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Endoscopic ultrasound-guided perivascular pancreatic radiofrequency ablation using a hydroxyethyl starch solution prior to pancreatectomy

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Authors

Julieta Montanelli[1], Leonardo Sosa-Valencia[2,3], Abdenor Badaoui[4], Gerlinde Averous[5], Lee Swanstrom[1], Didier Mutter[2,3], Patrick Pessaux[2,3], Barbara Seeliger[2,3]

Institutions

1 Innovation department, IHU Strasbourg, Strasbourg, France
2 Endoscopy department, IHU Strasbourg and Nouvel Hôpital Civil, Strasbourg, France
3 Department of Gastrointestinal and Endocrine Surgery, Hôpitaux Universitaires de Strasbourg, Nouvel Hôpital Civil, Strasbourg, France
4 Department of Gastroenterology and Hepatology , Université Catholique de Louvain, CHU UCL Namur, Yvoir, Belgium
5 Department of Pathology, Hôpitaux Universitaires de Strasbourg, Nouvel Hôpital Civil, Strasbourg, France

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Corresponding author

Leonardo Sosa-Valencia, Innovation department, IHU Strasbourg, Strasbourg, France
leonardo.sosa-valencia@ihu-strasbourg.eu

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ABSTRACT

Background and study aims Pancreatic surgery remains complex, particularly for borderline resectable and locally advanced tumors. Vascular invasion compromises resectability, and vascular resection entails increased morbidity and mortality. Following a feasibility and safety demonstration of augmented endoscopic ultrasound (EUS)-guided radiofrequency ablation (RFA) using hydroxyethyl starch (HES) in porcine pancreatic parenchyma, the present study assesses whether this approach (EUS-sugar-RFA) in the pancreatic perivascular space is safe and creates a controllable margin of necrosis to enable a vessel-sparing resection.

Methods EUS-sugar-RFA in the pancreatic parenchyma adjacent to the splenic artery and vein was performed in a live animal model. Following different survival periods (0–4 days) in the interventional group (n = 3), open pancreatectomy was carried out. The control group (n = 4) included open pancreatectomies in two pigs with non-treated pancreases and in two with pancreatic RFA alone on the same day.

Results All procedures were completed successfully, without intraoperative or postoperative complications. Survival periods were uncomplicated. Histopathological examination showed local necrosis and inflammatory reaction at the ablation sites. Vascular wall integrity was preserved in all specimens. The untreated pancreatic zones in the interventional group were no different from the normal pancreases in the control group.

Conclusions Preoperative perivascular augmented RFA using HES was safe, and in the pancreatic animal model, the best timeframe was within 24 hours before pancreatic surgery. This technique might improve resectability in selected borderline and locally advanced pancreatic cancers.

Introduction

Pancreatic surgery is complex, with a non-negligible risk of complications [1]. Although pancreatic surgery centralization in high-volume centers have helped reduced morbidity and mortality rates, up to 35% of these patients develop postoperative complications [1]. Because pancreatic resection offers the only curative option, its indications remain important. Peripancreatic vessel involvement has prompted vascular resection and reconstruction techniques, but with increased postoperative morbidity/mortality [2].

Patients with borderline resectable pancreatic cancer (BRPC) and locally advanced pancreatic cancer (LAPC) (i. e., unresectable non-metastatic pancreatic cancer), initially undergo chemotherapy ± radiotherapy for attempted downstaging and potential subsequent radical surgery. Nevertheless, only 12% of them proceed to surgery, and a R0 resection is achieved in 70% of these cases [3]. The main impediment is increasing the clean resection margin, particularly for arteries. Therefore, there is growing interest in complementary minimally invasive downstaging therapies.

Endoscopic ultrasound (EUS) enables high precision guidance of interventional therapies, and it can enhance radiofrequency ablation (RFA) by allowing real-time visualization for localized, controlled ablation while preserving surrounding structures. RFA causes coagulative necrosis and fibrotic changes. Its current indications include functional neuroendocrine tumors (NET) and percutaneous debulking in LAPC of pancreatic body after failed chemotherapy. Because the use of sucrose in isotonic solutions combined with RFA was shown to reduce conductivity and increase heating rates, we have tested the feasibility and safety of adding hydroxyethyl starch (HES) to pancreatic parenchyma RFA (EUS-sugar-RFA) in an animal model (n = 4), followed by pancreatic biopsies and resection [4, 5].

