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Efficacy and Safety of Endoscopic Ultrasonography-Guided Ethanol Injections of Small Pancreatic Neuroendocrine Neoplasms: a prospective, multicenter study

Kazuyuki Matsumoto, Hironari Kato, Takao Itoi, Masayuki Kitano, Kazuo Hara, Masaki Kuwatani, Mamoru Takenaka, REIKO ASHIDA, Shuntaro Mukai, Nozomi Okuno, Kazumichi Kawakubo, Tatsuhiro Yamazaki, Jun Sakurai, Yuki Nakatsuka, Michihiro Yoshida, Motoyuki Otsuka.

Affiliations below.

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Abstract:
Background and study aims: Endoscopic ultrasonography (EUS)-guided ethanol injection (EI) has recently been introduced as one of the management strategies for pancreatic neuroendocrine neoplasms (PNENs). However, its role as a surgical alternative is unclear. We evaluated the efficacy and safety of EUS-EI in treating small PNENs through a prospective multicentre study.
Patients and methods: Patients with Grade 1 tumours of ≤15 mm confirmed by pathology were included. The primary endpoint assessed efficacy and safety, measuring complete ablation using computed tomography at 1 and 6 months, prevention of adverse events (AEs) within 1 month, severe pancreatic fistula at 1 month, and diabetes mellitus (DM) incidence/worsening at 6 months. The composite endpoint of EUS-EI was compared with that of historical results of a study based on surgical treatment.
Results: Twenty-five patients with PNENs, with a median tumour size of 10.1 mm, were treated using EUS-EI. Seventy-six percent of the patients achieved the composite primary endpoint (19/25) (95% confidence interval [CI]=54.9%–90.6%), a proportion significantly higher than that of surgical treatment (P=0.0083). Regarding efficacy, 88% (22/25) of the patients achieved complete ablation at 1 and 6 months (95% CI=68.8%–97.5%). Regarding safety, 96% (24/25) of the patients had no severe AEs within 1 month (95% CI=79.7%–99.9%). No patients had severe pancreatic fistulas at 1 month, and 84% (21/25) of the patients had no incidence or exacerbation, or both, of DM at 6 months (95% CI=63.9%–95.5%).
Conclusion: EUS-EI is safe and could be a potent treatment option for patients with small PNENs.

Corresponding Author:
Dr. Hironari Kato, Okayama University Hospital, Gastroenterology and Hepatology, Okayama, Japan, katou-h@cc.okayama-u.ac.jp

Affiliations:
Kazuyuki Matsumoto, Okayama University Hospital, Gastroenterology and Hepatology, Okayama, Japan

Hironari Kato, Okayama University Hospital, Gastroenterology and Hepatology, Okayama, Japan
Takao Itoi, Tokyo Medical University, Gastroenterology and Hepatology, Shinjuku-ku, Japan
[...]
Motoyuki Otsuka, Okayama University Hospital, Gastroenterology and Hepatology, Okayama, Japan

Table 1. Patients' characteristics (*n*=25).

	All patients (*n*=25)
Age, median (IQR), years	62 (52–71)
Male/female, *n*	15/10
Tumour size, median (IQR), mm	10.1 (7.0–11.0)
Tumour location, *n* (%)	
Head	11 (44.0)
Body	8 (32.0)
Tail	6 (24.0)
Non-function/function, *n*	25/0
Performance status, *n* (%)	
0	22 (88.0)
1	3 (12.0)
Presence of DM, *n* (%)	8 (32.0)

DM: diabetes mellitus, IQR: interquartile range

Table 2. Details of achievement of the primary composite endpoint and the component endpoints using EUS-guided EI

