraging surgical outcomes. As mentioned previously, CR-POPF after DP remains an unresolved issue and despite the associated mortality, which is not very high if the complication is handled in a timely manner, it does represent an important source of further complications and a burden for the patient, caregivers and healthcare system. A recent multicenter, randomized clinical trial (DISPACT) failed to demonstrate greater efficacy associated with the mechanical stapler compared with the classical hand-sewn technique, with no differences observed between groups in the incidence of POPF.

Fistula rates as high as 36% in mechanical stapler group, and 37% in the hand-sewn group were reported[5], again highlighting the non-superiority of one transection technique over another to date.

Therefore, the pressing need to find an innovative surgical solution to the issue of POPF after DP is clear. RF energy has been employed for a long time in hepatic surgery and has demonstrated great effectiveness in achieving parenchymal hemostasis along with simultaneous significant reduction of intraoperative blood loss and biliary leak by inducing an obliterative effect on vascular and biliary structures on the liver[18,25]. Thus, it has been hypothesized in several experimental studies that it could have the same effect on the pancreas acini, producing a more severe macro and microscopic inflammatory response in the transection plane than occurs when a mechanical stapler is used[21]. Experimental studies showed promising outcomes in terms of both safety and feasibility[17,20]. The study by Dorcaratto et al.[17] on a porcine laparoscopic model, which represent the backbone of this current work, revealed an interesting histological pattern in the transection line with a central area of coagulative necrosis surrounded by a large capsule of connective tissue up to 1.8 mm when applying RF-assisted transection whereas the group stapler showed soft thin fibrosis on the stapler line. The rationale behind this finding is that this connective tissue barrier might prevent the leakage of pancreatic juice, due to the firm, fibrotic condition of the pancreatic remnant stump, such as that found in patients with chronic pancreatitis, which are believed to be less likely to leak and all techniques in these patients seem to have a higher rate of success[3]. Even more importantly, these experimental data demonstrated a common pattern of shrinkage of the main and secondary pancreatic ducts with no signs of pancreatitis in the remnant pancreas.

In line with these experimental findings, two previous clinical studies showed a decrease in the rate of CR-POPF when applying RF energy. Fronza et al.[16] reported their initial experience with RF energy for pancreatic transection using the Habib 4× device and even though it was a retrospective analysis involving only 14 patients, they showed a CR-POPF rate of 14.3% with only one surgical reintervention and no mortality, which suggests that RF energy is a safe and feasible method for pancreatic transection[16]. Shortly after, Blansfield et al.[15] published a retrospective study with 62 patients in which 29 of them underwent RF-assisted pancreatic transection with the TissueLink device, showing a reduction in POPF from 36 to 10% in the experimental group[15]. Although both studies had important limitations, both concluded that RF-assisted transection might be a promising technique for reduction of CR-POPF after DP. Interestingly, another recent RCT comparing stapler transection with ultrasonic sealing, which might be similar to RF transection to some extent as both imply thermic mechanism of action, showed no differences in terms of CR-POPF. However, this study has major drawbacks. On one side, the authors intentionally excluded patients with bulky pancreas (i.e. preoperatively measured > 17 mm), which supposes an important selection bias for the technique assessment since these are the pancreas more likely to crush after the stapler closure and the ones, which might benefit most of a thermal transection. In our study we did not perform any preoperatively selection according to the pancreas stiffness, precisely to assess the efficacy of RF in any setting and try to avoid this selection bias. On the other hand, it has been demonstrated that ultrasonic dissectors, such as Harmonic enable a coagulation margin much thinner than the one performed by a RF device and it might not generate enough connective tissue barrier to preclude the collection of a pancreatic juice and therefore not be decisive to avoid CR-POPF.

In the first stages, however, the potential value of a novel procedure is still hampered by heterogeneity in the patient population and an absence of standardization of the technique, which precludes wide acceptance. To minimize bias and achieve a balanced exposure of groups at baseline, a propensity score-matching model was developed and we were able to demonstrate the greater efficacy of the RF-assisted technique in terms of decreasing CR-POPF not only in the individual row data analysis but also in the PS matching. Therefore, given the absence of any improvement in CR-POPF rates in the last decade, which remain around 30%, stapler closure may no longer be regarded as the current state-of-the-art technique for distal pancreatectomy[5,26].

This study has however several limitations that warrant emphasis. First, the sample size is small, as there is a certain difficulty in recruiting patients for this surgical indication, and which becomes smaller still after PS matching to homogenize the study groups, which is one of the disadvantages of these type of studies. Moreover, the beginning of a new surgical technique usually faces many hurdles till it becomes widely accepted due to the initial doubts about its feasibility and safety, therefore the first preliminary reports published on this approach gathered no more than 20–30 patients. Although the study covers a long period of time, no significant variations in the technique of distal pancreatectomy have been implemented in the last two decades, besides perhaps the standardization of minimal invasive surgery; therefore we deemed that it has not a great impact on the outcomes. Second, despite adjusting for potential bias by using a rigorous propensity score-matching methodology, it is possible that unmeasured confounders were not accounted for, and sidelined as potential covariates. In spite of this, the AUC of our PS model was 0.847, indicating that the goodness-of-fit was very satisfactory which suggests that no important cofounders of the model were disregarded. In conclusion, we are aware that is the first step in the implementation of this novel technique, and a multicentric randomized clinical trial should be carried out to validate these results. In this regard, we are currently launching an RCT (clinicaltrials NCT04402346) to further strengthen our hypothesis.

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Author contributions
E.P.P. and C.T.M.: Both surgeons have equally contributed in the conception and design, acquisition and interpretation of data and participation in drafting the article. A.R.: Radiologist who has participated in drafting the article and interpretation of radiological images, contributing to the interpretation of results. O.M.: Surgeon who has participated in drafting the article and interpretation data. L.V.: Oncologist who has participated in the interpretation of the results and the preparation of the discussion by critically reviewing it. L.I.: Digestologist who has participated in interpretation of the results and the preparation of the discussion by critically reviewing it. E.B.: Engineer who has participated in the technological development of the radiofrequency device and in the discussion by critically reviewing it. E.D.V.: Surgeon who has participated in interpretation of the results and the preparation of the discussion by critically reviewing it. B.I.: Surgeon who has participated in the necessary modifications and who has contributed points of view and scientific information that have contributed to improving and adapting the article with scientific criteria. L.G.: Surgeon who has participated in the design of the study, in interpretation of the results and critical review and give the final approval of the version to be published. I.P., F.B. and P.S.V.: Surgeons who have contributed in conception and design, acquisition and interpretation of data and participation in drafting the article, and give the final approval of the version to be published.

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Competing interests
The authors declare no competing interests.

Additional information
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4.2 SECOND ARTICLE

Sánchez- Velázquez P, Pueyo-Périz E, Álamo JM, Suarez Artacho G, Gómez Bravo MA, Marcello M, et al.

Chromatography Radiofrequency-assisted transection of the pancreas versus stapler in distal pancreatectomy: study protocol for a multicentric randomised clinical trial (TRANSPAIRE). **BMJ Open.** 2022; 12(11):e062873. doi:10.1136/bmjopen-2022-062873.

Open access Protocol

BMJ Open

Radiofrequency-assisted transection of the pancreas versus stapler in distal pancreatectomy: study protocol for a multicentric randomised clinical trial (TRANSPAIRE)

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ABSTRACT

Introduction To date, no pancreatic stump closure technique has been shown to be superior to any other in distal pancreatectomy. Although several studies have shown a trend towards better results in transection using a radiofrequency device (radiofrequency-assisted transection (RFT)), no randomised trial for this purpose has been performed to date. Therefore, we designed a randomised clinical trial, with the hypothesis that this technique used in distal pancreatectomies is superior in reducing clinically relevant postoperative pancreatic fistula (CR-POPF) than mechanical closures.

Methods and analysis TRANSPAIRE is a multicentre randomised controlled trial conducted in seven Spanish pancreatic centres that includes 112 patients undergoing elective distal pancreatectomy for any indication who will be randomly assigned to RFT or classic stapler transections (control group) in a ratio of 1:1. The primary outcome is the CR-POPF percentage. Sample size is calculated with the following assumptions: 5% one-sided significance level (α), 80% power ($1-\beta$), expected POPF in control group of 32%, expected POPF in RFT group of 10% and a clinically relevant difference of 22%. Secondary outcomes include postoperative results, complications, radiological evaluation of the pancreatic stump, metabolomic profile of postoperative peritoneal fluid, survival and quality of life. Follow-ups will be carried out in the external consultation at 1, 6 and 12 months postoperatively.

Ethics and dissemination TRANSPAIRE has been approved by the CEIM-PSMAR Ethics Committee. This project is being carried out in accordance with national and international guidelines, the basic principles of protection of human rights and dignity established in the Declaration of Helsinki (64th General Assembly, Fortaleza, Brazil, October 2013), and in accordance with regulations in studies with biological samples, Law 14/2007 on Biomedical Research will be followed. We have defined a dissemination strategy, whose main objective is the participation of stakeholders and the transfer of knowledge to support the exploitation of activities.

Registration details ClinicalTrials.gov Registry (NCT04402346).

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ For the first time, a randomised clinical trial addresses specifically the unresolved problem of the pancreatic transection after distal pancreatectomy assessing the efficacy of radiofrequency in this setting.
⇒ Despite the novelty of the technique, TRANSPAIRE trial is a multicentric study which has been implemented in several different specialised pancreatic centres.
⇒ The trial also evaluates the metabolic phenotype in peritoneal liquid from the patients in each arm in order to identify inflammatory changes secondary to the treatment applied.
⇒ One limitation would be that tumours close to the pancreatic neck should be excluded and therefore reducing the generalisability of the results.

INTRODUCTION

Pancreatic surgery is currently the gold-standard option for curative treatment not only in neoplastic diseases but also in benign diseases and mucinous cystic neoplasms. Distal pancreatectomy consists of resecting the portion of the pancreas on the left aspect of the superior mesenteric vein and inevitably leads to a pancreatic stump, as no anastomosis is performed between the pancreatic remnant and the bowel. The most feared and potentially serious complication after

distal pancreatectomy is a postoperative pancreatic fistula (POPF), which consists of the leakage of pancreatic juice from the main and secondary branches of the duct to the peripancreatic space or peritoneal cavity.[1] Although different surgical techniques have been applied to seal the pancreatic stump throughout the history of pancreatic surgery, and with the centralisation of surgery and the multidisciplinary approach, we have witnessed a considerable reduction in postoperative mortality and morbidity,[2] the POPF rate remains however unchanged, around 30%–40%.[3] Historically, the closure of the pancreatic stump by manual suture (hand-sewn) was the standard of care[3] but with later technological developments and the implementation of the minimally invasive approach, staplers, ultrasonic scalpels,[4] biological glues[5] and even fatty tissue patches attached to the pancreatic stump[6] have been widely accepted.

Since none of the previously mentioned techniques have been able to reduce the incidence of POPF, energy-assisted and radiofrequency-assisted devices have been implemented in both experimental studies[7 8] and clinical settings to try to reduce the POPF rate. The preliminary data from retrospective studies showed promising results, with a significant reduction of POPF of up to 10%–14%,[9 10] and despite their major limitation of being retrospective uncontrolled studies with few patients, they provided an insight into the efficacy of the technique for solving a serious clinical dilemma.

In a recent retrospective propensity-score matched analysis of 89 patients, we suggested that the use of the *Coolingbis* radiofrequency device was associated with a significant reduction of POPF rates compared with stapler closure.[11] Under these premises, in a randomised trial, we aim to evaluate the effectiveness of radiofrequency-assisted transection (RFT) of the pancreas in terms of duct sealing compared with the classic method of (stapler) transection (ST) to significantly reduce POPF rates in distal pancreatectomy.

METHODS AND ANALYSIS

Study design

The TRANSPAIRE trial is a multicentric randomised controlled parallel-group trial carried out in seven Spanish pancreatic centres to compare two different methods of pancreatic transection in distal pancreatectomy (DP), that is, RFT (study group) versus ST (control group). Local approval was required for the individual participating centres and the study was registered at ClinicalTrials.gov (NCT04402346). The patients eligible to participate in the study will be approached by the investigators and recorded, even if they did not decide to participate. All the patients will sign a written informed consent form before randomisation.[12]

Study population and eligibility criteria

All consecutive patients requiring distal pancreatectomy for any cause will be considered eligible if they complied with all of the following at randomisation (figure 1—flow chart):

Inclusion criteria

- Over 18 years old.
- Patients with benign or malignant solid or cystic pancreatic neoplasms.
- Transection of the pancreas performed at least >2 cm on the left from the medial aspect of the superior mesenteric vein (assessed by CT or magnetic resonance at least 2 months before the surgical intervention) to avoid potential iatrogenic lesions of the intrapancreatic common bile duct.
- Either spleen-preserving or espleno-pancreatectomy is accepted.
- Either open or minimally invasive approach (laparoscopic or robotic) is acceptable.

Exclusion criteria

- Any other system of pancreatic transection in the control group apart from stapling will be excluded.
- American Society of Anesthesiologists (ASA) physical status >3.
- Inability to sign the informed consent and under 18 years old.
- Pregnancy.
- Emergent surgery (ie, post-traumatic).

Patient and public involvement

Patients were not directly involved in the design and conduct of this research. However, patients will be asked in setting the outcome measures for the quality of life questionnaires and help to decide about the most appropriate ones. Once the trial has been published, results will be communicated to keep people informed throughout the project, reporting negative and positive results.

Calculation and justification of the sample size

The sample size was calculated following Delgado and Domenech[13] and hypothesising that RFT was superior to ST. Assumptions were made considering a POPF rate of 32% for ST[14] and 10% for RFT, respectively, so that there was a clinically relevant difference of 22%. At 5% one-sided significance level (α), 80% power (1–β), the required sample size was 56 patients per arm, including a 10% drop-out rate after randomisation (patients who underwent no surgery after randomisation) led to a total number of 112 patients to be randomised.

Trial-specific interventions

- *RFT group:* the technique will be conducted with either an open or minimally invasive approach (robotic or laparoscopic). All procedures will be performed by a pancreatic surgeon with at least 5 years of experience in the field and having completed the learning curve with the performance of more than 10 pancreatic transections using the radiofrequency device. All the surgeons are familiar with both techniques of stump closure after pancreatectomy. After examination of

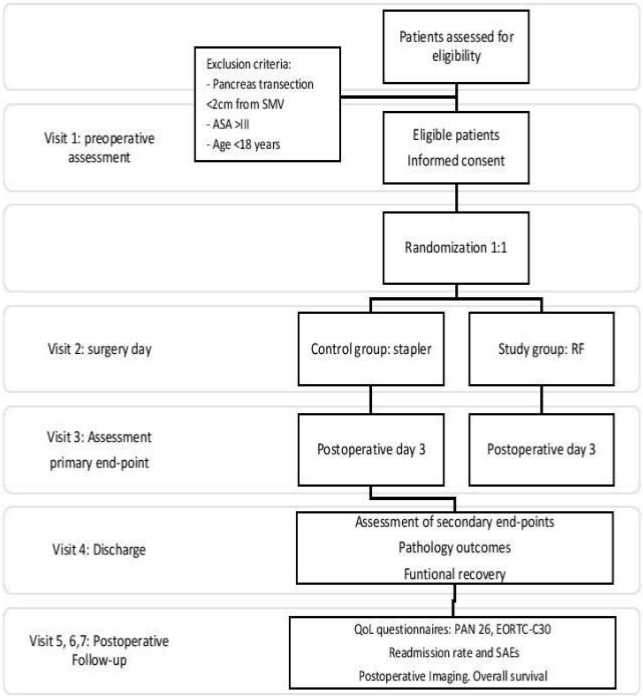

Figure 1 Flow chart followed by patients once they meet inclusion criteria and can be randomised. ASA, American Society of Anesthesiologists; QoL, quality of life; RF radiofrequency; SAEs, serious adverse effects. SMV: superior mesenteric vein; PAN-26/ EORTC-C30: EORT Quality of life Questionnarie - Pancreatic Cancer Module

the abdominal cavity, the gastrocolic ligament will be divided to allow correct visualisation of the upper border of the pancreatic gland and the course of the splenic vessels. In case of splenic preservation, these vessels must be spared. The position of the pancreas division line will be selected in the proximal normal pancreas according to the position of the lesion and intraoperatively guided by ultrasonography to ensure correct margins. In all cases, pancreatic transection will be performed in the RFT group with a 10 mm diameter version of the *Coolingbis* device (Vec Medical, Valencia, Spain). By applying the device and moving it backwards over the surface of the parenchyma, the blunt section of the device coagulates the tissue and the blade cuts through the portion of coagulated tissue. If transection with RFT is impossible, the surgeon will be free to cross over to perform any other transection technique. The specific techniques used will be recorded together with the consequent data analysis.