The present study assessed the effects of EUS-sugar-RFA applied to the perivascular space of the splenic vessels before pancreatectomy, with the objective of causing targeted necrosis while maintaining vascular integrity.

The primary outcomes were safety of EUS-sugar-RFA applied to the perivascular pancreatic space, capacity to create a controllable margin of perivascular necrosis to facilitate R0 resection, best treatment timing before pancreatectomy, and histopathological effect on pancreas and perivascular space. The secondary outcome was effect visibility in post-interventional imaging studies.

Methods

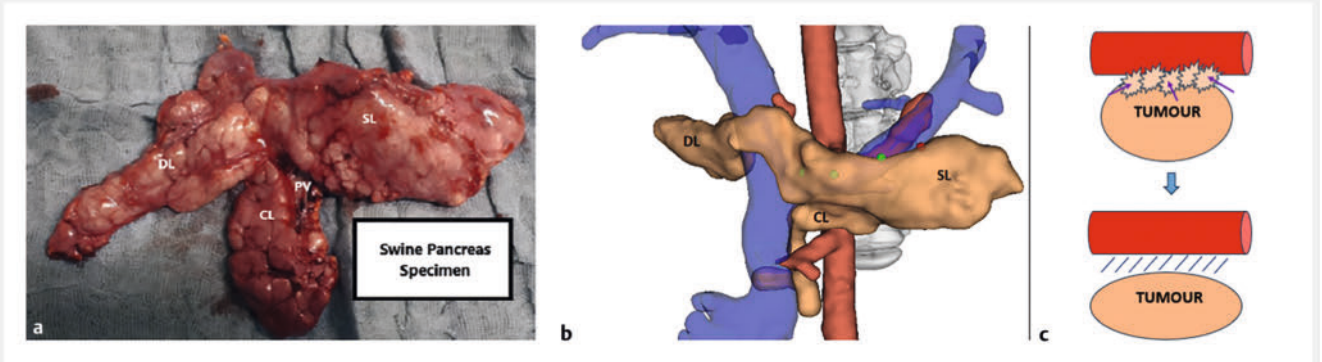
The study was approved by the institutional Animal Care and Ethics Committee (reference #28599–2020121012122760 v2). Seven pigs (sus scrofa domesticus) were included (interventional group n = 3; control group n = 4, 2x normal pancreas and 2x standard RFA), and managed according to French regulations, European Community Council directives (2010/63/EU), and ARRIVE guidelines [6].

Under anesthesia, a blood collection (hemogram, creatinine, amylase, lipase) and a contrast-enhanced triphasic thoraco-abdominal CT scan were done.

EUS-sugar-RFA

The three porcine pancreatic segments (duodenal lobe [DL], connecting lobe [CL], and splenic lobe [SL]) and vascular landmarks were identified using a EUS therapeutic linear scope (EG38-J10UT, Pentax, Japan; processor Arietta V70, Hitachi, Japan) [7] (▶ **Fig. 1a**). Two to three target zones (TZs) according to the individual anatomy were defined adjacent to the splenic artery (SA) and to the portal vein (PV) and splenic vein (SV), respectively. The first site was 15 mm distal to the spleno-porto-mesenteric confluence (SPMC). The second and third TZs were determined in a caudal direction, leaving a 10- to 15-mm distance between them (▶ **Fig. 1b** and ▶ **Fig. 1c**).

A 22G needle (Expect Slimline, Boston Scientific Corporation, United States; SonoTip ProFlex, MediGlobe GmbH, Germa-



▶ **Fig. 1** **a** Porcine pancreas specimen with the duodenal (DL), connecting (CL) and splenic lobe (SL) as well as the portal vein (PV) section. **b** Latero-anterior vision of 3 D CT reconstruction showing the planned EUS-sugar-RFA ablation strategy. Orange transparent: Porcine pancreas; red: arterial system, blue: venous system; green points: Sites of injection that were both planned and performed; red point: Site injection planned, not performed after considering the individual anatomy of the pig. **c** Schematic design of the hypothesized EUS-sugar-RFA's effect. Superior image: Pancreatic tumor compromising the adjacent vessel. Purple arrows: EUS-sugar-RFA application in the perivascular space. Inferior image: Necrotic effect with vascular wall preservation, allowing vessel-preserving dissection and tumor resection.

ny) was used to inject 1 to 1.5 cc of HES 130/0.4. Then, 50 watts were applied through a 19G EUSRA needle (Taewoong Medical, United States) placed in the TZs for 6 seconds (VIVA COMBO RF Generator System). Color Doppler was routinely used to determine the TZ and to check for bleeding. Finally, an adapted GAPS-EUS assessment tool was completed [8].