Endpoints	Treatment	Number of patients	Number of achievements	Number of non-achievements	Number of unevaluable patients*	Percentage of achievement (%)			Exact binomial test
						Point estimation	90% CI**	95% CI	*P* value***
Primary composite endpoint (ITT analysis)[1)]	EUS-EI	25	19	6	0	76.0	58.0–89.0	54.9–90.6	.0083
	Surgery	23	11	12	0	47.8	29.6-66.5	26.8-69.4	
Component endpoints									
1. Efficacy									
Complete ablation at 1 month[2)]	EUS-EI	25	22	3	0	88.0	71.8–96.6	68.8–97.5	-
	Surgery	23	23	0	0	100.0	87.8-100.0	85.2-100.0	
Complete ablation at 6 months[3)]	EUS-EI	25	22	2	1	88.0	71.8–96.6	68.8–97.5	-
	Surgery	23	23	0	0	100.0	87.8-100.0	85.2-100.0	
2. Safety									
Avoidance of severe AEs within 1 month[4)]	EUS-EI	25	24	1	0	96.0	82.4–99.8	79.7–99.9	-
	Surgery	23	15	8	0	65.2	46.0-81.4	42.7-83.6	
Avoidance of pancreatic fistula at 1 month[5)]	EUS-EI	25	25	0	0	100.0	88.7–100.0	86.3–100.0	-
	Surgery	23	15	8	0	65.2	46.0-81.4	42.7-83.6	
Avoidance of incidence and/or exacerbation of DM at 6 months[6)]	EUS-EI	25	21	3	1	84.0	67.0–94.3	63.9–95.5	-
	Surgery	23	20	3	0	87.0	69.6-96.4	66.4-97.2	

AE: adverse event, CI: confidence interval, DM: diabetes mellitus, EI: ethanol injection, ITT: intention-to-treat

* One patient died due to cardiac infarction 5 months after the procedure

**For scientific publications, statistical significance level was set at 5% (two-sided). The corresponding two-sided 90% CI was shown purely as a reference because the s ample size was calculated with a two-sided significance level of 10%.

***The null hypothesis was set using the historical results of a study based on surgical treatment

Table 3. Details of secondary endpoints

	All patients (*n*=25)
Efficacy	
Complete ablation at 1 month, % (*n*)	88.0 (22)
Complete ablation at 6 months, % (*n*)	88.0 (22)
Six-month overall survival, % (*n*)	96.0 (24*)
Safety	
Prevalence of total adverse events, % (*n*)	68.0 (17)
Prevalence of severe AEs within 1 month, % (*n*)	4.0 (1)
Prevalence of severe pancreatic fistula at 1 month, % (*n*)	0.0 (0)
Prevalence of DM incidence and/or exacerbation at 6 months, % (*n*)	12.0(3)
Prevalence of device failures, % (*n*)	4.0 (1)
Prevalence of conversion to surgery, % (*n*)	0.0 (0)

AE: adverse event, DM: diabetes mellitus, IQR: interquartile range

* One patient died due to a cardiac infarction 5 months after treatment

Table 4. Procedure-related adverse events

	Number of patients (%)		
	Any grade	**Grade 1 or 2**	**Grade 3 or 4**
Total adverse events	15 (60.0)	14 (56.0)	1 (4.0)
Post-procedure			
Hyperamylasaemia	8 (32.0)	8 (32.0)	0 (0.0)
Pancreatitis	5 (20.0)	4 (16.0)	1 (4.0)
Nausea	1 (4.0)	1 (4.0)	0 (0.0)
Abdominal pain	1 (4.0)	1 (4.0)	0 (0.0)
Vomiting	1 (4.0)	1 (4.0)	0 (0.0)
During procedure			
Hypotension	1 (4.0)	1 (4.0)	0 (0.0)
Needle obstruction	1 (4.0)	1 (4.0)	0 (0.0)

Procedure-related adverse events were evaluated based on the American Society for Gastrointestinal Endoscopy (ASGE) guideline 2010, and other adverse events were assessed based on Common Terminology Criteria for Adverse Events (CTCAE) V.5.0.

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Kazuyuki Matsumoto M.D., Ph.D.[1], Hironari Kato M.D., Ph.D.[1], Takao Itoi M.D., Ph.D.[2], Masayuki Kitano M.D., Ph.D.[3], Kazuo Hara M.D., Ph.D.[4], Masaki Kuwatani M.D., Ph.D.[5], Mamoru Takenaka M.D., Ph.D.[6], Reiko Ashida M.D., Ph.D.[3], Shuntaro Mukai M.D., Ph.D.[2], Nozomi Okuno M.D.[4], Kazumichi Kawakubo M.D., Ph.D.[5], Tatsuhiro Yamazaki M.D., Ph.D.[1], Jun Sakurai M.D., Ph.D.[7], Yuki Nakatsuka MPH[7], Michihiro Yoshida Ph.D.[7] and Motoyuki Otsuka M.D., Ph.D.[1]

[1]Department of Gastroenterology and Hepatology, Okayama University Hospital, Okayama, Japan

[2]Department of Gastroenterology and Hepatology, Tokyo Medical University, Tokyo, Japan

[3]Second Department of Internal Medicine, Wakayama Medical University, Wakayama, Japan

[4]Department of Gastroenterology, Aichi Cancer Center, Nagoya, Japan

[5]Department of Gastroenterology and Hepatology, Hokkaido University Hospital, Hokkaido, Japan

[6]Department of Gastroenterology and Hepatology, Kindai University Faculty of Medicine, Osaka-Sayama, Osaka, Japan

[7]Center for Innovative Clinical Medicine, Okayama University Hospital, Okayama, Japan

Corresponding Author: Hironari Kato, M.D., Ph.D.