- *ST group*: the surgical procedure will be performed in essentially the same way as in the RFT group, except for the step of pancreatic transection, which will be carried out with a stapler. As the aim is to compare the technique itself with RFT, no restrictions were set concerning the stapler load/cartridge or the use of Bioabsorbable Staple Line Reinforcement. A gradual compression will be applied for 5–10 min, the stapler will be then fired and slowly released after transection. Hand-sewn or other transection methods such as the harmonic dissector are absolute exclusion criteria.

As the TRANSPAIRE trial is pragmatic, no extra effort will be focused on standardising the patients' postoperative care, as long as the same protocol will be applied to both RFT and ST groups in each individual centre. Participants will receive postoperative care according to the centre's daily routine; however, all surgical techniques, materials and medical devices used were reported in detail to detect any differences among the participants, identify potential confounders and to register any imbalance among the treatment groups.

Data capture and trial endpoints

Primary endpoint(s)

The primary endpoint of the study is clinically relevant POPF (CR-POPF) rate according to the updated guidelines recently published by the International Study Group of Pancreatic Fistula, that is, a drainage output of any measurable volume of fluid with an amylase level >3 times the institutional upper limit of normal serum amylase activity, associated with a clinically relevant development/condition directly related to the POPF.[15]

Pancreatic amylase will be measured in the peritoneal fluid of the drain at postoperative days 3 and 5 (if drain still in place). Any type of fistula (biochemical leak or clinically relevant B or C) will be assessed.

Table 1 Secondary endpoints

Endpoint	Definition	Timeline
Intraoperative		
Blood loss	Millilitres	Day of the surgery
Operative time	Minutes	Day of the surgery
Surgical approach	Open/minimal invasive	Day of the surgery
Spleen preservation	Yes/no	Day of the surgery
Postoperative endpoints		
CR-POPF	According to ISGPF definition[15]	Within 90 days after surgery
DGE	According to ISGPF definition[20]	Within 90 days after surgery
PPH	According to ISGPF definition[21]	Within 90 days after surgery
QoL questionnaires	PAN-26, EORTC-30 (PAN-26/ EORTC-C30: European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire -Core Questionnaire (C30) - Pancreatic Cancer Module)[22]	Until 12 months after surgery
Readmission rate	Any readmission in the hospital	Within 90 days after surgery
Reoperation rate	Any surgery after index surgery	Within 90 days after surgery
Overall survival	Time from surgery to last follow-up	Within 12 months after surgery

CR-POPF, clinically relevant postoperative pancreatic fistula; DGE, delayed gastric emptying; ISGPF, Internation Study Group Pancreatic Fistula; ISGPF, International Study Group of Pancreatic Fistula; PPH, postpancreatectomy haemorrhage; QoL, quality of life.

Secondary endpoint(s)

The most important secondary endpoints are in-hospital mortality, postoperative complications until discharge and long-term postoperative endpoints (see table 1).

Complications will be graded by the Clavien-Dindo classification,[16] which groups the complication according to the treatment received and the Comprehensive Complication Index,[17] a value which measures overall cumulative morbidity on a scale from 0 (no complications) to 100 (death) and will be applied to cover the total number of complications by severity for individual patients. Other variables include patients' clinical demographic characteristics (ie, sex, age, ASA classification, jaundice level), variables associated with the type of procedure (open or laparoscopic surgery, intraoperative bleeding, duration of the intervention, size of the pancreatic duct) and oncological outcomes such as quality of lymphatic resection. Pathological assessment of the specimen will be performed as standard in both groups.

Metabolic phenotyping will be carried out on the peritoneal fluid on the third postoperative day to assess the inflammatory changes secondary to the treatment applied. The possibility of generating metabolic phenotypes from large patient samples can thus identify candidates for metabolic biomarkers, certain disease risks or the result of a certain treatment.[18] Specifically, a battery of inflammatory cytokines is measured with the Proteome Profiler Human XL Protein array, which can test a battery of up to 105 different cytokines. The remnants of biological samples not used for this determination will be destroyed.

Patients' quality of life will be evaluated by QLQ-C30 (European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire - Core Questionnaire) and PAN-26 (EORT Quality of life Questionnarie - Pancreatic Cancer) questionnaires sent to the participants at baseline, 30, 180, and 365 days after surgery.

Long-term endpoints

- Evaluating the postoperative morbidity of patients in the follow-up in the first year (late complications, presence of endocrine and/or exocrine insufficiency) as well as overall and disease-free survival in patients with cancer.
- Radiological assessment of pancreatic stump evolution in the first month and first year after surgery. Volume of the ablation lesion created in the transection margin according to digital reconstruction with CT or MRI 1 month and 1 year after surgery using a segmented injury manual with appropriate software (3D Doctor, Able Software Corp, Massachusetts, USA) measured in cubic centimetres.[19]

Patient timeline and trial visits

All patients scheduled for elective DP in all the centres will be considered to participate in the trial and assessed for eligibility. Reasons for non-inclusion and all those who refuse to take part must be reported. Patients will be enrolled by their ability to understand the extent and nature of the trial and provided written informed consent after receiving detailed information and by fulfilling all inclusion criteria. Baseline data together with the first quality of life questionnaire will be recorded during the baseline visit. The mentioned surgical data will be collected in visit 2, that is, surgery day. Primary and secondary outcome parameters will be collected from visit 3 to discharge date (visit 4). Diagnostic and any

Table 2 Trial visits and documented parameters

Assessment	V1 Prestudy screening/consent/ randomisation	V2 Surgery day	V3 POD 3	V4 Discharge	V5 1 month	V6 6 months	V7 1 year
Eligible criteria	x						
Informed consent	x						
Demographics and baseline characteristics	x						
Randomisation	x						
QoL assessment	x				x	x	x
Primary outcome assessment			x	x			
Metabolomics analysis (peritoneal fluid)			x				
Secondary outcomes (CCI, complications)				x	x	x	x

CCI, Comprehensive Complication Index; POD, postoperative day; QoL, quality of life; V, visit.

ensuing therapeutic procedures caused by postoperative complications will be collected and reported. Table 2 summarises the visits.

Randomisation

Patients who meet the inclusion and exclusion criteria and sign the informed consent in the outpatient clinic are eligible for randomisation. They will be given a code or identification number in strict sequential order. Randomisation will be performed before surgery so that specific devices can be prepared for the pancreatic transection. Patients will be allocated to the RFT or ST group in the centre by the study promoter on an online computer-controlled Permuted-Block Randomization Module (Castor EDC, CIWIT, Amsterdam, the Netherlands) in a 1:1 ratio without reposition and block sizes vary between two and four patients. Randomisation will be stratified by centre.

Blinding

The study will be single blind since blinding the surgeon is not possible. Therefore, the surgeon will know and must apply the technique to be used. However, the patient will not be informed of the instruments and technical details to be used in their case, since they are common techniques. Blinding will be reported according to the standards of surgical trial methodology.

Patients are blinded to the intervention for as long as possible.Therefore, the outcome assessment will be as free from detection bias as possible. No attempt will be made to blind trial statisticians; however, they will not have access to unblinded data during the study and will perform analyses according to a predefined statistical analysis plan.

DATA MANAGEMENT, STATISTICAL ANALYSES AND QUALITY ASSURANCE

Data management

All the variables collected in the study will be stored in the electronic case report form (eCRF) to be automatically transferred to a database by the study coordinators, as described in the eCRF. Each researcher and study monitor will have digital access to the eCRF and database to include new patients and review any data during the follow-up. Any addition or correction in the remote data entry system will be automatically protocolled in an audit file. At least one backup copy of the database will be made monthly. Both the eCRF and a copy of the prospective database will be kept up to 5 years after completion of the study and will be treated with the same degree of confidentiality as the rest of the patients' clinical history data.

Data analyses

The main analysis will be performed following the principle of intention to treat. Both groups will be compared initially according to the POPF percentage and number of serious adverse effects (SAEs), as in relation to secondary variables already described according to a conventional univariate analysis. To adjust confounding variables, a multivariate analysis will be considered for the CR-POPF study. Time to event endpoints, such as survival, will be calculated by Kaplan-Meier estimations. A Cox regression analysis will be performed to investigate postoperative survival predictors. All parameters with a $p<0.1$ in a univariable analysis will be included in the multivariable Cox regression analysis. A specific subanalysis will be considered in the following variables: surgical approach, histological types of tumours treated, pancreas stiffness and size of the pancreatic duct. Regression lines will be created between Di (length total pancreas) and Df (distance from the superior mesenteric vein (SMV) to the transection zone of the pancreas) to assess differences in resection margins between groups and length of pancreatic remnant.

An interim analysis will be performed on the primary endpoint when 50% of the patients have been randomised and completed the 6-month follow-up by an independent statistician blinded for the treatment allocation.

Serious adverse effect

An SAE is an adverse effect and should meet one or more of the following requirements: (1) it leads to the patient's death; (2) there is an imminent risk of death; (3) the patient requires hospitalisation or prolongation of hospitalisation; (4) it involves a disability or a significant persistent sequel; (5) it is a major medical life-threatening event or may require medical intervention to prevent any of the above-mentioned effects.

Any SAE will be noted on the patient's eCRF including start time, action taken and whether it constitutes an SAE. The committee will evaluate the SAEs and will continue with the project if more than 10% of the patients treated in the first phase have SAEs.

Quality assurance

Independent-qualified Hospital del Mar Medical Research Institute monitors will provide risk-based clinical monitoring according to the standard operating procedures. Before initiation of the trial, interactive training will be conducted and an electronic test database will be created for familiarisation with the system and entering test data. All investigators will grant the monitors access to trial-specific patient data and agree to being visited before, during and after completion of the study to ensure that the study is conducted, recorded and reported according to the study protocol. The monitoring strategy will consist of a combination of centralised and on-site monitoring. Monitoring visits will be scheduled according to the number of visits ready for verification. On-site monitoring will focus on patient-informed consent and safety, inclusion and exclusion criteria, surgical procedures, randomisation and correct recording and documentation of primary and secondary endpoints by source data verification. Data will be entered into an eCRF, and visits will be marked as 'complete data' after monitoring. The data's completeness, validity and plausibility will be checked when entering data (edit checks) and by using validating programmes that generate queries. The completed eCRF must be reviewed and signed by the investigator named in the trial protocol or a designated subinvestigator. The investigator or the designated representative will be obliged to complete the eCRF as soon as possible after information is collected and to clarify or explain any queries.

Duration and schedule

The duration of the trial for each patient is 12 months. The overall trial is expected to take 3 years to complete, including study preparation and analysis. The first patient was recruited in February 2021 at the Hospital Universitario del Mar.

ETHICS AND DISSEMINATION

The approach can be minimally invasive or open, and the surgical procedure will be described and standardised. There will be no special handling of patients outside of normal medical practice.

This project will be carried out in accordance with national and international guidelines, the basic principles of protection of human rights and dignity established in the Declaration of Helsinki (64th General Assembly, Fortaleza, Brazil, October 2013), and in accordance with the regulations in studies with biological samples, Law 14/2007 on Biomedical Research will be followed.

The CEIM-PSMAR has previously approved the study, the patient information sheet and the informed consent. It is essential to obtain the signature of the informed consent, which must be signed by both the researcher and the participant, who will receive a copy. The study promoter is responsible for obtaining the approval of each Institutional Ethics Committee involved in the study. Given that in neither of the two groups is the surgical procedure modified by the clinical trial, the usual informed consent will be used in each centre to perform the surgical procedure. However, once signed, the patient will be asked to participate in the study and will be informed of the possibility of being part of one or another group through the specific informed consent of the study in question. The principal investigator is responsible for informing the Ethics Committee of any amendment to the protocol in accordance with local requirements.

Civil liability insurance will be available.

The study protocol has been approved by the Institutional Review Board (IRB) of the Hospital del Mar (2020/9390/I) and that a list of IRB approvals from the other participating centres can be found in the online supplemental file.

The confidentiality of the data is guaranteed in accordance with current regulations. All information obtained is treated confidentially in compliance with Organic Law 3/2018, of 5 December, 'Protection of Personal Data and guarantee of digital rights' in compliance with Regulation European Union 2016/679 of the European Parliament and of the Council of 27 April 2016 of Data Protection.

We have defined a dissemination strategy, whose main objective is the engagement of the stakeholders and the transfer of knowledge to support the exploitation of the activities. Our first target audiences will be health organisations and the medical research community. Beyond this, we will target the medical device industry and other social stakeholders such as a policymakers and/or key opinion leaders. In this context, we will develop a dissemination strategy that will be crucial to provide the broadest distribution of our clinical results.

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Correction notice The article has been updated since it was first published for change in one of the author's affiliation.

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4.3 BRIEF SUMMARY OF THE METHODOLOGY

This doctoral thesis is presented as a compendium of publications. Accordingly, the detailed methodology corresponding to the published studies is fully described in the articles included in section 4 and is not repeated in this chapter. However, given the complexity of the research project and the incorporation of complementary analyses that are not described in full detail in the published manuscripts, this section provides an integrated and clarifying summary of the overall methodological framework.

Importantly, a clear distinction is made between:

- methodological aspects already described and peer-reviewed within the published articles (including the pilot study and the clinical trial protocol), and
- additional exploratory and translational analyses developed within the context of this doctoral thesis, whose results have not yet been published.

The research project comprises two complementary clinical phases addressing the unresolved problem of CR-POPF following DP.

The first phase consisted of a single-center retrospective matched cohort study using propensity score matching (PSM) to explore the feasibility, safety, and preliminary efficacy of RF-assisted pancreatic transection compared with conventional stapler closure. This pilot study was hypothesis-generating and provided the rationale for subsequent prospective evaluation.

Built on these preliminary findings, the second phase corresponds to the TRANSPAIRE trial, a multicenter, prospective, randomized, controlled, parallel-group clinical trial designed to compare RF-assisted pancreatic transection versus ST based transection in DP. The study was conducted across eight Spanish pancreatic surgery centers.

The RCT was conducted between February 2021 and April 2025**,** encompassing patient recruitment, surgical interventions, postoperative follow-up, and completion of the predefined study timeline. The trial protocol was developed in accordance with CONSORT guidelines for CT, registered in ClinicalTrials.gov (NCT04402346), and obtained ethical approval at all participating institutions prior to patient inclusion.

The complete design, eligibility criteria, randomization procedures, and predefined primary and secondary endpoints are fully detailed in the published protocol article included in this thesis and are therefore not reiterated here.

All patients included in the clinical studies underwent DP performed via an open or minimally invasive approach (laparoscopic or robotic), according to institutional practice and surgeon expertise.

In the RF group, pancreatic transection was performed using the Coolingbis® device, following a standardized technique aimed at achieving controlled coagulative necrosis and sealing of pancreatic parenchyma and ductal structures. In the control group, pancreatic transection was performed using a mechanical ST. Apart from the transection method itself, all other surgical steps were comparable between groups.