Pancreatectomy

Under anesthesia, the blood sample was repeated. A thoracoabdominal computed tomography (CT) scan and a diagnostic EUS were done to document changes.

Open pancreatectomy was performed en bloc with vascular axes, using a vessel-sealing device (Ligasure, Covidien, Ireland). Then, an Objective Structured Assessment of Technical Skills (OSATS) and a questionnaire created by us evaluating the subjective perception regarding the difficulty of pancreatectomy between interventional and control groups were completed (Annex 1) [9].

The control pancreatectomy group included two normal specimens and two after RFA alone (50 watts applied in the splenic vessel perivascular space, 10 to 15 seconds), obtained from educational courses.

Statistics

Due to the pilot character of the study with purposely low sample size and variable survival period durations, no statistical analysis was performed. Descriptive results are provided as mean standard deviation.

Results

All procedures were successfully completed. Pigs 1 and 2 had a survival of 4 and 1 days, respectively, between both procedures. They had appetite and tolerated a liquid diet. No vital sign alterations occurred. On the second follow-up day, pig 1 showed mild abdominal tenderness during palpation, with a soft abdomen, which resolved within 1 day. No abnormalities were found in the blood samples. Pig 3 underwent a non-survival protocol (both procedures on the same day).

EUS-sugar-RFA

The first TZ was 15 mm distal to the SMPC, and the following at 10 to 15 mm in the direction of the SL. With the SA as the landmark, three TZs were defined for pigs 1 & 3, and 2 TZs for pig 2, varying according to the pancreas length; and three TZs adjacent to the SV. Consequently, five to six injections/RFAs were performed in each pig. The mean procedure duration was 48.3 ± 10.89 minutes. Classic hyperechogenic bubbles were observed during RFA. No bleeding was observed under color Doppler control. Two EUS experts performed the procedures, one with extensive (pig 1) and one with less experience on animal models (pigs 2 and 3). Completion of EUS-sugar-RFA was represented by a GAPS-EUS overall score of 71 of 75 for the interventional group (▶ **Fig. 2**).

Pancreatectomy

Normal pancreas control

For the 30- and 45-minute procedures: In the longest, metal stent gastrojejunostomies (EUS-GJ) had been placed during an EUS course, which reduced maneuverability.

Surgical difficulty (Annex 1) was normal complexity for one (score 0), harder exposure for the EUS-GJ sample (score 3).

RFA alone control

For the 20- and 55-minute procedures: In the longer one, splenic vessels dissection was more difficult due to previous educational coil + glue treatment, without entailing complications. Surgical difficulty was normal complexity (score 0), slightly increased difficulty with the coiled vessel (score 1).

The control OSATS [9] score was 35 of 35 for all but one pig (EUS-GJ: 28/35).

Interventional group

Mean duration of the pancreatectomies = 54 ± 27 minutes. There were no signs of bleeding or peritonitis during exploration. The pancreas had a regular consistency. There were no visible signs of inflammation, neovascularization or tissue scarring in pig 3 (acute study), which allowed the selective use of vessel-sealing device. Pigs 2 and 1 (1 and 4-day survival respectively) had neovascularization around the TZ.

OSATS score: 35/35 (all cases). Surgical difficulty was normal for two pigs (score 0); harder dissection of TZ for pig 1(score 1). The pre-EUS CT scan was normal. On the presurgical scan, hypodense areas were visible adjacent to the splenic vessels, corresponding to the TZ (▶ **Fig. 3**). On the post-interventional EUS, a Doppler-negative hypoechogenic zone was visible adjacent to the splenic vessels, which were slightly larger when compared to the initial EUS control after EUS-sugar-RFA (▶ **Fig. 3**).