Department of Gastroenterology and Hepatology, Okayama University Hospital

Address: 2-5-1 Shikata-cho, Okayama 700-8558, Japan

E-mail: katou-h@cc.okayama-u.ac.jp

Specific Author Contributions:

Kazuyuki Matsumoto (Conceptualisation: Lead; Data curation: Equal; Investigation: Lead; Methodology: Equal; Writing the -original draft: Lead; Grant Acquisition: Supporting; Writing, -review, and & editing: Equal)

Hironari Kato (Conceptualisation: Equal; Data curation: Equal; Investigation: Equal; Methodology: Equal; Writing-original draft: Equal; Grant Acquisition: Lead; Writing, -review, and & editing: Lead)

Takao Ito (Data curation: Equal; Investigation: Equal; Grant Acquisition: Supporting)

Masayuki Kitano (Data curation: Equal; Investigation: Equal; Grant Acquisition: Supporting)

Kazuo Hara (Data curation: Equal; Investigation: Equal; Grant Acquisition: Supporting)

Masaki Kuwatani (Data curation: Equal; Investigation: Equal; Grant Acquisition: Supporting)

Mamoru Takenaka (Data curation: Equal; Investigation: Equal; Grant Acquisition: Supporting)

Reiko Ashida (Data curation: Equal; Investigation: Equal; Grant Acquisition: Supporting)

Tatsuhiro Yamazaki (Data curation: Lead; Investigation: Equal)

Jun Sakurai (Data curation: Equal; Methodology: Lead).

Yuki Nakatsuka (Formal analysis: Lead; Methodology: Equal)

Michihiro Yoshida (Formal analysis: Equal; Methodology: Equal)

Motoyuki Otsuka (Writing-original draft: Supporting; Writing, -review, and & editing: Equal).

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The study protocol was approved by the Okayama University Certified Review Board (approval no.: CRB19-007) and registered in the Japan Registry of Clinical Trial Registration (trial number: jRCTs061200016).

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Potential Competing Interests:

All authors disclose no conflicts.

Data Transparency Statement:

The datasets either used or analysed or both in the current study are available from the corresponding author upon reasonable request. Data collection forms are available from the UMIN Internet Data and Information System for Clinical and Epidemiological Research, Cloud version (https://www.umin.ac.jp/indice/cloud.html). The study protocol has been included as a supplementary data supplement in the online version of this article.

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Abstract

***Background and study aims*:** Endoscopic ultrasonography (EUS)-guided ethanol injection (EI) has recently been introduced as one of the management strategies for pancreatic neuroendocrine neoplasms (PNENs). However, its role as a surgical alternative is unclear. We evaluated the efficacy and safety of EUS-EI in treating small PNENs through a prospective multicentre study.

***Patients and methods*:** Patients with Grade 1 tumours of ≤15 mm confirmed by pathology were included. The primary endpoint assessed efficacy and safety, measuring complete ablation using computed tomography at 1 and 6 months, prevention of adverse events (AEs) within 1 month, severe pancreatic fistula at 1 month, and diabetes mellitus (DM) incidence/worsening at 6 months. The composite endpoint of EUS-EI was compared with that of historical results of a study based on surgical treatment.

***Results*:** Twenty-five patients with PNENs, with a median tumour size of 10.1 mm, were treated using EUS-EI. Seventy-six percent of the patients achieved the composite primary endpoint (19/25) (95% confidence interval [CI]=54.9%–90.6%), a proportion significantly higher than that of surgical treatment (*P*=0.0083). Regarding efficacy, 88% (22/25) of the patients achieved complete ablation at 1 and 6 months (95% CI=68.8%–97.5%). Regarding safety, 96% (24/25) of the patients had no severe AEs within 1 month (95% CI=79.7%–99.9%). No patients had severe pancreatic fistulas at 1 month, and 84% (21/25) of the patients had no incidence or exacerbation, or both, of DM at 6 months (95% CI=63.9%–95.5%).