Perioperative management, including postoperative care and complication management, followed routine practice at each participating center. As the TRANSPAIRE trial was designed as a pragmatic study, no additional standardization was imposed beyond ensuring that both treatment arms were managed according to the same institutional protocols within each participating center.

Across the clinical phases, the primary outcome was the incidence of POPF, classified according to the updated ISGPS definition. Both biochemical leak and CR-POPF (grades B and C) were recorded.

Secondary outcomes included intraoperative variables (blood loss, operative time, surgical approach and spleen preservation), postoperative morbidity like DGE or postpancreatectomy hemorrhage (PPH) and mortality. Also includes need for reintervention, readmission rate, and PO complications graded according to the Clavien-Dindo classification. Overall morbidity burden was summarized using the Comprehensive Complication Index (CCI). All the variables are detailed and systematically presented in table 3 below.

Table 3. Secondary endpoints

Endpoint	*Definition*	*Timeline*
Intraoperative		
Blood loss	Millilitres	Day of the surgery
Operative time	Minutes	Day of the surgery
Surgical approach	Open/minimal invasive	Day of the surgery
Spleen preservation	Yes/no	Day of the surgery
Postoperative		
CR-POPF	According to ISGPF definition	Within 90 days after surgery
DGE	According to ISGPF definition	Within 90 days after surgery
PPH	According to ISGPF definition	Within 90 days after surgery
Readmission rate	Any readmission in the hospital	Within 90 days after surgery
Reoperation rate	Any surgery after index surgery	Within 90 days after surgery
QoL questionaries*	EORTC-30 PAN-26	Until 12 months after surgery
Overall survival	Time from surgery to last follow-up	Within 12 months after surgery

* EORTC-30: European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire-Cancer 30
PAN-26: Pancreatic Cancer-specific module of the EORTC

Beyond the core variables reported in the publications, the doctoral project incorporated a broader set of demographics, clinical, surgical, and pathological parameters to allow comprehensive characterization of the study population. These included patient demographic characteristics (BMI, American Society of Anesthesiologists (ASA) classification) and operative variables (surgical approach, operative time, intraoperative blood loss, pancreatic duct diameter).

Quality-of-life (Qol) assessment was initially planned in the clinical trial protocol; however, since these data were not ultimately analyzed, quality-of-life outcomes are not included in this doctoral thesis

Clinical follow-up was conducted for up to 12 months after surgery.

In addition to the clinical outcomes reported in the published articles, this doctoral thesis incorporates a structured radiological evaluation of the pancreatic remnant and postoperative peripancreatic collections, which is not fully described in the published protocol.

A radiological follow-up protocol was developed to evaluate morphological changes at the pancreatic transection margin. Contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI) studies were reviewed during the first postoperative month and throughout the first postoperative year (at 1, 3, 6, and 12 months), focusing on the evolution of the transection/ablation zone and the presence of postoperative peripancreatic collections.

Preoperative pancreatic morphology was assessed by measuring pancreatic thickness on axial contrast-enhanced CT images at standardized anatomical landmarks:

(1) at the level of the portal vein and

(2) 1 cm from the planned transection line. The mean of these two measurements was recorded for analysis.

The mean value of these two measurements was used for analysis, as it was considered the most robust variable. This approach accounts for the known heterogeneity in pancreatic thickness along the gland, which may vary significantly between different anatomical points, as previously reported in studies evaluating pancreatic morphology and risk factors for POPF (122). In addition, thickness measurements closer to the distal transection line may be influenced by the surgical transection technique; therefore, relying on a single distal measurement could introduce bias. Moreover, preoperative CT imaging does not reliably predict the exact transection site in all cases.

CT was used as the reference imaging modality for the detection and characterization of postoperative peripancreatic collections after pancreatic surgery and played a central role in the assessment of clinically relevant postoperative pancreatic fistula. In the specific setting of DP, CT-based evaluation of peripancreatic fluid collections allows objective characterization of postoperative changes and provides relevant information regarding the extent, morphology, and evolution of collections. Accordingly, standardized CT-derived morphometric parameters, including collection size, wall thickness, shape descriptors, and attenuation, were applied to characterize postoperative peripancreatic collections and correlate radiological findings with clinical outcomes

When postoperative collections were identified, morphometric analysis was performed on axial images at the slice showing maximal extension. Collection size was quantified by

measuring area (cm^2) and perimeter (cm) using a region of interest (ROI) at the largest axial diameter, with the minimum diameter measured on the same axial slice where the maximum diameter was identified. Maximum wall thickness (cm) was defined as the greatest distance between inner and outer margins of the collection wall/capsule.

Density measurements were obtained using ROIs placed on the capsule of the collection (Hounsfield Units (HU_capsule), the fluid content of the collection (HU_collection), and adjacent pancreatic parenchyma during the portal venous phase. To reduce inter-scan variability and differences in contrast enhancement, adjusted density ratios were calculated by normalizing collection and capsule density values to pancreatic parenchymal density in the portal phase (HU_collection/portal ratio and HU_capsule/portal ratio).

When postoperative collections were identified, morphometric analysis was performed on axial images at the slice showing maximal extension. Collection size was quantified by measuring area (cm^2) and perimeter (cm) using a region of interest (ROI 1cm2) at the largest axial diameter. Maximum wall thickness (cm) was defined as the greatest distance between inner and outer margins of the collection wall/capsule.

To further characterize the morphology of postoperative collections, shape descriptors were calculated, including circularity and sphericity (Ψ). Sphericity was derived assuming an ellipsoidal geometry and computed using predefined SPSS syntax to ensure standardized and reproducible calculation. All radiological variables were treated as continuous variables for statistical analysis. All measurements were independently performed by two investigators (FB and EP) using RadiAnt DICOM Viewer (64-bit) software. Quantitative analyses were conducted by placing standardized circular ROIs of 1 cm^2 within the predefined anatomical areas. Care was taken to avoid adjacent structures, vessels, and artifacts to ensure measurement consistency and reproducibility.

These radiological analyses were designed to provide mechanistic insight into stump healing and postoperative collection behavior and should be regarded as exploratory.

An exploratory metabolomic analysis was incorporated into the doctoral project to assess postoperative metabolic and inflammatory changes associated with different pancreatic transection techniques. Peritoneal fluid samples were collected on postoperative day 3 and processed for metabolic phenotyping using mass spectrometry–based platforms. Metabolomic profiling was performed using a liquid chromatography–mass spectrometry

(LC–MS) approach, following a standardized analytical workflow previously applied to pancreatic and peripancreatic fluids.

This analysis was conceived as a translational and hypothesis-generating tool to explore metabolic patterns related to pancreatic healing and the development of clinically relevant postoperative pancreatic fistula. Metabolomic profiling enables the detection of small-molecule metabolites that reflect local and systemic biological responses to surgical injury, including inflammation, tissue damage, and repair processes. Differences in transection technique may induce distinct metabolic responses at the pancreatic remnant, which can be captured at a molecular level through metabolomic analysis.

In addition, metabolomic profiling provides an integrative readout of postoperative biological responses and allows the identification of metabolic pathways potentially involved in fistula development. By capturing both local and systemic metabolic changes associated with surgical stress and postoperative inflammation, this approach supports the investigation of postoperative complications beyond conventional clinical, biochemical, or radiological parameters.

Statistical analyses were performed according to predefined analysis plans. In the pilot retrospective study, propensity score matching was used to reduce selection bias inherent to the study design. In the randomized clinical trial, analyses were conducted primarily on an intention-to-treat basis.

Continuous variables were summarized as mean ± standard deviation (SD) or median (interquartile range), as appropriate, and categorical variables as frequencies and percentages. Normality of continuous variables was assessed using the Shapiro–Wilk test. Between-group comparisons were performed using Student's *t* test or Mann-Whitney U test for continuous variables, and the chi-square test or Fisher's exact test for categorical variables, as appropriate.

Univariate analyses were first conducted to explore associations between experimental variables and study outcomes. Variables showing statistical significance in univariate analyses were subsequently entered into multivariable models to adjust for potential confounders, including in the evaluation of CR-POPF. Logistic regression models were used when outcomes were binary; results are reported as odds ratios (ORs) with 95% confidence intervals (CIs). To account for potential confounders in the metabolomic

component, adjusted logistic regression models included age, sex, BMI, and DM status as covariates. Both standard logistic regression and Firth penalized regression were applied to reduce bias related to small sample size and rare events.

The discriminative performance of relevant models was assessed using receiver operating characteristic (ROC) curve analysis**.** All statistical tests were two-sided, and differences were considered statistically significant at $p < 0.05$.

Data were collected and managed using secure electronic databases, with regular monitoring and quality control procedures in accordance with Good Clinical Practice (GCP) standards and applicable data protection regulations.

OVERALL SUMMARY OF RESULTS

5 OVERALL SUMMARY OF RESULTS

This doctoral thesis investigated the efficacy and safety of RF-assisted pancreatic parenchymal transection as an alternative to conventional ST based closure during DP. The research combined clinical, radiological and translational approaches and was developed through an initial pilot experience followed by a prospective multicenter RCT. Following the promising pilot results, the RCT was initiated in 2021 with funding from the Fondo de Investigación Sanitaria (FIS) and endorsement by the Spanish Ministry of Science and Innovation (PI20/0008). To date, no RCTs specifically addressing this objective had been reported. Complementary analyses included the assessment of postoperative pancreatic morphology and exploratory metabolomic profiling.

In TRANSPAIRE study a total of 112 patients enrolled and underwent DP across eight participating centers between February 10th, 2021, and April 25th, 2025. Following 16 dropouts, 96 patients were included in the final analysis: 50 in the ST group and 46 in the RF group.

In the ST group, 8 patients were excluded prior to the intention-to-treat (ITT) analysis: 6 because a left-sided pancreatic resection was ultimately not performed and 2 due to the presence of distant metastases. Among the 50 patients included in the ITT analysis, 11 exhibited protocol deviations, including 9 crossovers to the RF group, resulting in a final cohort of 39 patients who underwent ST transection per protocol.

In the RF group, 8 patients were also excluded (7 patients did not undergo left-sided resection and 1 did not provide informed consent). Among the 46 patients included in the ITT analysis, 6 presented protocol deviations: 4 crossed over to the stapler group and in 2 cases the RF device was not available in the operating room. Consequently, 40 patients ultimately underwent RF-assisted transection per protocol. The entire study workflow, including patient enrollment, exclusions, group allocation, protocol deviations, and final per-protocol analysis, is summarized in the algorithm presented in figure 13.

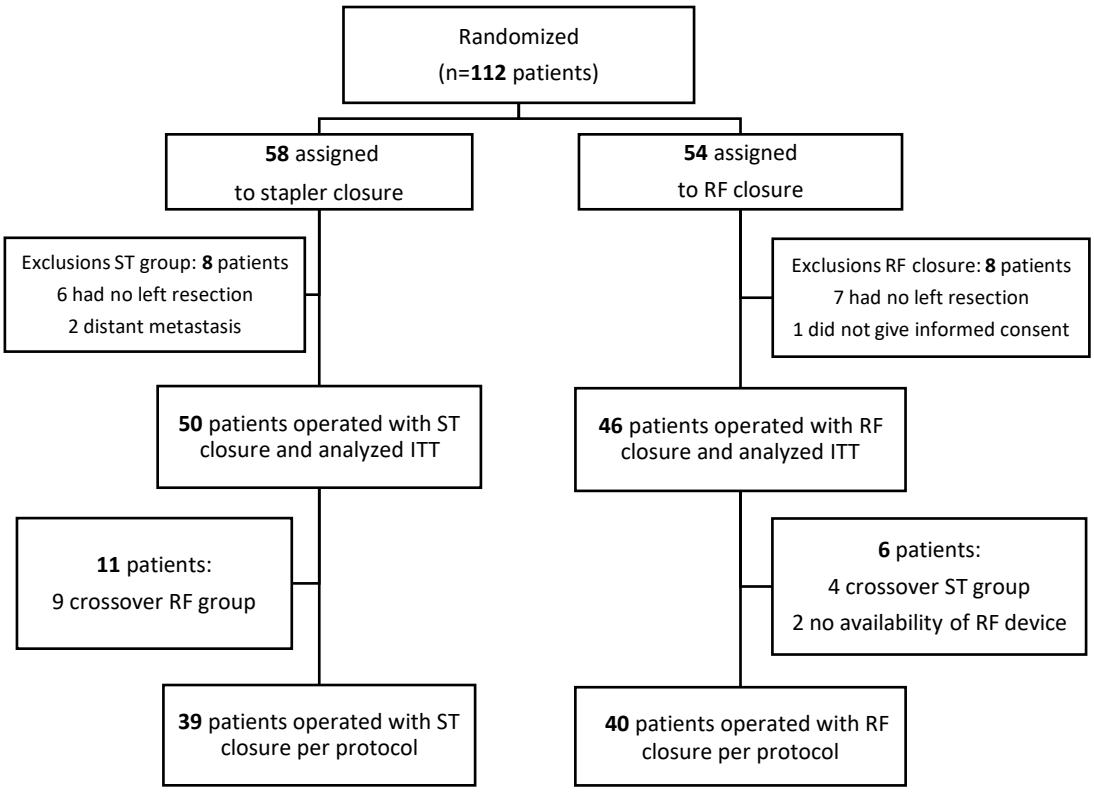


Figure 13. Flow chart of patient recruitment, randomization, and analysis in the multicenter TRANSPAIRE trial.

When the study reached its halfway point, an *interim analysis* was performed, yielding the following results. At the time of the analysis, 44 patients had completed the eCRF (electronic Case Report Form) correctly. The preliminary results indicated a higher rate of CR-POPF in the control group compared with the RF group (29.6% vs. 19.6%), as well as a higher reoperation rate in the control group (14.3% vs. 0%). Although statistical significance was not achieved, these findings suggested a clear trend toward reduced complications with RF-assisted transection compared with mechanical closure of the pancreatic stump. The independent data safety and monitoring board recommended continuation of patient recruitment up to the initially planned sample size.

Baseline demographic and clinical characteristics were well balanced between the two study groups (table 4). Median age, sex distribution, BMI and ASA classification did not differ significantly between groups. Most patients were classified as ASA II. The distribution of surgical approaches (open, laparoscopic, robotic) was also comparable. Tumor location, pancreatic duct diameter, and pancreatic texture were evenly distributed,

confirming the adequacy of randomization. Operative parameters, including surgical duration, intraoperative blood loss, and transfusion requirements did not differ significantly between the groups. The rate of concomitant splenectomy and the proportion of patients with neoplastic histology were comparable. These findings indicate that both groups were appropriately matched in demographic and surgical baseline variables.

Table 4. Baseline demographic and clinical characteristics of the study population

	RF (n=50)	**ST** (n=46)	p
Demographic characteristics			
Sex			0.097
Female (n, %)	25 (50%)	30 (65.2%)	
Male (n, %)	25 (50%)	16 (34.8%)	
Age (years), (mean, SD)	63.6 (4.7)	62.4 (5.3)	0.644
BMI (kg/m²), (mean, SD)	28.7 (2.1)	27.4 (2.4)	0.344
ASA			0.310
I, (n, %)	1 (2.0%)	2 (4.3%)	
II, (n, %)	27 (54.0%)	29 (63%)	
III, (n, %)	21 (42.0%)	15 (32.6%)	
IV, (n, %)	1 (2.0%)	0 (0%)	
DM (n, %)	15 (30%)	12 (26.1%)	0.913
Cardiovascular disease (n, %)	11 (22%)	15 (32.6%)	0.269
History of smoker			0.125
Former smoker (n, %)	9 (18%)	12 (26.1%)	
Current smoker (n, %)	9 (18%)	2 (4.3%)	
No smoker (n, %)	31 (62%)	32 (69.6%)	
Indication for operation			0.816
Malignant disease (n, %)	36 (72%)	30 (65.25)	
Benign tumors (n, %)	14 (28%)	16 (34.8%)	
Technical considerations			
Surgical Approach			0.461
Open (n, %)	7 (14%)	8 (17.4%)	
Laparoscopic (n, %)	25 (50%)	20 (43%)	
Robotic (n, %)	16 (32%)	18 (39.1%)	
Conversion (n, %)	2 (4%)	0 (0%)	
Type of surgery			0.188
Spleen preserving DP, (n, %)	12 (24%)	16 (34.8%)	
DP with splenectomy (n, %)	38 (76%)	30 (65.2%)	
Pancreatic specific features			
Adjusted pancreatic density (HU), (mean, SD)	0.58 (0.01)	0.56 (0.02)	0.810
P. thickness (cm), (mean, SD)	1.89 (0.54)	1.7 (0.35)	0.290
Pancreatic Duct, (n, %)			0.919
Dilated (3mm), (n, %)	9 (21.4%)	8 (20.5%)	
No dilated (<=3mm), (n, %)	33 (78.6%)	31 (79.5%)	

5.1 PRIMARY OUTCOMES RESULTS

The primary outcome of the multicenter TRANSPAIRE trial was the rate of CR-POPF according to the revised ISGPF classification. In the intention-to-treat analysis, the CR-POPF rate was significantly lower in the RF group than in the ST group (17.4% vs. 36.0%, OR 2.67, CI 95% 1.027- 6.953), p = 0.044). The results are summarized in table 5.