Pathology

Normal pancreas specimens

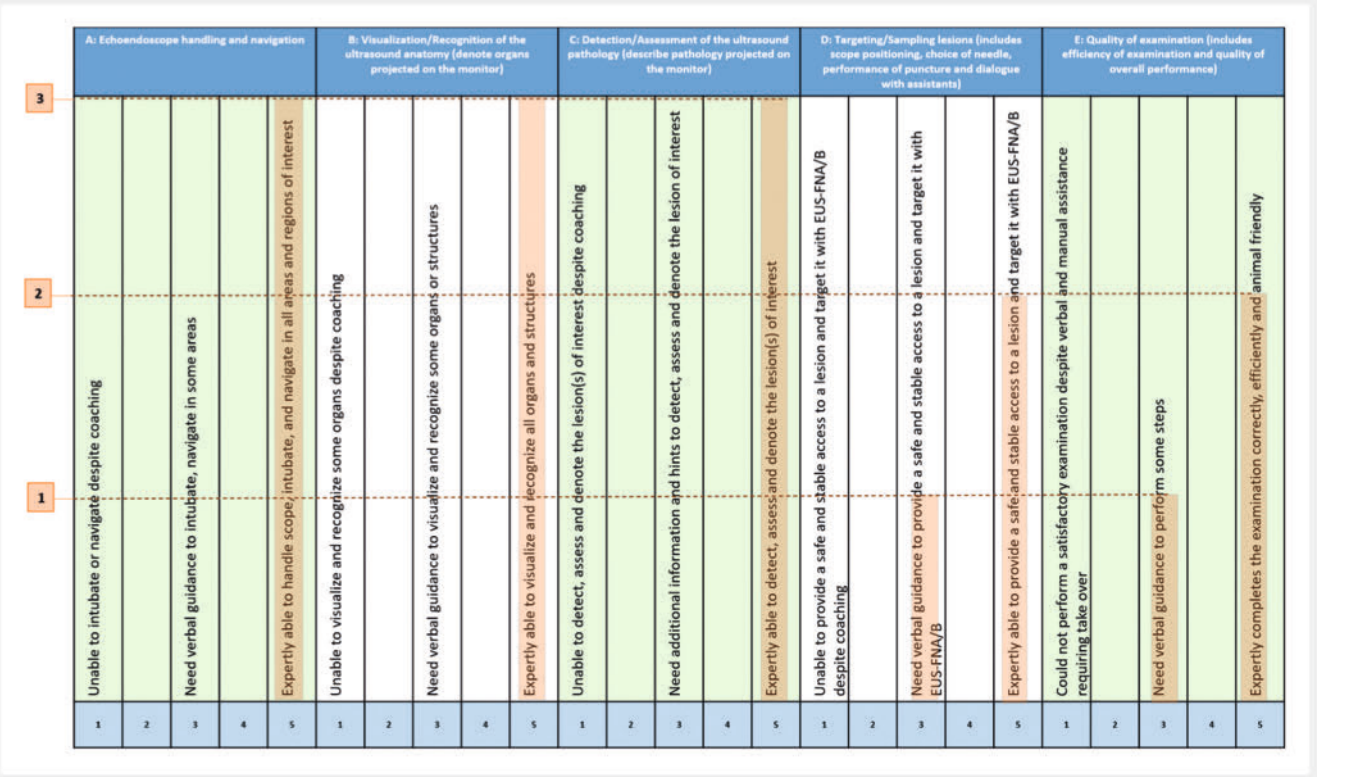
Signs of peripancreatic adiponecrosis and slight coagulation necrosis at the pancreatic margins, consistent with the use of monopolar cautery and a vessel-sealing device during dissection. There were minimal foci of acute lymphadenitis. Overall, the pancreatic parenchyma was homogeneous and served as a reference for comparison with the study group and RFA controls.