***Conclusion*:** EUS-EI is safe and could be a potent treatment option for patients with small PNENs.

Introduction

Pancreatic neuroendocrine neoplasms (PNENs) are rare, accounting for 1%–2% of primary pancreatic malignancies.[1] However, their incidence has increased substantially owing to the widespread use of advanced endoscopic and radiological imaging techniques.[2]

Treatment options for PNENs depend on hormone-related symptoms and tumour size.[3,4] Specifically, surgical resection is usually performed in patients with symptomatic disease or tumours >2 cm in diameter. However, the optimal treatment approach for patients with small non-functional low-grade PNENs (≤2 cm diameter) remains controversial. The complication rate of pancreatic surgery is higher than that of other gastrointestinal surgeries. Additionally, decreased pancreatic endocrine and exocrine functions may occur after pancreatic resection. Therefore, the benefits of surgery must be balanced against potential postoperative complications.[3-5]

When treating PNENs, a watch-and-wait approach is generally chosen for small-sized low-grade malignant tumours.[3, 4] A recent study about patients with non-functional small pancreatic endocrine tumours (PNETs) who underwent surgical resection reported a similar 5-year cancer-specific survival to those under observation.[4] However, a longer 5-year prognosis and the tumours among the small-sized tumours that are likely to grow in the future remain unclear. Furthermore, opting for surveillance in a wait-and-watch approach requires annual contrast-enhanced examinations, potentially raising concerns related to allergies to contrast media, renal dysfunction, or radiation exposure.

Recently, advances in EUS-guided ablative techniques have enabled a possible alternative to surgical resection. The advantages of the EUS-guided local ablation therapy include reduced complications and preserved pancreatic functions. The two most commonly described techniques are radiofrequency ablation (RFA) and ethanol injection (EI).[6-22] EUS-EI, which involves direct EI into a tumour to induce coagulation necrosis, was reported in 2006;

[16] however, most related studies were single-centre retrospective studies. Therefore, we planned a multicentre single-arm prospective study to evaluate the efficacy and safety of EUS-EI for low-grade small PNENs.

Patients and Methods

Study design and participants

This multicentre, single-arm prospective study was conducted at six high-volume medical centres in Japan between September 2020 and July 2023. Figure 1 shows a flow diagram of patient enrolment and the study's protocol overview. The eligibility criteria included: (1) age 20–75 years, (2) provision of informed consent, (3) grade 1 PNEN diagnosed pathologically using EUS-fine-needle aspiration (FNA) specimens (World Health Organisation 2017 classification), (4) well-enhanced tumour (diameter, ≤15 mm) in the arterial phase on contrast enhanced (CE)-CT, and (5) PNEN diagnosed as a non-functional tumour or insulinoma. The exclusion criteria included: (1) allergy to contrast media or ethanol, (2) distance between the tumour and main pancreatic duct of ≤2 mm, (3) administration of ≥2 antithrombotic agents, and (4) poor prognosis (<5 years) predicted as described in the protocol article.[23]

Written informed consent was obtained from all patients before initiation of procedures. The study protocol was approved by the Okayama University Certified Review Board (approval no.: CRB19-007), registered in the Japan Registry of Clinical Trial Registration (trial number: jRCTs061200016), and followed the principles of the Declaration of Helsinki. Monitoring and auditing were conducted during the trial. We also established an independent data monitoring committee comprising three additional doctors (R.Y., R.H., and M.F.) who were not associated with the study to determine whether the study should continue if severe adverse events (AEs) occurred. All authors had access to the study data and reviewed and approved the final manuscript.

Endpoints

Primary endpoint

To clarify the rationale to be presented in the Japanese regulatory submission, the primary composite endpoint was established as proportion of participants who achieved all of the following clinical efficacy and safety component endpoints: (1) Efficacy: complete ablation on CE-CT at 1 and 6 months after treatment and (2) Safety: (a) no severe AEs within 1 month after treatment, (b) no severe pancreatic fistula at 1 month after the treatment, and (c) no incidence or exacerbation, or both, of diabetes mellitus (DM) at 6 months after treatment.

Secondary endpoints

The following secondary endpoints were evaluated.