The distribution of PF grade shown in table 5 illustrates a reduction in grade B/C fistula in the RF group and highlights the occurrence of a 6% of CR-POPF grade C in the ST group compared to 0% in the study group.

Table 5. Univariate Analysis in primary endpoints (Intention-to-Treat analysis)

	RF (n=46)	**ST** (n=50)	OR (CI 95%)	p
CR_POPF	8 (17.4%)	18 (36.0%)	2.67 (1.027- 6.953)	**0.044**
Clinical grading				**0.042**
Biochemical leak (n, %)	12 (26.1%)	15 (30%)		
Grade B, (n, %)	8 (17.4%)	16 (32%)		
Grade C, (n, %)	0	3 (6%)		
Severity CR_POPF*				**0.037***
Clavien 0, (n, %)	35 (76.1%)	28 (56%)		
Clavien I, (n, %)	2 (4.34%)	3 (6%)		
Clavien II, (n, %)	1 (2.17%)	5 (10%)		
Clavien IIIa, (n, %)	8 (17.39%)	10 (20%)		
Clavien IIIb, (n, %)	0 (0%)	3 (6%)		
Clavien Iva, (n, %)	0 (0%)	1 (2%)		
Clavien IVb, (n, %)	0 (0%)	0 (0%)		
Clavien V, (n, %)	0 (0%)	0 (0%)		

* Clavien-Dindo classification
Date at number (% of group total), man (SD), odds ratio (OR 95% CI), or
*p value linear trend

Aligned with the previous findings, postoperative day-3 amylase levels were significantly higher in the ST group compared with the RF group (10.000- 12.000 vs 1000- 1500 U/L respectively; p= 0.04). Patients in the RF group demonstrated consistently low and homogeneous amylase values, whereas those in the stapler group exhibited markedly elevated levels with substantial variability, including peaks suggestive of pancreatic leakage as illustrated in figure 14.

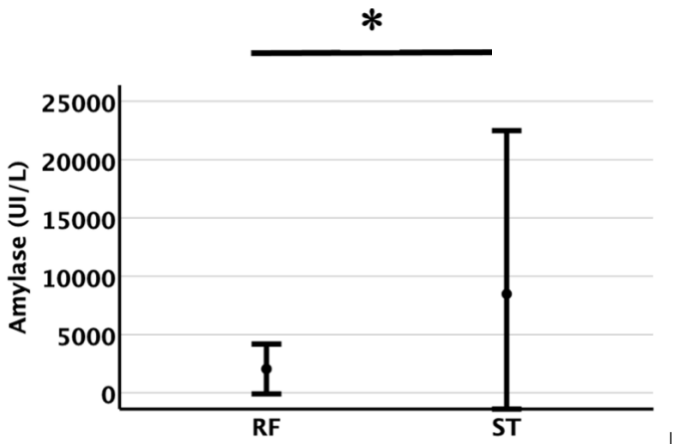


Figure 14. Comparison of postoperative day-3 drain amylase levels between RF and ST pancreatic transection techniques, showing a statistically significant difference (p = 0.04).

These results indicate a reduced efficacy of pancreatic secretion control in the ST cohort.

The occurrence of any postoperative pancreatic fistula (any POPF) was significantly associated with the pancreatic transection technique, showing a statistically significant linear trend (linear-by-linear association test, p = 0.037). The incidence of any POPF was higher in the stapler (ST) group (68%) compared with the RF group (43.48%).

Besides the group other predictors of the development of CR-POPF (B-C POPF) were analyzed, such as soft pancreatic consistency (expressed by the HU), preoperative CT small Wirsung diameter, and pancreatic thickness (in mm). Only pancreatic thickness out of them, demonstrated to be independent risk factors for CR-POPF and therefore, considered for the multivariate analyses (table 6).

Table 6. Univariable and multivariable predictors of CR-POPF. Study group and pancreatic thickness remained significant independent predictors

	OR	(95%CI)	p	β	OR (95%CI)	p
	UNIVARIABLE			**MULTIVARIABLE**		
Study Group	**2.67**	**(1.027-6.953)**	**0.044**	**1.76**	**5.82 (1.058- 31.97)**	**0.043**
Pancreatic Thickness	**10.02**	**1.96 – 51.2**	**0.006**	**2.05**	**7.73 (1.46-40.99)**	**0.016**
Pancreatic density (HU)	34.6	0.2 – 6091.2	0.179			
Age (years)	0.99	0.96 - 1.02	0.412			
Wirsung diameter (mm)	1.15	0.35 - 3.76	0.814			
BMI ((kg/m²)	1.07	0.98 – 1.18	0.136			
Histology (malignancy)	2.33	0.78 – 6.95	0.128			

These findings confirm that RF-assisted transection effectively reduced the incidence of CR-POPF compared with mechanical ST closure.

5.2 SECONDARY OUTCOMES RESULTS

Regarding secondary endpoints, PO morbidity during the first year of follow-up, including late complications and the development of endocrine and exocrine pancreatic insufficiency, was systematically evaluated and no statistically significant differences were observed between the two study groups (table 7). Overall PO morbidity at 90 days tended to be lower in the RF than in the ST group (21.7% vs. 38.0%), though not statistically significant ($p = 0.08$). However, these findings suggest that RF-assisted transection does not adversely affect pancreatic function when compared with ST-based closure.

Reintervention rates also showed a non-significant trend favoring the RF group (2.2% vs. 14.0%; $p = 0.068$). Readmission rates were similar between the two groups (32.6% vs. 28.0%; $p = 0.623$), as was the median length of hospital stay (10 vs. 13 days; $p = 0.179$) respectively.

No significant differences were detected in DGE (6.5% vs. 10%; $p = 0.538$) or severe PPH (grade C; 4.3% vs. 6%). Long-term outcomes were comparable between groups, including diabetes mellitus (DM) (23.9% vs. 24%; $p = 0.960$) and exocrine pancreatic insufficiency (15.2% vs. 20%; $p = 0.573$).

Collectively, these findings indicate that RF-assisted pancreatic transection was associated with lower overall morbidity and reintervention rates compared with ST closure. While statistical significance was not reached for all secondary endpoints, significant differences were observed in the most clinically relevant outcomes, supporting a consistent trend toward improved postoperative results without increasing the risk of complications or long-term pancreatic dysfunction.

No PO mortality was observed in either study group during the 90-day follow-up period. Specifically, there were no deaths in patients undergoing DP with RF-assisted pancreatic stump management nor in those treated with conventional ST closure.

Table 7. Multivariate Analysis in secondary endpoints

	RF (n=46)	**Stapler** (n=50)	OR (CI 95%)	p value
Secondary Endpoints				
Intraoperative				
Intraoperative transfusion, (mean, SD)	6 (13%)	6 (12%)	0.9 (0.271- 3.048)	0.877
Surgical approach: Splenectomy, (mean, SD)	31 (67.4%)	38 (76%)	1.53 (0.626- 3.750)	0.350
Operative time (min), (mean, SD)	286 (104)	288 (98)		0.472
Postoperative				
Overall morbidity (90d) *, (mean, SD)	10 (21.7%)	19 (38%)	2.2 (0.894- 5.448)	0.08
CCI (median)	8.7	20.9		
DGE, (n, %)	3 (6.5%)	5 (10%)	1.59 (0.359- 7.06)	0.538
PPH severe (grade C), (n, %)	2 (4.3%)	3 (6%)		0.561
Hospital stays (days), (n, %)	10 (7)	13 (12)		0.179
Readmission rate (n, %)	15 (32.6%)	14 (28%)	0.80 (0.336- 1.923)	0.623
Reintervention rate, (n, %)	1 (2.2%)	7 (14%)	7.33 (0.865- 62.5)	0.068
Mortality	0	0		
One-year outcomes				
DM, (n, %)	11 (23.9%)	12 (24%)	0.98 (0.381- 2.498)	0.960
Exocrine pancreatic Insufficiency, (n, %)	7 (15.2%)	10 (20%)	1.36 (0.469- 3.929)	0.573

*chi-square

5.2.1 DESIGN OF A MULTIVARIABLE MODEL FOR PREDICTING POPF

A multivariable logistic regression model was constructed to identify independent predictors of POPF. Model performance and goodness of fit were assessed using pseudo–R^2 statistics, classification accuracy, and receiver ROC curve analysis.

The Cox & Snell R^2 value was 0.259, and the Nagelkerke R^2 was 0.366, indicating that the model explained approximately 26- 36% of the variability in POPF risk. Given the clinical context and the inclusion of anatomical and technical variables derived from imaging, this represents a reasonably good explanatory performance.

Classification analysis showed that 88.2% of patients without POPF were correctly classified, whereas 53.3% of patients with POPF were correctly identified. The overall

classification accuracy of the model was 77.6%, indicating good global predictive capacity, particularly for ruling out POPF.

Two variables remained independently associated with POPF in the multivariable model: the pancreatic transection technique and pancreatic width measured at 1 cm from the transection line at the portal vein level. Use of the stapler technique (ST) was significantly associated with a higher risk of POPF compared with RF-assisted transection (B = 1.761, p = 0.043), corresponding to an odds ratio (OR) of 5.82. This finding indicates that stapler closure increased the odds of developing POPF by nearly sixfold compared with RF, independently of pancreatic morphology.

Pancreatic width**,** defined as the mean value of two measurements obtained at the transection level (as detailed in the Methodology section), was also identified as an independent predictor of POPF (B = 2.045, p = 0.016; OR = 7.73). Each 1 cm increase in pancreatic width was associated with an almost eightfold increase in the risk of POPF, highlighting the strong influence of pancreatic stump morphology on fistula development.

The discriminative performance of the multivariable model was further evaluated using ROC curve analysis. The model demonstrated good discriminative ability, with an area under the curve (AUC) of 0.818 (figure 15), indicating an adequate capacity to distinguish between patients who developed POPF and those who did not. An AUC of 0.818 suggests a favorable balance between sensitivity and specificity, supporting the ability of the multivariable model to identify patients at increased risk of POPF in the PO setting.

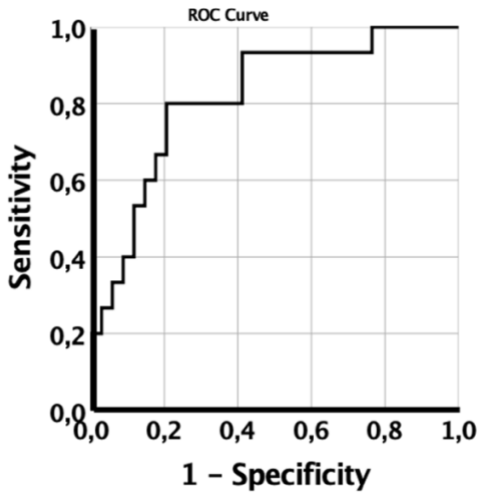


Figure 15. Receiver operating characteristic (ROC) curve illustrating the discriminative performance of the multivariable model for postoperative pancreatic fistula (POPF) prediction (AUC = 0.818).

5.2.2 RADIOLOGICAL EVALUATION

PO radiological evaluation of peripancreatic fluid collections allowed a systematic description of the morphological characteristics of the pancreatic stump and associated collections following pancreatic transection, according to both the technique used and the presence or absence of CR-POPF. The results of this analysis are presented comparatively in figure 16 and table 8.

The radiological protocol included contrast-enhanced imaging studies used for standardized measurement of several morphological parameters, including collection wall thickness, collection area, sphericity, diameter ratios, and adjusted density values of both the collection content and its wall, allowing an objective characterization of PO peripancreatic changes.

Postoperative imaging follow-up was available for approximately 60% of the total study population, with assessments performed at 1 and 6 months after surgery. Extended follow-up up to 12 months was available in a subset of 23 patients, enabling longitudinal documentation of pancreatic stump morphology during the first postoperative year.

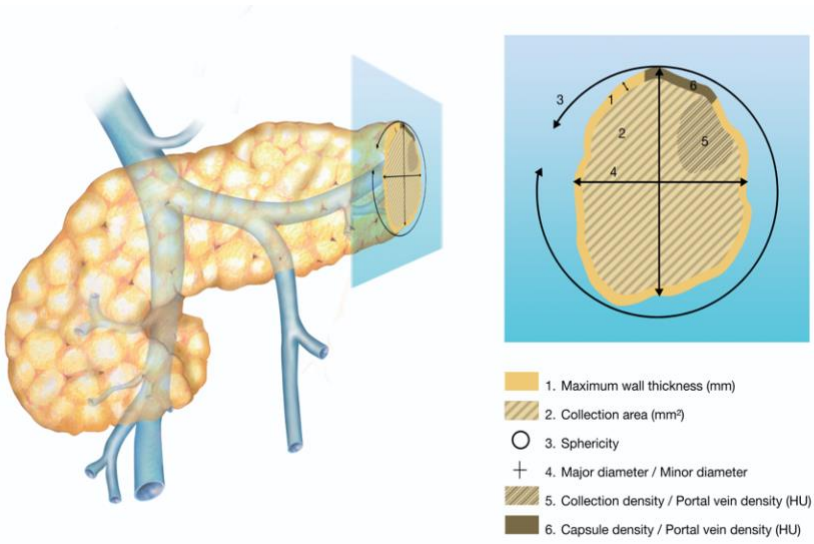


Figure 16. Schematic representation of radiological assessment of peripancreatic fluid collections. The illustration depicts the measurement of morphological parameters, including maximum wall thickness, collection area, sphericity, major-to-minor diameter ratio, collection density, and capsule density, obtained from postoperative imaging. Source: Original artwork by Julia Anaya commissioned for this doctoral thesis.

In continuation, a detailed comparative analysis of radiological parameters is presented between the RF-assisted transection and the ST group, focusing on the morphological characteristics of the pancreatic stump and the associated postoperative peripancreatic fluid collections. In addition, the same radiological variables are analyzed according to the presence or absence of CR-POPF, allowing evaluation of their potential association with fistula development.