RFA-alone specimens

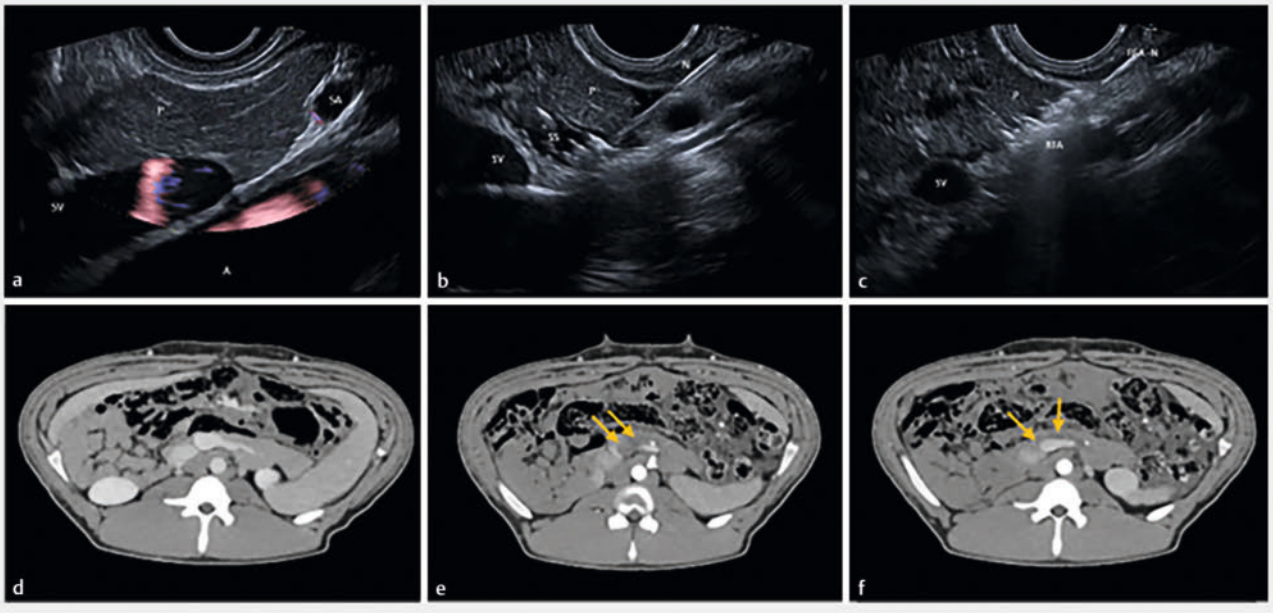
The vascular coil + glue treatment sample showed a SL hematoma and local peritonitis consistent with the splenic vessel injury.

Interventional group

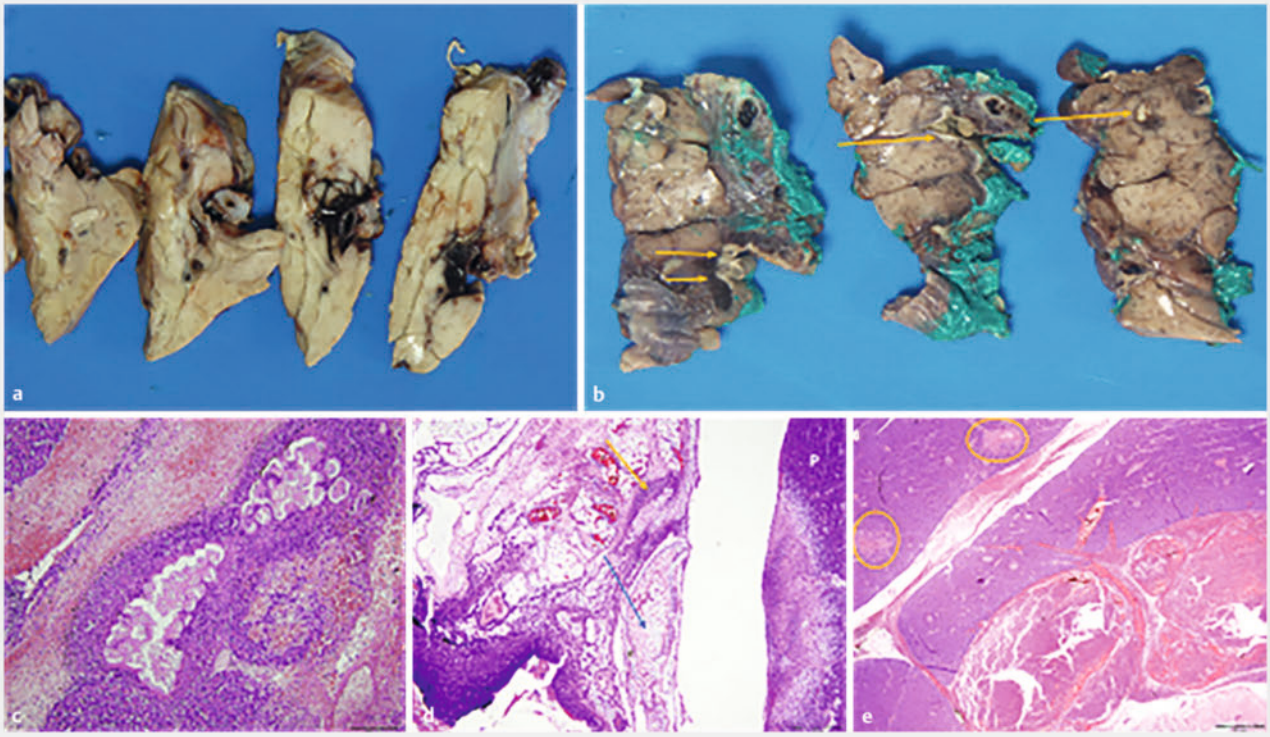
Specimen #1 had a 3 × 4 cm yellowish, necrotic zone in the posterior part of the pancreas. Specimen #2 had a 9 × 3 mm congestive zone in perivascular pancreatic tissue. Specimen #3 had 1-cm pancreatic haematoma adjacent to treated vessels, and a 1–2 mm necrotic area (▶ **Fig. 4**). The vessel walls revealed