Table 8. Radiological characteristics of peripancreatic fluid collections and pancreatic stump morphology according to transection technique (RF vs. ST) and the presence or absence of CR-POPF

	RF	**Stapler**	P*	**CR-POPF**		P*
				Yes	**No**	
1. Collection's wall thickness (cm), (mean, SD)	**0.28 (0.13)**	**0.21 (0.11)**	**0.033**	**0.19 (0.79)**	**0.25 (0.23)**	**0.045**
2. Fluid Collection Area (cm^2), (mean, SD)	15.45 (17.56)	21.13 (18,37)	0.299	**33.82 (21.9)**	**12.85 (12.27)**	**0.005**
3.Fluid's Sphericity (mean, SD)	**0.81 (0.14)**	**0.67 (0.16)**	**0.04**	**0.62 (0.12)**	**0.77 (0.16)**	**0.002**
4.Max. diameter/min diameter (mean, SD)	0.66 (0.26)	0.57 (0.26)	0,213	0,64 (0,23)	0.59 (0.27)	0.543
5.Density collection adjusted, HU (mean, SD)	22.67 (14.64)	28.9	0.32	23.31 (26.99)	27.67 (20.89)	0.566
6.Density collection's wall adjusted, HU, (mean, SD)	56.18 (25.24)	52.53 (28.99)	0.66	58.00 (29.7)	52.23 (26.53)	0.55
7. Perimeter of the fluid collection (mean/SD)	13.83 (9.64)	18.12 (9.79)	0.382	**13.08 (8.10)**	**24.64 (9.25)**	**0.001**

*t Student, mean (SD)

Collection's wall thickness

Mean wall thickness was significantly greater in fluid collections observed in the RF group compared with the ST group (0.28 ± 0.13 vs. 0.21 ± 0.11; p = 0.033) as shown in figure 17. When stratified by fistula status, collections associated with CR-POPF exhibited thinner walls than those observed in patients without CR-POPF (0.19 ± 0.79 vs. 0.25 ± 0.23; p = 0.045) as observed in figure 18.

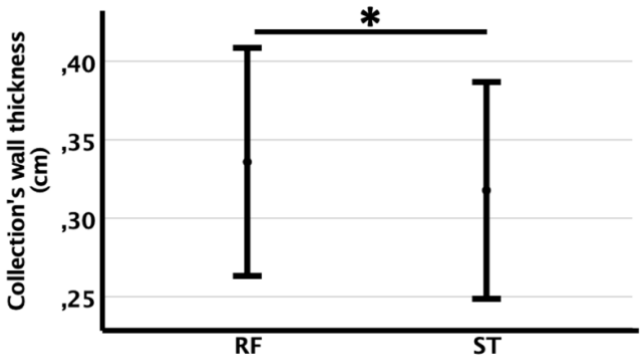


Figure 17. Maximum wall thickness of peripancreatic fluid collections according to transection technique. Collections in the RF group showed a significantly greater wall thickness compared with those in the stapler (ST) group. Data are presented as mean with 95% confidence intervals. ***p= 0.033**

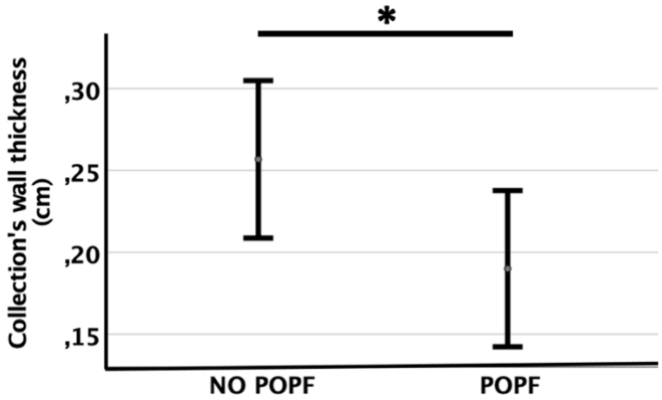


Figure 18. Collection wall thickness according to CR-POPF status. Comparison of peripancreatic fluid collection wall thickness between patients with and without CR-POPF. Data are presented as mean values with 95% confidence intervals. A statistically significant difference was observed between groups, with thinner collection walls in patients who developed CR-POPF. ***p = 0.045**

Peripancreatic Collection Area

No statistically significant difference in peripancreatic collection area was identified between RF (15.45 ± 17.56 mm²) vs. ST group (21.13 ± 18.37 respectively; p = 0.299) as shown in figure 19.

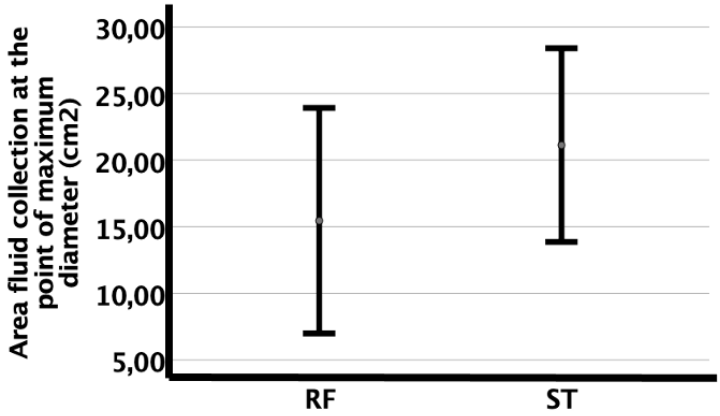


Figure 19. Peripancreatic fluid collection area measured at the level of maximum diameter according to transection technique. No statistically significant differences were observed between the radiofrequency (RF) and stapler (ST) groups. Data are presented as mean with 95% confidence intervals.

In contrast, collections in patients who developed CR-POPF were significantly larger than those in patients without CR-POPF (33.82 ± 21.90 vs. 12.85 ± 12.27 mm², respectively; p = 0.005) as observed in figure 20.

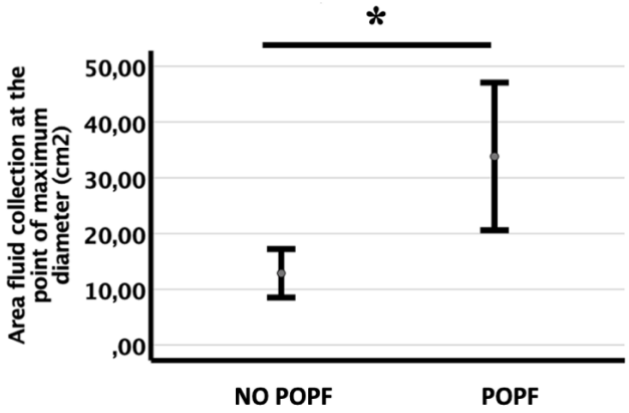


Figure 20. Maximum peripancreatic fluid collection area according to CR-POPF status**.** Comparison of the maximum area of peripancreatic fluid collections at the point of greatest diameter between patients with and without CR-POPF. Data are presented as mean values with 95% confidence intervals. Collections were significantly larger in patients who developed CR-POPF. ***p = 0.005**

Peripancreatic Collection Sphericity

Peripancreatic collections in the RF group demonstrated a significantly higher sphericity index compared with those in the ST (0.81 ± 0.14 vs. 0.67 ± 0.16; p = 0.040). Conversely, collections associated with CR-POPF were significantly less spherical than those observed in patients without CR-POPF (0.62 ± 0.12 vs. 0.77 ± 0.16; p = 0.002)(figure 21).

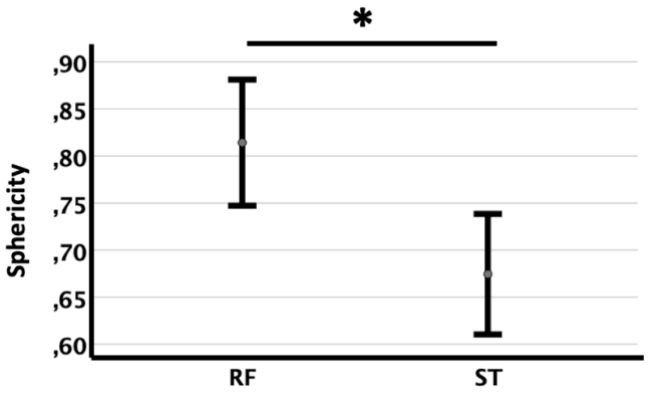


Figure 21. Sphericity of peripancreatic fluid collections according to transection technique. Collections in the radiofrequency (RF) group exhibited a significantly higher sphericity index compared with those in the stapler (ST) group. Data are presented as mean with 95% confidence intervals ***p=0.04**

Maximal-to-minimal diameter ratio

The ratio between maximal and minimal diameters did not differ significantly between the RF and ST groups (0.66 ± 0.26 vs. 0.57 ± 0.26; p = 0.213), nor when stratified according to the presence or absence of CR-POPF (0.64 ± 0.23 vs. 0.59 ± 0.27; p = 0.543) as illustrated in figure 22.

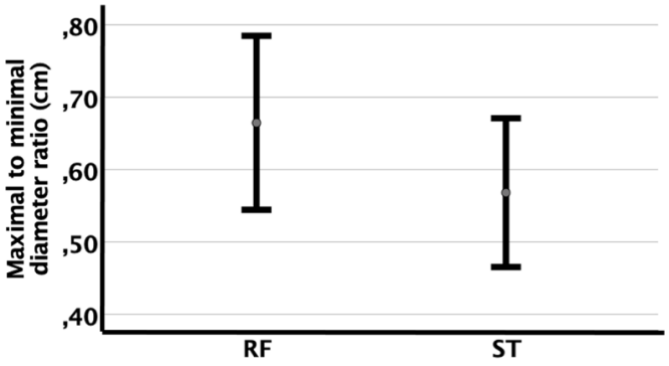


Figure 22. Shape descriptor of peripancreatic fluid collections expressed as the maximal-to-minimal diameter ratio according to transection technique. Collections in the radiofrequency (RF) group showed a geometry closer to circularity compared with those in the stapler (ST) group. Data are presented as mean with 95% confidence intervals.

Adjusted density of the fluid collection

No significant differences in adjusted radiological density of the fluid content were observed between transection techniques (0.13 ± 0.08 vs. 0.16 ± 0.13; p = 0.410) or according to CR-POPF status (0.14 ± 0.12 vs. 0.15 ± 0.10; p = 0.878) as depicted in figure 23.

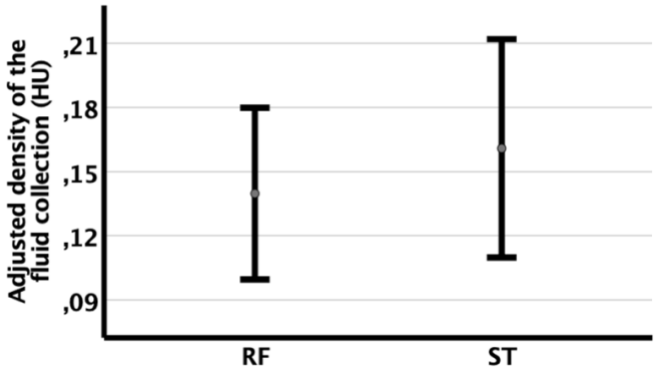


Figure 23. Adjusted radiological density of peripancreatic fluid collections according to transection technique. Density was expressed as the fluid-to-portal vein attenuation ratio (HU). No significant differences were observed between the radiofrequency (RF) and stapler (ST) groups. Data are presented as mean with 95% confidence intervals.

Adjusted density of the collection wall

Similarly, adjusted density of the collection wall did not differ significantly between the RF and ST groups (0.34 ± 0.15 vs. 0.32 ± 0.17; p = 0.687), nor between patients with and without CR-POPF (0.39 ± 0.16 vs. 0.29 ± 0.16; p = 0.878) as shown in figure 24.

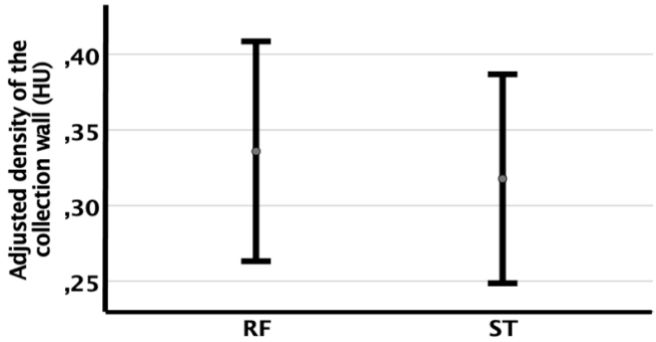


Figure 24. Adjusted radiological density of the peripancreatic collection wall according to transection technique. Wall density was expressed as the capsule-to–portal vein attenuation ratio (HU). No significant differences were observed between the radiofrequency (RF) and stapler (ST) groups. Data are presented as mean with 95% confidence intervals.

Circularity

Peripancreatic fluid collections associated with radiofrequency-assisted (RF) transection exhibited significantly higher circularity values compared with those observed after mechanical stapler (ST) closure.

This finding indicates that collections in the RF group tend to adopt a more regular and rounded morphology, whereas collections in the ST group show a more irregular or elongated configuration. The difference observed between both groups was statistically significant ($p = 0.0038$), suggesting that the pancreatic transection technique influences not only the occurrence of postoperative collections but also their radiological morphology.

From a pathophysiological perspective, increased circularity may reflect a more homogeneous and well-contained delineation of the collection, potentially associated with a more controlled tissue response at the pancreatic stump following the application of radiofrequency energy.

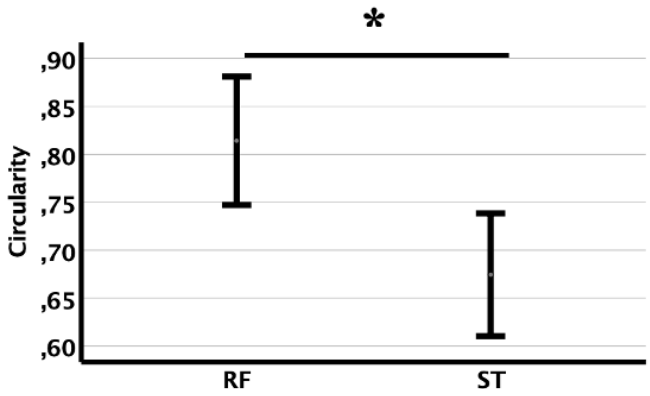


Figure 25. Circularity of peripancreatic fluid collections according to pancreatic transection technique. Comparison of circularity values between radiofrequency-assisted (RF) and stapler-based (ST) pancreatic transection. Data are shown as mean ± 95% confidence intervals. A statistically significant difference was observed between groups ***p = 0.0038.**

Perimeter of the fluid collection

No statistically significant differences in collection perimeter were observed between transection techniques, with mean values of 13.83 ± 9.64 in the RF group and 18.12 ± 9.79 in the ST group (p = 0.382) as observed in figure 26.

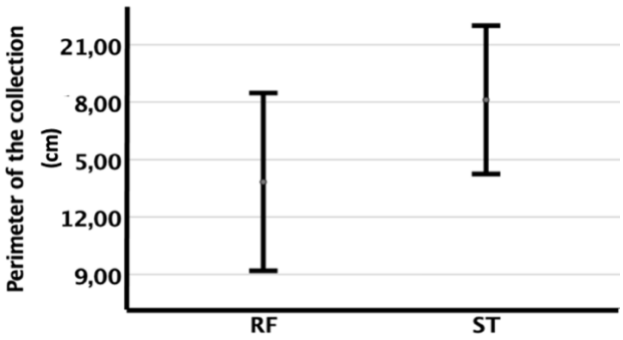


Figure 26. Perimeter of peripancreatic fluid collections according to pancreatic transection technique. Comparison of the peripancreatic fluid collection perimeter between radiofrequency-assisted (RF) and stapler-based (ST) pancreatic transection. Mean values with 95% confidence intervals are shown. No statistically significant differences were observed between groups

In contrast, when stratified according to clinically relevant postoperative pancreatic fistula (CR-POPF) status, patients who developed CR-POPF exhibited a significantly larger collection perimeter compared with those without CR-POPF (24.64 ± 9.25 vs. 13.08 ± 8.10, respectively; p = 0.001) as see in figure 27.

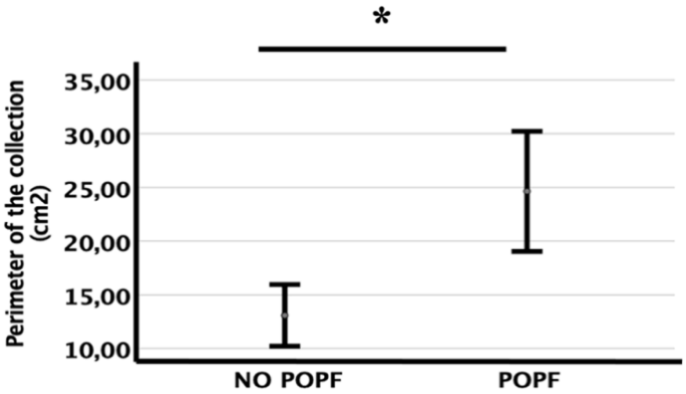


Figure 27. Peripancreatic fluid collection perimeter stratified by CR-POPF status. Mean collection perimeter with 95% confidence intervals in patients with and without clinically relevant postoperative pancreatic fistula (CR-POPF). A statistically significant difference was observed between groups. ***p = 0.001**

Evolution of the collections

To complement the quantitative radiological findings, a visual chronological comparison was performed to evaluate the postoperative evolution of the pancreatic stump and peripancreatic changes in both the RF and ST groups. This qualitative assessment was based on the sequential review of representative contrast-enhanced CT images obtained at 1, 6, and 12 months after surgery, which are illustrated in figures 28, 29 and 30.

At the 1-month evaluation, the RF-treated patient exhibited a more compact and regular pancreatic stump morphology, with limited peripancreatic fluid and well-defined margins. In contrast, the ST-treated patient demonstrated a larger and more heterogeneous peripancreatic collection, characterized by irregular contours and a less clearly delineated stump.

By the 6-month follow-up, patients in the RF group showed a marked reduction in peripancreatic inflammatory changes, accompanied by progressive normalization of stump morphology. Conversely, the ST group continued to exhibit residual peripancreatic fluid and persistent irregularity of the pancreatic stump, indicating a slower radiological recovery process.

At 12 months, the RF-treated stump appeared fully stabilized, with minimal or no residual inflammatory changes, whereas the ST-treated stump still demonstrated subtle but appreciable peripancreatic alterations

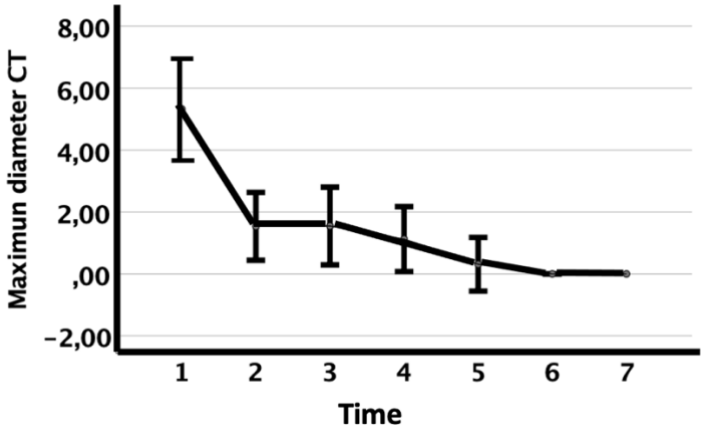


Figure 28. **Evolution of maximum diameter over time.** The graph illustrates the evolution of the maximum diameter measured at different follow-up time points. Data are presented as mean ± standard deviation. A progressive decrease in maximum diameter is observed over time, with a more pronounced reduction between the first and second time points, followed by a gradual decline in subsequent measurements, reaching values close to zero at the final assessments.

Overall, this longitudinal visual assessment is consistent with the quantitative radiological findings, which demonstrated smaller collection areas and perimeters and more favorable stump morphology in the RF group throughout the postoperative course.

Visual comparison of the chronological radiological progression in the radiofrequency versus stapler groups **CR-POPF**

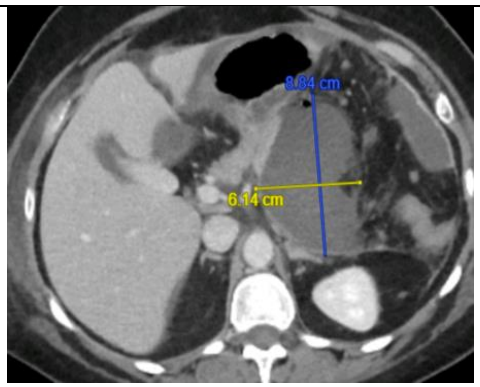
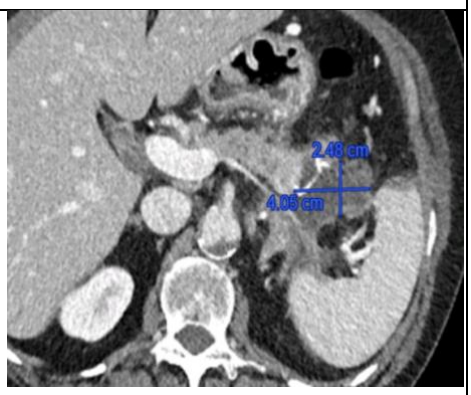
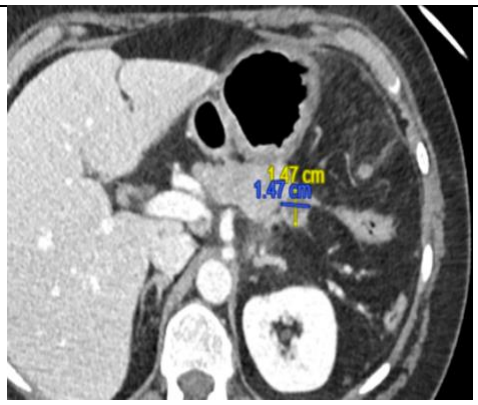
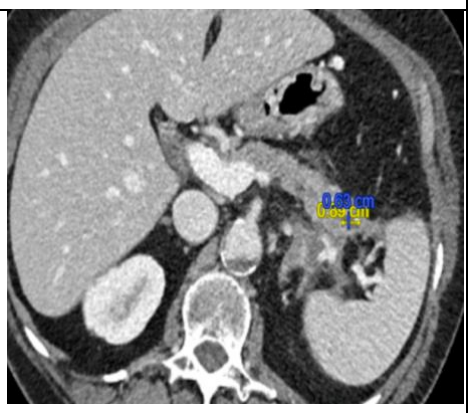
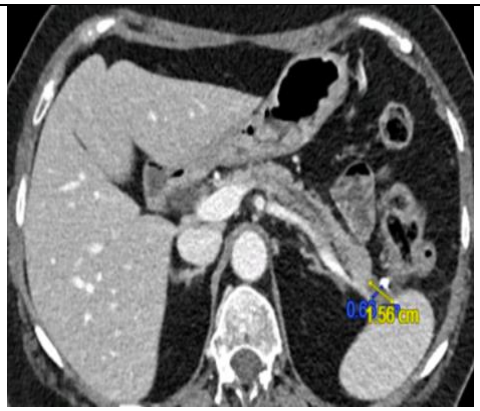
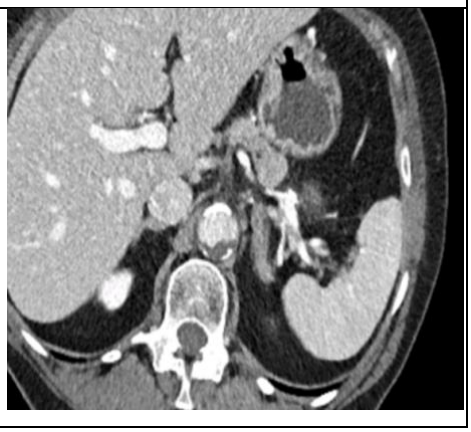
TAC	**RF**	**ST**
1st Month		
6th Month		
12th Month		

Figure 29. Chronological radiological comparison of the pancreatic stump and peripancreatic changes in patients treated with radiofrequency versus stapler transection at 1, 6, and 12 months postoperatively in CR-POPF.

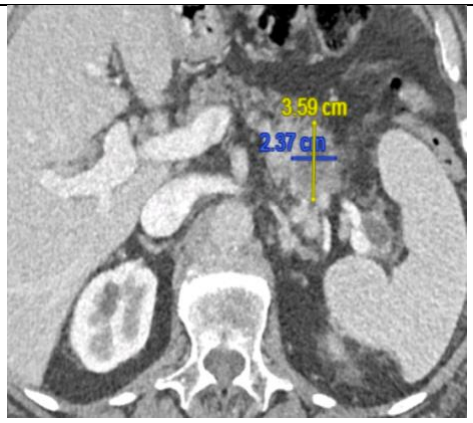
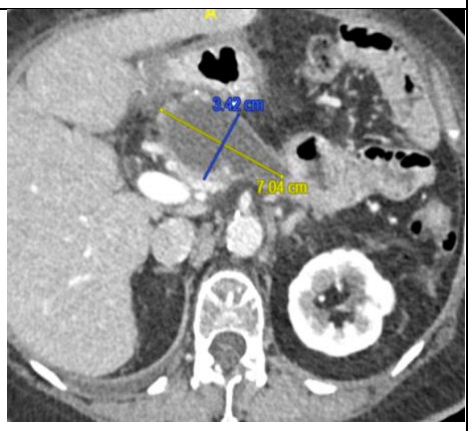
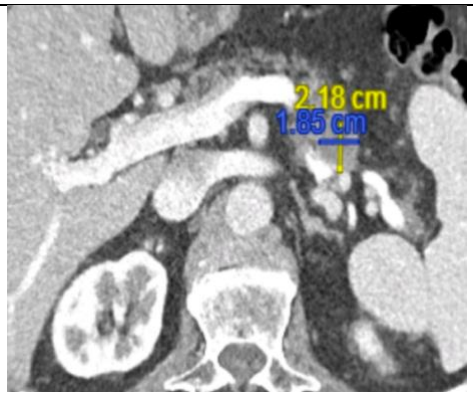
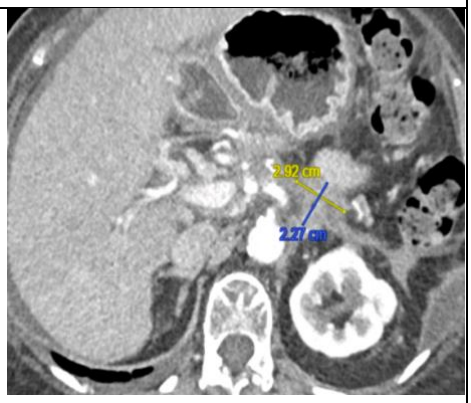
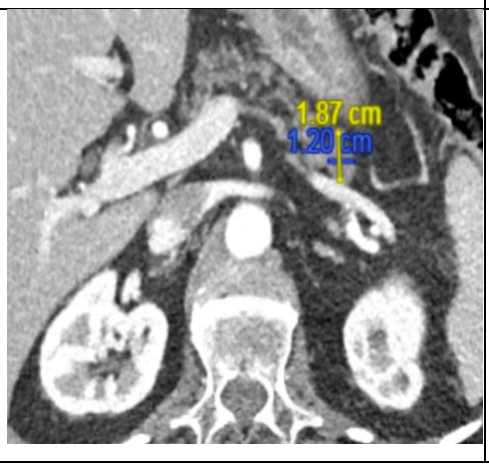
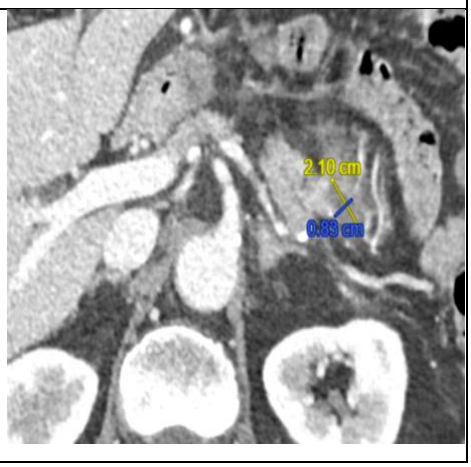
Visual comparison of the chronological radiological progression in the radiofrequency versus stapler groups **NO CR-POPF**		
TAC	**RF**	**ST**
1st Month		
6th Month		
12th Month		

Figure 30. Chronological radiological comparison of the pancreatic stump and peripancreatic changes in patients treated with radiofrequency versus stapler transection at 1, 6, and 12 months postoperatively in NO CR-POPF. Source: Authors owns clinical material

5.2.3 EXPLORATORY TRANSLATIONAL RESULTS: METABOLOMIC ANALYSIS

Patients in the ST transection group exhibited metabolic patterns compatible with a higher degree of systemic inflammatory response and tissue disruption. In contrast, RF-assisted transection was associated with a metabolic profile suggestive of a more controlled biological response to pancreatic injury. These findings are consistent with the known biological effects of RF energy, which induces localized coagulative necrosis and fibrosis at the transection margin, potentially limiting pancreatic juice leakage and secondary inflammatory activation.

An exploratory metabolomic analysis was performed to investigate potential metabolic patterns associated with pancreatic transection technique and the development of CR-POPF. This analysis was conceived as a hypothesis-generating component of the doctoral project and was conducted independently of the published clinical outcomes.

Metabolomic profiling was performed on peritoneal fluid samples collected on PO day 3. After data inspection and curation, only true missing values were retained as missing, while values below the limit of detection were imputed using half of the corresponding detection limit when appropriate. Sixteen initially available metabolite ratios were excluded due to redundancy, and ceramide and hexosylceramide ratios with overlapping information were removed to avoid collinearity. These steps were performed to ensure data robustness and to minimize spurious associations derived from technical or redundant variables. Visual inspection of individual metabolite distributions suggested subtle differences between transection techniques (figure 31) with an exploratory, hypothesis-generating analysis revealing a rightward shift in the distribution of peritoneal fluid 3-hydroxykynurenine levels in patients undergoing RF-assisted pancreatic transection compared with ST-based transection, despite substantial overlap between groups.

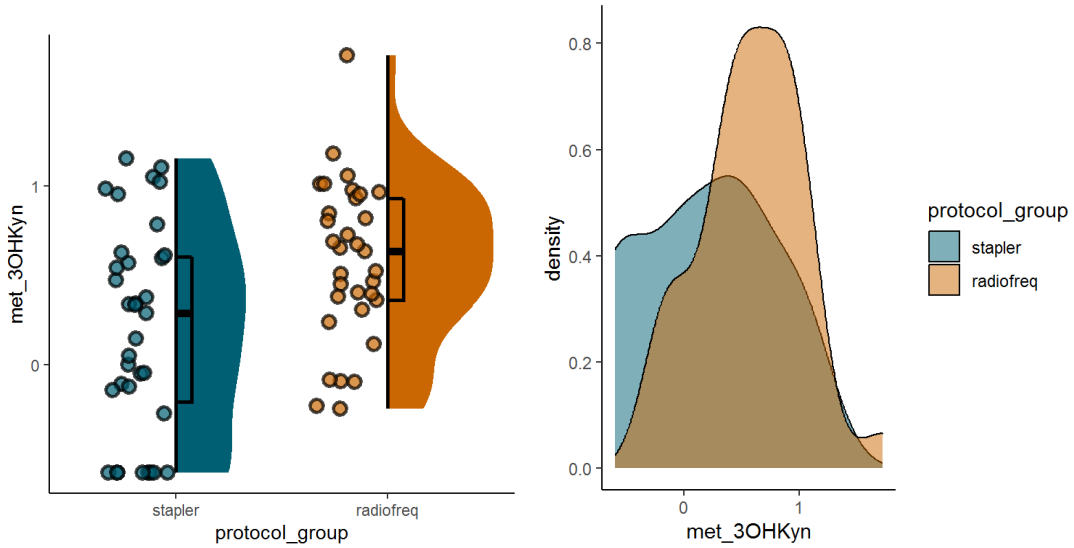


Figure 31. Exploratory visualization of peritoneal fluid 3-hydroxykynurenine levels according to pancreatic transection technique. Raincloud plots (left) display individual values, median, interquartile range, and density distribution, while kernel density curves (right) illustrate group-level distributional shifts. Although considerable overlap is observed, visual inspection suggests a rightward displacement of the distribution in the radiofrequency group compared with the stapler group.