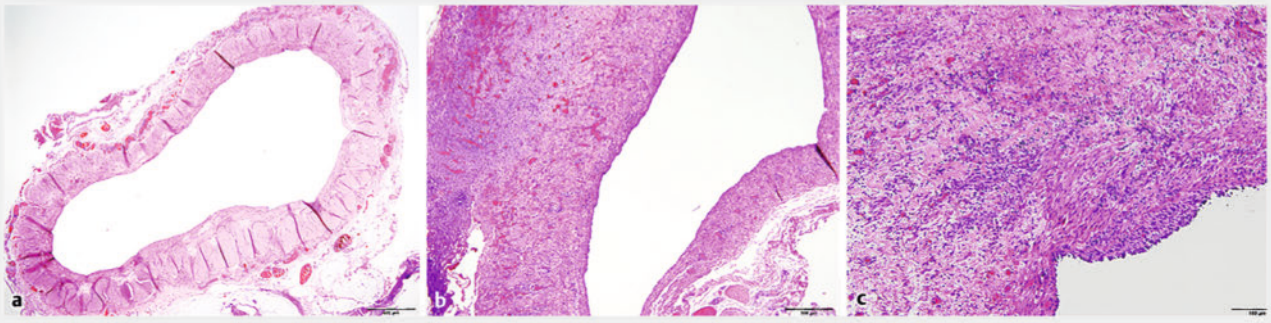
▶ **Fig. 2** GAPS-EUS assessment tool adapted from [8]. Ratings are shown as orange bars representing the absolute numbers for the three interventional group procedures.



▶ **Fig. 3** Treatment sites, visualized in EUS and contrast-enhanced CT before (a,d), during (b), and after (c,e,f) the EUS-sugar-RFA procedure in pig 1. **a** EUS assessment prior to the EUS-sugar-RFA with identification of the course of the splenic vessels' course, and choice of the target zones. **b** First part of the EUS-sugar-RFA treatment. Injection in the target zone adjacent to the splenic vein. **c** Second step of the EUS-sugar-RFA treatment: After needle retrieval, the RFA probe is inserted in the target zone and 50 watts are applied for 6 seconds. **d** Contrast-enhanced CT scan prior to EUS-sugar-RFA: The pancreas is normal. **e,f** Contrast-enhanced CT scan prior to pancreatectomy (4 days after the EUS-sugar-RFA). The hypodense area adjacent to the splenic vessels is indicated by the yellow arrows. P, pancreas; SV, splenic vein; SA, splenic artery; a, aorta; N, needle; SS, sugar solution; RFA-N, radiofrequency ablation needle; RFA, radiofrequency ablation effect; HES, hydroxyethyl starch.