Initial exploratory analyses included raincloud and density plots to visualize metabolite distributions according to treatment group and CR-POPF status. Exploratory analyses identified several metabolites showing nominal associations with CR-POPF, predominantly involving the tryptophan–kynurenine pathway. Specifically, 3-hydroxykynurenine levels, as well as the 3-hydroxykynurenine-to-kynurenine and 3-hydroxykynurenine-to-tryptophan ratios, were significantly associated with CR-POPF status in unadjusted analyses. In addition, a ceramide-related metabolite (ceramide d18:1/18:1) showed a nominal association with CR-POPF, suggesting concurrent alterations in sphingolipid metabolism.

Given its central position within the kynurenine pathway and its biological relevance, 3-hydroxykynurenine was selected for illustrative purposes (figure 32) therefore depicts the exploratory distribution of peritoneal fluid 3-hydroxykynurenine levels according to CR-POPF status, highlighting a shift in the distribution despite substantial overlap between groups.

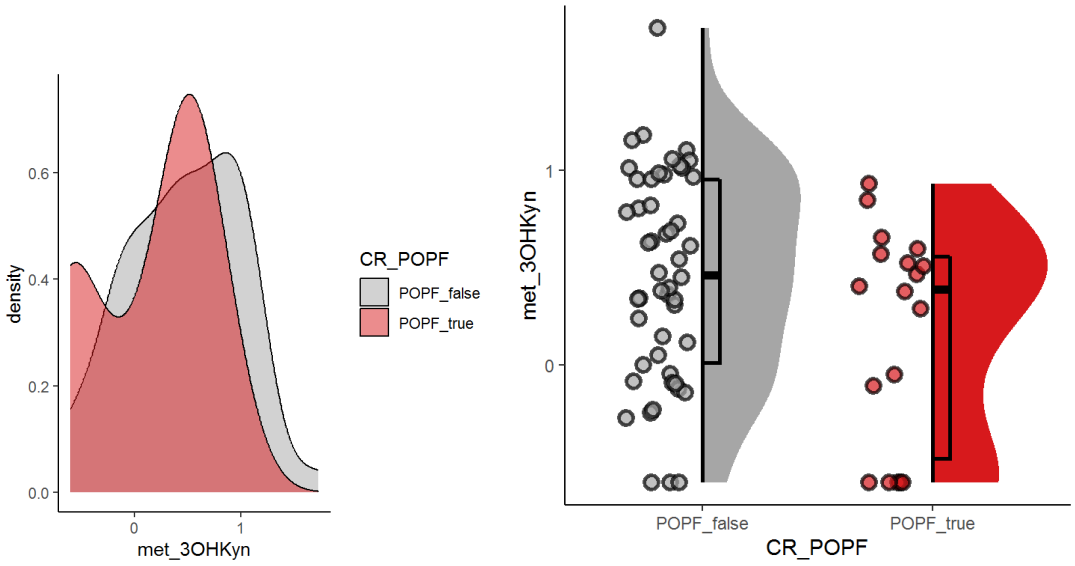


Figure 32. Exploratory visualization of peritoneal fluid 3-hydroxykynurenine levels according to clinically relevant postoperative pancreatic fistula (CR-POPF) status. Raincloud plots (left) display individual values, median, interquartile range, and distribution shape, while kernel density plots (right) illustrate group-level distributional patterns. Visual inspection suggests a shift in the distribution of 3-hydroxykynurenine levels in patients with CR-POPF compared with those without CR-POPF, with substantial overlap between groups.

Non-parametric Mann–Whitney U tests were used to compare metabolite levels between groups, with log10 transformation applied to all metabolomic variables. Several metabolites and metabolite ratios showed nominal associations with CR-POPF, particularly within amino acid metabolism and sphingolipid pathways. Specifically, metabolites related to the tryptophan–kynurenine pathway, including 3-hydroxykynurenine (p=0.008), the 3-hydroxykynurenine-to-kynurenine ratio (p=0.003), and the 3-hydroxykynurenine-to-tryptophan ratio (p = 0.006), showed significant associations with CR-POPF in unadjusted analyses. A ceramide-related metabolite (ceramide d18:1/18:1) was also nominally associated with CR-POPF (p=0.019). After adjustment for multiple testing using false discovery rate correction, a subset of metabolites, mainly kynurenine–tryptophan pathway ratios and 18-carbon ceramides, remained significantly associated with CR-POPF.

The metabolomic analysis revealed significant alterations involving amino acid metabolism and sphingolipid pathways. In addition, metabolites related to the kynurenine pathway, including kynurenine, kynurenic acid, and 3-hydroxykynurenine, were markedly altered, along with multiple metabolite ratios reflecting shifts in tryptophan catabolism (e.g., kynurenine/tryptophan, 3-hydroxykynurenine/kynurenine).

Overall, these findings indicate a coordinated metabolic response characterized by disturbances in amino acid turnover, particularly involving branched-chain amino acids and the tryptophan–kynurenine pathway, and concomitant alterations in ceramide metabolism, pointing to a broader impact on energy, inflammatory, and lipid signaling pathways. Several downstream kynurenine metabolites, including kynurenic acid and 3-hydroxykynurenine, exert immunomodulatory effects. While kynurenic acid has been associated with anti-inflammatory and cytoprotective properties, 3-hydroxykynurenine is a pro-oxidant metabolite capable of promoting oxidative stress and tissue injury. The imbalance between these metabolites may therefore reflect a shift toward a pro-inflammatory and oxidative microenvironment.

Consequently, the observed perturbations in kynurenine pathway metabolites are consistent with an inflammatory metabolic phenotype, supporting the notion that activation of tryptophan catabolism through the kynurenine pathway represents a key link between metabolic reprogramming and inflammation.

Adjusted and multivariable analyses

After adjustment for relevant clinical covariates (age, sex, body mass index, and diabetes mellitus), the association between CR-POPF and metabolites related to the kynurenine pathway, including 3-hydroxykynurenine and related metabolic ratios, as well as sphingolipid metabolism, remained detectable. Although these associations persisted in adjusted analyses, the observed effect sizes were modest and confidence intervals wide, reflecting limited precision and underscoring the exploratory nature of these findings.

These adjusted analyses were performed to ensure that the observed associations were not solely explained by baseline patient characteristics and therefore could reflect postoperative biological differences.

Multivariate analysis

Variance distribution across principal components was assessed to support dimensionality reduction prior to supervised multivariate analysis.

Prior to supervised multivariate modeling, variance distribution across principal components was examined to assess the suitability of dimensionality reduction in the metabolomic dataset. The first two principal components accounted for approximately

46% of the total variance, supporting their use for subsequent exploratory and supervised analyses.

An unsupervised principal component analysis (PCA) was then performed to explore the intrinsic structure of the postoperative peritoneal fluid metabolomic data without imposing any group labels (figure 33). Although the first two principal components captured a substantial proportion of the overall metabolic variability, unsupervised PCA did not reveal a clear spontaneous separation between pancreatic transection techniques, with considerable overlap between groups. This finding suggests that global metabolic differences between transection techniques are subtle and not driven by dominant variance components.

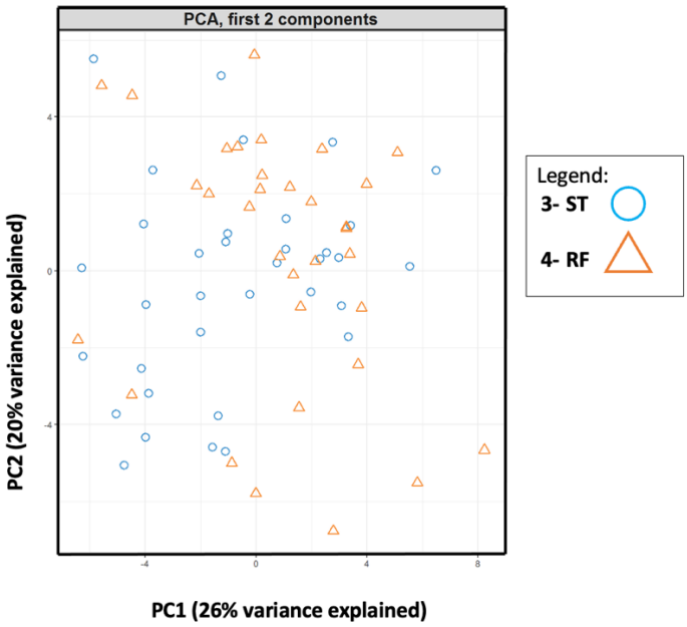


Figure 33. Unsupervised principal component analysis (PCA) according to pancreatic transection technique. Score plot of the first two principal components (PC1 and PC2) derived from unsupervised PCA of postoperative peritoneal fluid metabolomic profiles, stratified by pancreatic transection technique (stapler vs radiofrequency). PC1 and PC2 explain 26% and 20% of the total variance, respectively.

Subsequently, a supervised sparse partial least squares discriminant analysis (sPLS-DA) was applied, integrating metabolomic profiles and clinical group information to identify multivariate patterns associated with pancreatic transection technique and CR-POPF status (figure 34). This supervised approach revealed partial group discrimination, indicating the presence of subtle but coordinated metabolic differences between groups.

However, substantial overlap persisted, underscoring the limited discriminative performance of the model and reinforcing its exploratory, hypothesis-generating nature.

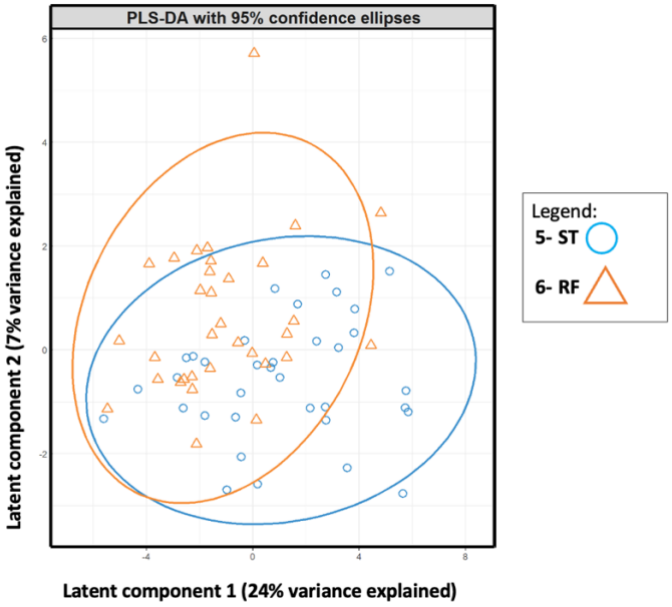


Figure 34. Supervised sparse partial least squares discriminant analysis (sPLS-DA) score plot. Score plot of the first two latent components obtained from sparse partial least squares discriminant analysis (sPLS-DA) of postoperative peritoneal fluid metabolomic data, stratified by pancreatic transection technique. The first two components explain 24% and 7% of the variance, respectively. The supervised model shows partial separation between groups; however, considerable overlap persists, consistent with limited discriminative performance and the exploratory nature of the analysis.

The optimal sPLS-DA model retained two latent components, and a limited set of variables selected through repeated cross-validation. Metabolites with the highest stability across validation folds included compounds related to the tryptophan–kynurenine pathway (notably 3-hydroxykynurenine and related ratios), selected ceramides, as well as tyrosine and nicotinamide. These metabolites overlap with those identified in univariate analyses, supporting their potential biological relevance despite the absence of strong multivariate separation.

Overall, this exploratory multivariate metabolomic analysis suggests that postoperative inflammatory and metabolic responses, particularly involving amino acid and sphingolipid metabolism, may differ according to pancreatic healing and the development of CR-POPF). Patients undergoing stapler-based transection exhibited metabolic patterns compatible with a higher degree of systemic inflammatory response and tissue disruption, whereas radiofrequency-assisted transection was associated with a metabolic profile suggestive of a more controlled biological response to pancreatic injury. These

observations are consistent with the known biological effects of radiofrequency energy, which induces localized coagulative necrosis and fibrosis at the transection margin, potentially limiting pancreatic juice leakage and secondary inflammatory activation.

These findings should be interpreted with caution and do not establish causal relationships. Rather, they provide preliminary biological insight into metabolic processes associated with pancreatic stump healing and serve to generate hypotheses for future studies with larger cohorts and external validation.

OVERALL SUMMARY OF THE DISCUSSION

6 OVERALL SUMMARY OF THE DISCUSSION

To our knowledge, this thesis presents the first comprehensive evaluation of RF-assisted pancreatic transection compared with conventional ST closure, combining results from an initial pilot study and a subsequent multicenter RCT (TRANSPAIRE). Together, these studies provide consistent evidence supporting the efficacy and safety of RF energy as an alternative for pancreatic stump closure after DP, with the potential to significantly reduce the incidence of CR-POPF and improve postoperative outcomes.

The pilot study, designed using a PSM methodology, demonstrated a marked reduction in the incidence of CR-POPF in the RF group compared with the ST group, both before and after adjustment. These preliminary findings suggested that the use of RF energy for pancreatic transection was safe and feasible, with encouraging short-term outcomes. The multicenter TRANSPAIRE trial later confirmed these observations under a randomized design across eight Spanish centers, revealing a significant decrease in CR-POPF rates in the RF group (17.4%) compared with the ST group (36.0%; $p = 0.04$) (see Table 5).

Beyond the reduction in CR-POPF, RF-assisted pancreatic transection was associated with a lower severity of postoperative complications, as assessed by the Clavien–Dindo classification. Together, the decrease in CR-POPF and the reduced complication burden constitute the most CR findings of this thesis and highlight the impact of RF-assisted transection on overall PO morbidity.

This consistent reduction in CR-POPF across studies reinforces the hypothesis that RF energy induces a controlled coagulative effect on the transection plane, producing localized necrosis and subsequent fibrosis that act as a mechanical and biological barrier to pancreatic juice leakage. Histological findings from preclinical models by Burdío et al. demonstrated a distinctive pattern of coagulative necrosis surrounded by a dense fibrotic capsule up to 1.8 mm thick, supporting the concept of a "biological seal" effect unique to RF-based transection. This mechanism contrasts with the thin and often discontinuous fibrotic line generated by mechanical staplers, which may fail to provide adequate closure in soft pancreatic tissue.

A key contribution of this thesis is the detailed radiological characterization of the pancreatic stump and the peripancreatic inflammatory response following transection, an aspect seldom explored in previous clinical trials. Quantitative imaging analysis revealed

meaningful structural differences that help explain the mechanisms underlying POPF formation and the protective effect of RF-assisted transection.

Radiofrequency-assisted pancreatic transection resulted in a more regular and rounded pancreatic stump compared with stapler closure. This was reflected by significantly higher fluid collection sphericity in the RF group (0.81 ± 0.14 vs. 0.67 ± 0.16; p = 0.04), together with a greater collection wall thickness at the transection margin (0.28 ± 0.13 cm vs. 0.21 ± 0.11 cm; p = 0.033). These radiological features are compatible with deeper protein denaturation, collagen contraction, and early formation of an organized connective barrier induced by radiofrequency energy. This imaging profile is consistent with the expected biological response to RF and provides a mechanistic explanation for the improved stump stability and the reduced incidence and severity of postoperative complications observed clinically.

In contrast, stapler closure resulted in a more irregular and elongated stump, lacking the biological reinforcement induced by thermal coagulation. This irregularity may create points of structural weakness that predispose to microleaks, particularly in soft pancreatic glands. This observation also highlights a more fundamental limitation of stapler-based pancreatic transection. Mechanical staplers were originally conceived and engineered for the closure and division of hollow viscera, such as the gastrointestinal tract, where tissue compression and linear staple formation are well suited to the biomechanical properties of the bowel wall. In contrast, the pancreas is a solid, highly vascularized, and enzymatically active gland with heterogeneous texture and a complex ductal architecture. Applying a device designed for bowel tissue to pancreatic parenchyma represents a conceptual compromise rather than a tissue-specific solution. The lack of biological adaptation to pancreatic tissue characteristics may contribute to the irregular stump morphology observed after stapler closure and help explain the persistently high rates of POPF reported with this technique.