▶ **Fig. 4** Histopathological pancreatic findings of the interventional group. Macroscopy: Hematomas in the perivascular treatment zones (**a,** pig 3), and necrotic area (yellow arrows) developed in the 4-day period between procedures 1 and 2 (**b,** pig 1). Microscopy (H&E x 100): Acute pancreatitis with necrosis of pancreatic and peripancreatic tissues (**c,** pig 1). Omentum with acute inflammatory reaction (yellow arrow) and adipose tissue necrosis (blue arrow) adjacent to the pancreas (P) (**d,** pig 2); early perivascular pancreatic cell necrosis with microscopic foci of perivascular hematomas (yellow circles) (**e,** pig 3).



▶ **Fig. 5** Microscopic vascular findings in the interventional group (pig 1). Transversal cut of the portal vein (PV) (a, H&E x 40); mesenterico-portal phlebitis with thickening of the PV showing polymorphonuclear infiltration and angiogenetic foci (B, H&E x 100).; and polymorphonuclear infiltration extending up to the tunica intima of the PV (C, H&E x 200).

mild mesenterico-portal phlebitis in specimen #1; in the other pigs, the walls were normal. Wall integrity was maintained in all specimens (▶ **Fig. 5**).

Microscopic specimens from pigs 1 and 2 showed acute pancreatitis and peripancreatic fat necrosis. The mesenterico-portal phlebitis (pig 1) revealed polymorphonuclear infiltration up to the tunica intima and foci of neoangiogenesis. Pig 2 had signs of acute peritonitis and Pig 3 presented a perivascular hematoma as well as early perivascular pancreatic and fat cell necrosis (▶ **Fig. 4** and ▶ **Fig. 5**).

Detailed histopathological results are shown in ▶ **Table 1**.

▶ **Table 1** Histopathological findings from the study and control groups.

	Specimen	Procedure done	Macroscopic description	Microscopic evaluation
Control group	Non-treated pancreas 1	No particularities	25 × 3 × 4 cm specimen, no visible lesion. 1.5-cm lymph node	Slight pancreatic coagulation necrosis (specimen margins), rest normal
				Peri-pancreatic adiponecrosis
				Minimal acute lymphadenitis
	Non-treated pancreas 2	Modified anatomy (lumen-apposing metal stents with electrocautery enhanced system for EUS-GJ)	15 × 4 × 3 cm specimen, with 1,5 cm^2 pancreatic gray/white lesion + focal congestion. 1.7-cm lymph node	Slight pancreatic coagulation necrosis (specimen margins) + focus of isolated coagulative necrosis (consequence of EUS-GJ)
				Peri-pancreatic adiponecrosis
				Minimal acute lymphadenitis
	RFA 1	RFA and pancreatectomy (D0); Modified anatomy (artificial attached fluid-filled collections, and a coil + glue treatment in the splenic vessel)	19 × 4 × 3 cm specimen 2 × 1 cm gray pancreatic lesion 6 mm hematoma in the tail Coil near splenic vessel	▪ Pancreatic coagulation necrosis
				▪ Acute lymphadenitis
				▪ Acute peritonitis
				▪ Hematoma in the tail
				▪ Splenic vessel injury
	RFA 2	RFA and pancreatectomy (D0) Modified anatomy (artificial attached fluid-filled collections)	13 × 5,5 × 3 cm specimen, no visible lesion	▪ Pancreatic coagulation necrosis
				Acute lymphadenitis
				Acute peritonitis
Interventional group	EUS-sugar-RFA 1	EUS-sugar-RFA (D0); Pancreatectomy (D4)	15 × 5 × 4 cm specimen. 3 × 4 cm yellowish necrotic zone	Pancreatic & peri-pancreatic coagulation necrosis, nerval and fat tissue necrosis
				Pancreatitis and peripancreatitis
				Focal mesenterico-portal phlebitis
	EUS-sugar-RFA 2	EUS-sugar-RFA (D0); Pancreatectomy (D1)	Congestive perivascular pancreatic tissue. Gastric submucosal hematoma	▪ Pancreatic coagulation necrosis
				Pancreatic and peri-pancreatic adiponecrosis
				Acute pancreatitis
				Acute peritonitis
	EUS-sugar-RFA 3	EUS-sugar-RFA + pancreatectomy (D0)	17 × 5 × 4 cm specimen 1 cm hematoma around treated vessels, with no other pancreatic lesion	Focal early perivascular pancreatic necrosis, minimal adiponecrosis
				Hematoma

D0, day of procedure 1; D1, day after procedure 1; D4, 4 days after procedure 1; EUS-GJ, endoscopic ultrasound-guided gastrojejunostomy; RFA, radiofrequency ablation.

Discussion

This study assessed the impact of adding a starch solution to RFA (EUS-sugar-RFA) applied to the perivascular space of the splenic vessels before pancreatectomy. After proving feasibility and safety along a 4-day survival period (pig 1), the following survival periods were shortened to minimize local inflammatory response. The best timeframe for EUS-sugar-RFA was within 24 hours prior to pancreatectomy, where the reduced inflammatory response and neovascularization limited the use of a vessel-sealing device during dissection. Vascular wall integrity was maintained for all specimens.

Our previous study suggested that the interaction between starch and RFA generated a demarcated necrosis that allowed a clear separation of necrotic from normal tissue, but that study was performed within the parenchyma [5]. The present study focused on assessing the effect when applied to the perivascular space, specifically on the vascular axes adjacent to the pancreas, for potential application in pancreatic cancer with vascular compromise.

Because the present study targeted the perivascular space, in contrast to pancreatic parenchyma or neoplasia, the energy was applied for a shorter time to avoid potential vascular complications. The result was a perivascular 5-mm charred layer composed by fibrin and granulation tissue.

The interaction between sugar solutions and RFA has not been extensively explored in in vivo, but an ex vivo study using a porcine vascular model concluded that the addition of carbohydrates to a solution enabled a selective higher cell death rate and lower conductivity when exposing a tissue to RF energy [4]. Therefore, we have subsequently assessed sugar-boosted RFA, targeting a specific zone in which a circumscribed, augmented effect is desired.

Perivascular space injection may benefit from the local fluid spread adjacent to the initial injection site, dissecting the space, and thereby supporting energy transmission to the perivascular space. Moreover, the sugar/RF interaction allows an augmented ablation while remaining limited to the TZ.

As observed in our previous study, the addition of starch allowed the delivery of a lower amount of energy to achieve the desired effect.

The present study is limited by the absence of pancreatic neoplasia. The proof of concept, therefore, was achieved without assessing its capacity to downstage pancreatic tumors. Survival after pancreatectomy was omitted due to the expected complex management of insufficiencies in accordance with ethical considerations.

A detailed assessment of the vascular area is essential prior to treatment and avoiding areas close to the pancreatic ducts. The maintenance of a stable position during HES injection, needle retrieval, and RFA catheter insertion is also fundamental. Such technical precision requires a high level of EUS expertise and the assistance of a second operator, which is a disadvantage. However, the high overall GAPS-EUS score obtained by the second operator indicates that the procedure can be quickly learned by expert endoscopists.

The small number of animals is also a limitation, as it does not allow taking significant conclusions as to the ideal timeframe of application. Also, the control animals were taken from educational courses for ethic reasons, but still hinder comparison. However, feasibility, safety and histopathological findings are consistent along the previous and present study, with an overall of seven animals treated with EUS-sugar-RFA. A multicentric study with several experts and higher number of procedures is required to assess generalisability of the procedure and further biological aspects before clinical translation.

Conclusions

In conclusion, perivascular EUS-sugar-RFA of the pancreas is an emerging neoadjuvant supportive technique. Although not conclusive, the best observed time to perform it was on the day of surgery, because it efficiently induced perivascular necrosis without complicating inflammation/hemorrhage. Potential applications are preoperative treatment before distal/partial pancreatectomies for NETs, in selected patients with BRPC and LAPC, and for treatment of metastases in the body/tail. Larger studies, with follow-up periods after EUS-sugar-RFA and surgery, are needed to evaluate the impact on the residual parenchyma. Only then can further clinical protocols be planned. If this approach is also shown to be safe and feasible in clinical contexts, it may become part of the multidisciplinary treatment of pancreatic disease in the future.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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