Across the cohort, patients who developed POPF showed wider, denser, and structurally thinner stumps, consistent with acute edema, inflammatory infiltration, and early tissue degradation triggered by enzymatic leakage. Higher HU values in these patients likely reflect increased inflammatory cellularity and interstitial fluid accumulation. These findings support the concept that POPF is not only a biochemical phenomenon but also a radiologically quantifiable structural failure of the pancreatic stump.

In line with these findings, the RF group showed lower and more homogeneous drain amylase levels on postoperative day 3, whereas the stapler group exhibited markedly higher values with greater variability, including peaks compatible with pancreatic leakage, suggesting less effective control of pancreatic secretion following mechanical stump closure.

The multivariable model developed in this study showed good discriminative performance for predicting postoperative pancreatic fistula (POPF), with an AUC of 0.818, supporting the relevance of combining technical and anatomical variables for postoperative risk stratification. Although not intended as a standalone predictive tool, the model demonstrated adequate internal performance within the clinical context of DP.

Both the pancreatic transection technique and pancreatic stump morphology were independently associated with POPF. ST closure was linked to a significantly higher risk of POPF compared with radiofrequency-assisted transection, even after adjustment for pancreatic width, suggesting a protective effect of RF beyond anatomical factors. Increased pancreatic width at 1 cm from the transection line also emerged as a strong predictor of POPF, likely reflecting greater parenchymal reactivity and susceptibility to leakage.

Overall, these findings support a multifactorial pathophysiological mechanism underlying POPF development and highlight the potential value of integrating surgical technique and pancreatic morphology in risk assessment after DP.

Peripancreatic collections exhibited the most striking differences. Patients with POPF developed significantly larger and more extensive collections, with increases in both area and perimeter, radiological manifestations of widespread enzymatic inflammation and autodigestive injury. The strong correlation between collection size and POPF severity highlights the utility of CT imaging as an objective biomarker for early identification and monitoring of postoperative pancreatic complications.

Although the differences in collection dimensions between RF and ST groups did not reach statistical significance, a consistent trend toward smaller volumes and perimeters in RF-treated patients was observed, supporting the hypothesis that RF reduces the initial leakage of pancreatic secretions and limits the inflammatory cascade.

Longitudinal evaluation further reinforced these findings. RF-treated stumps exhibited faster radiological recovery, with earlier resolution of inflammatory changes and progressive morphological normalization at 1, 6, and 12 months. In contrast, stapler-treated stumps showed slower regression of edema and more persistent irregularity, suggesting delayed or incomplete biological healing. This temporal pattern aligns with the histological evidence of progressive collagen deposition and maturation following RF energy application.

The qualitative chronological radiological comparison further reinforces the interpretation of the quantitative imaging findings and provides insight into the temporal dynamics of pancreatic stump healing. Visual assessment of representative contrast-enhanced CT images obtained at 1, 6, and 12 months postoperatively revealed clearly divergent radiological trajectories between radiofrequency- and stapler-treated pancreatic stumps, both in patients with and without CR-POPF.

In patients who developed CR-POPF, RF-treated stumps demonstrated a more compact and regular morphology as early as the first postoperative month, with relatively well-defined margins and limited peripancreatic extension. Over time, these collections showed a progressive reduction in size and improved structural organization, with substantial resolution by 6 months and near-complete stabilization at 12 months. In contrast, stapler-treated stumps in the presence of CR-POPF were characterized by larger, more irregular peripancreatic collections at early follow-up, with slower regression and persistent morphological heterogeneity at later time points, suggesting prolonged inflammatory activity and delayed tissue remodeling.

A similar, though less pronounced, pattern was observed in patients without CR-POPF. In this subgroup, RF-treated stumps displayed faster radiological normalization, with earlier reduction of peripancreatic fluid and progressive restoration of a smooth, stable stump contour. Stapler-treated stumps, while not associated with CR-POPF, nonetheless exhibited more persistent peripancreatic changes and delayed morphological stabilization over time.

These longitudinal imaging patterns are fully consistent with the quantitative findings, which demonstrated thicker collection walls, higher sphericity, and smaller collection areas in RF-treated patients, as well as larger, thinner-walled, and less spherical collections in patients who developed CR-POPF. Together, these data suggest that RF-assisted

transection promotes a more rapid transition from acute postoperative inflammation to organized fibrosis, resulting in a mechanically and biologically more stable pancreatic stump.

Importantly, the concordance between visual chronological assessment and quantitative radiological metrics supports the reliability of CT-derived morphological parameters as meaningful surrogates of stump healing quality. This integrated radiological approach underscores the concept that pancreatic fistula formation represents not only a biochemical leak but also a temporally evolving structural failure that can be objectively monitored through imaging.

Collectively, the radiological findings not only corroborate the clinical reduction in CR-POPF but also provide mechanistic insight into the biological sealing effect of RF. They also illustrate how early CT-derived markers, such as stump thickness, circularity, density, and collection perimeter, may serve as objective, quantifiable predictors of fistula risk. This integration of imaging biomarkers, clinical outcomes, and physiological mechanisms represents a novel contribution of the TRANSPAIRE study.

The inflammatory response underlying this process should be interpreted as a double-edged sword. While sustained or excessive inflammation may be detrimental and potentially pro-oncogenic, an acute and self-limited inflammatory response appears to be a necessary driver of effective fibrotic sealing and tissue repair.

In this regard, experimental evidence from preclinical models provides important complementary insights. In a porcine model, RF-assisted pancreatic transection induced an early postoperative increase in inflammatory markers, including interleukin-6 (IL-6), followed by a clear normalization at one month postoperatively. This temporal profile suggests that RF energy triggers a controlled and transient inflammatory response rather than a persistent proinflammatory state.

In parallel with these structural and inflammatory findings, an exploratory metabolomic analysis of postoperative peritoneal fluid provided additional biological context to the observed differences in pancreatic healing. Specifically, patients who developed CR-POPF showed distinct metabolic profiles characterized by alterations in amino acid metabolism and sphingolipid pathways, consistent with enhanced inflammatory activation and tissue injury, whereas patients without CR-POPF exhibited more homogeneous

metabolic patterns. Although hypothesis-generating in nature, these findings support a biological link between postoperative metabolic responses and fistula development.

Metabolites related to the kynurenine–tryptophan pathway and selected ceramides remained associated with CR-POPF after adjustment for baseline clinical variables, supporting the concept that fistula formation is accompanied by specific inflammatory and metabolic responses beyond purely mechanical failure of the stump. These pathways have been previously linked to immune activation, oxidative stress, and tissue injury, processes that are biologically plausible contributors to impaired pancreatic healing.

Importantly, multivariate analyses did not reveal a clear spontaneous separation according to transection technique, but supervised models demonstrated partial discrimination between patients with and without CR-POPF. This pattern is consistent with subtle, group-level biological differences rather than a deterministic metabolic profile and aligns with the concept of RF-induced modulation of the postoperative inflammatory milieu rather than complete metabolic reprogramming.

Taken together, the metabolomic findings complement the radiological and clinical data, indicating that RF-assisted transection is associated with a more homogeneous postoperative metabolic profile, characterized by lower variability and fewer extreme inflammatory-related metabolic patterns compared with stapler closure. This metabolic homogeneity is consistent with the more uniform radiological characteristics of the pancreatic stump and the lower severity of postoperative complications observed in the RF group. Although these results do not establish causal relationships and should be interpreted with caution due to the exploratory design and limited sample size, they provide preliminary biological insight into postoperative pancreatic healing and generate hypotheses for future translational studies integrating metabolic, imaging, and clinical biomarkers.

Two retrospective clinical series have suggested favorable outcomes with RF-assisted pancreatic transection. Fronza et al. reported an initial experience using RF energy (Habib 4×) for pancreatic stump closure, with a CR-POPF rate of approximately 14.3% and no associated mortality, while Blansfield et al. described a reduction in POPF incidence to around 10% using the TissueLink device compared with standard techniques. However, the absolute POPF rates reported in these studies were lower than those observed in the present trial in both treatment groups. This difference is likely explained by variations in

pancreatic transection level and baseline anatomical risk, as more proximal transection planes have consistently been associated with a higher risk of POPF due to increased pancreatic thickness, higher exocrine output, and more complex ductal anatomy, as previously reported in the literature (Bassi et al., Kawai et al., Frozanpor et al.). In contrast, many earlier series predominantly included more distal transection sites, which are known to be associated with a lower intrinsic fistula risk. Within this context, the results of the TRANSPAIRE randomized trial are particularly relevant, as they demonstrate a significant reduction in CR-POPF with RF-assisted transection despite a higher-risk anatomical setting, thereby reinforcing the clinical value of the technique.

Conversely, other randomized clinical trials evaluating mechanical staplers and hand-sewn closure techniques, such as the DISPACT trial, failed to demonstrate superiority of any method, with reported POPF rates around 36–37%. These findings emphasize the persistent challenge of preventing fistula formation after distal pancreatectomy and the pressing need for innovative strategies, an unmet need that RF-assisted transection may begin to address.

Experimental and translational data also provide mechanistic support for RF use. Studies in porcine and ex vivo pancreatic models showed that RF application produces uniform parenchymal coagulation, contraction of both main and secondary ducts, and minimal risk of upstream pancreatitis, indicating a favorable histopathological response. This combination of effective sealing and tissue preservation may explain the consistent reduction in POPF observed clinically.

In contrast, energy-based devices using ultrasonic or harmonic mechanisms, despite achieving hemostasis, generate a narrower coagulation margin than radiofrequency. This limited thermal effect may be insufficient to create the collagenous barrier required to contain pancreatic secretions. Notably, the internally cooled RF device used in the present study has demonstrated a substantially greater depth of tissue coagulation in experimental models (approximately 6 mm vs. 3 mm with other RF systems), a property that is consistent with the increased stump regularity, higher sphericity, and greater wall thickness observed on postoperative imaging. In line with this concept, recent trials comparing stapler versus ultrasonic transection have reported no significant differences in CR-POPF rates, likely related to inadequate sealing depth at the transection margin.

From a clinical standpoint, the reduction of CR-POPF has important implications beyond the immediate postoperative period. Lower fistula rates translate into fewer major complications, reduced reintervention rates, and shorter recovery times, as suggested by the trends observed in our secondary outcomes (see Table 5). Although not all differences reached statistical significance, the consistent direction of effect across variables (overall morbidity, reintervention rate, and hospital stay) underscores the potential clinical benefit of RF-assisted transection.

In addition, the use of RF may have positive economic implications by reducing costs related to prolonged hospitalization and postoperative management of pancreatic leaks. Given that CR-POPF remains one of the most resource-demanding complications in pancreatic surgery, the adoption of a safer and more effective closure technique could have a substantial impact on healthcare systems.

6.1 STRENGTHS AND LIMITATIONS

This thesis integrates a methodologically rigorous pilot study with a multicenter randomized clinical trial, providing a robust translational framework linking experimental evidence with clinical outcomes. The use of propensity score matching in the pilot study improved comparability between groups, while the randomized design of the TRANSPAIRE trial strengthened causal inference. This triangulation of evidence enhances both internal and external validity.

In addition, the study incorporates a detailed radiological evaluation of postoperative findings, allowing comprehensive characterization of pancreatic stump morphology and postoperative collections, together with an exploratory metabolomic analysis that adds a biological dimension to the interpretation of clinical outcomes. These complementary approaches increase the translational depth of the work and provide mechanistic insight into pancreatic healing and fistula development. The thesis is further supported by the extensive prior experience of the research group with radiofrequency-assisted pancreatic transection, including a matched non-randomized clinical series previously reported in the literature and more than a decade of experimental research in preclinical models. This cumulative experience supports the technical consistency of the intervention and situates the randomized trial within a sustained and progressive research trajectory.

Nonetheless, several limitations should be acknowledged. The pilot study included a relatively small sample size, inherent to the early clinical evaluation of a novel technique. Although propensity score matching improved internal validity, residual confounding cannot be completely excluded. In the randomized trial, recruitment challenges and the need for surgical standardization across multiple centers may have introduced variability. However, the participation of eight centers enhances generalizability and reflects routine clinical practice. Finally, longer follow-up would be valuable to assess the durability of radiofrequency-induced fibrosis and its potential impact on long-term pancreatic function.

Despite these limitations, the consistency of the findings across clinical, radiological, and biological analyses supports the robustness of the conclusions and the overall validity of the study.

CONCLUSIONS

7 CONCLUSIONS

- RF-assisted pancreatic transection is a safe and effective technique that is associated with a significant reduction in the incidence of CR-POPF after DP compared with conventional ST closure. In the multicenter TRANSPAIRE randomized clinical trial, RF-assisted transection reduced the rate of CR-POPF from 36.0% to 17.4%, corresponding to an OR of 2.67 in favor of RF-assisted transection ($p = 0.044$)

- PO morbidity during the first year of follow-up was lower in patients undergoing radiofrequency-assisted pancreatic transection. RF-assisted transection was associated with a significant reduction in the severity of postoperative complications**,** as assessed by the Clavien-Dindo classification, with fewer high-grade complications compared with stapler closure, supporting a favorable postoperative safety profile**.**

- No differences were observed in in-hospital, 90-day, or one-year mortality between radiofrequency-assisted transection and stapler-based closure, confirming an equivalent safety profile.

- Radiological follow-up demonstrated predictable postoperative morphological changes of the pancreatic stump over time, supporting a stable healing process after radiofrequency-assisted transection.

- Exploratory metabolomic analysis demonstrated that pancreatic transection technique is associated with different postoperative metabolic responses, and that metabolic patterns linked to inflammation and tissue injury were more frequently observed in patients who developed clinically relevant postoperative pancreatic fistula. These findings support the biological plausibility of the clinical results.

FUTURE PERSPECTIVES

8 FUTURE PERSPECTIVES

Future research should focus on validating these findings in larger, international multicenter RCT, as the current evidence, although encouraging, remains derived from single-country experiences. A similar large-scale effort would be essential to definitively establish the role of RF-assisted transection in routine clinical practice.

Another crucial research avenue is the histological and radiological evolution of the RF-treated pancreatic remnant**.** Although preclinical models have shown a characteristic pattern of coagulative necrosis followed by a thick collagen-rich capsule, the maturation, stability, and long-term remodeling of this fibrosis in humans remain unexplored. Advanced imaging modalities, including radiomics, textural analysis, and quantitative MRI, have recently been proposed as tools to characterize PO pancreatic healing and may help elucidate the structural and functional trajectory of the RF-induced seal.

Further progress will also depend on optimizing device parameters. RF technology offers substantial room for refinement: energy dose, application time, impedance-based feedback algorithms, cooling systems, and electrode geometry could be tailored to pancreatic tissue characteristics, which vary greatly between soft glands, fibrotic glands, and those affected by chronic pancreatitis.

In parallel, the incorporation of RF technology into minimally invasive and robotic surgery represents an important future direction. Robotic platforms, with enhanced dexterity and stable energy application, could provide optimal conditions for delivering RF energy with high precision. Early reports in robotic DP suggest that device-based stump sealing may be more reproducible in this setting. Dedicated RF instruments compatible with robotic systems could accelerate adoption and improve standardization.

Finally, an emerging area with substantial potential is the identification of biomarkers associated with pancreatic stump healing and the risk of fistula, including metabolomic, proteomic, and inflammatory profiles. Recent studies have shown that specific cytokine patterns, peritoneal fluid markers, and metabolomic signatures correlate with early anastomotic failure. Applying multi-omics analyses to the RF-treated stump could enable the identification of biological correlates of effective sealing and guide personalized strategies for postoperative monitoring.

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9. BIBLIOGRAPHY

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