1. Efficacy: (a) complete ablation on CE-CT at 1 month after treatment; (b) complete ablation on CE-CT at 6 months after treatment; and (c) 6-month overall survival.
2. Safety: prevalence of (a) total AEs, (b) severe AEs within 1 month after treatment, (c) severe pancreatic fistulas at 1 month after treatment, (d) DM exacerbation at 6 months after treatment, (e) device failure, and (f) conversion to surgery.

Definition

Complete ablation was defined as the absence of enhanced areas within the tumour on arterial-phase CE-CT images with a 1–2-mm thick slice. Two expert gastroenterologists independently reviewed the CE-CT images based on the radiologist's findings. If a judgement could not be made using CE-CT, CE-EUS with perflubutane (Daiichi-Sankyo Co., Ltd., Tokyo, Japan) was performed to assess the enhanced areas within the tumour. Procedure-related AEs were evaluated based on the 2010 guideline of the American Society for Gastrointestinal Endoscopy (ASGE),[24] and other AEs were evaluated using the Common Terminology Criteria for

Adverse Events (CTCAE) v 5.0. Severe AEs were defined as moderate or higher in ASGE and grade ≥3 in CTCAE. Severe pancreatic fistula was defined as the continuation of any treatment for pancreatic fistula (percutaneous or endoscopic drainage tube or medication or both) at 1 month after treatment. DM was defined as fasting or occasional blood glucose levels of 126 or 200 mg/dL, respectively, and glycated haemoglobin (HbA1c) levels of ≥6.5 (National Glycohemoglobin Standardization Program value). New-onset DM referred to a patient without DM at the time of registration. However, DM exacerbation referred to a patient who qualified as having DM at the time of registration but subsequently started or increased medication for DM owing to poor glycaemic control or whose HbA1c level increased by approximately 0.2%.

Study procedure

The procedure was performed on the patients in the prone or semi-prone position under conscious sedation in an endoscopy room using an intravenous anaesthetic. Before the procedure, a 50-mg Diclofenac suppository was used to prevent pancreatitis. Regarding treatment, a 25-gauge FNA needle (EZ-shot 3; Olympus Medical Systems, Tokyo, Japan) filled with ethanol was advanced into the tumour under EUS. Pure ethanol (100%) (Mylan Seiyaku Ltd., Tokyo, Japan) was injected until a hyperechoic blush extended to the tumour edge margin, and the needle was kept inside the tumour for at least 1 min to prevent ethanol backflow. The injection was initiated from the deeper tumour side on the EUS image because a spread hyperechoic bush prevents recognition of tumour's low-echoic parts on the far side. After removing the needle, we looked for the low-echoic tumour parts, which, if detected, were injected with ethanol. For safety, the amount of ethanol per puncture, total number of punctures per session, and maximum volume per session were set to 1 mL, 3, and 2 mL, respectively (Figure 2, Video 1, and Supplemental Figure 1).

CE-CT was performed 3–5 days posttreatment to evaluate tumour viability and procedure-related AEs. If enhanced areas of the tumour were observed on postprocedural CE-CT, an additional ablation session was performed within the same hospitalisation period. If it was difficult to identify the tumour's viable part on B-mode, CE-EUS was performed to locate any residual tumours [25]. The patient was discharged a day after the additional session or when no enhanced tumour areas were observed on postprocedural CE-CT (Supplemental Figures 2 and 3).

Follow-up

To assess the acute and sub-acute posttreatment course of patients with PNENs with EUS-EI in this study, patients were followed up postoperatively for 6 months. Follow-up examinations were scheduled at 1, 3, and 6 months to evaluate the patient's general condition and perform blood tests. The patients were scheduled to undergo follow-up CE-CT imaging at 1 and 6 months after discharge. Salvage surgical resection was suggested to the patient when incomplete ablation of the treated lesion was observed based on follow-up CE-CT.

Sample size calculation

Because this study was related to an orphan disease, it was designed as a single-arm study. For the interpretation of the study results, known historical results of surgical treatment in 23 patients with PNENs (diameter, ≤15 mm) who underwent treatment at Okayama University Hospital between November 2007 and January 2018 were referred (Supplemental Table 1). The result showed that 47.8% (11/23) of the patients met the primary endpoint. In our previous pilot study of EUS-EI in a similar population, 75.0% (6/8) of the patients achieved the primary endpoint. Therefore, the null and alternative hypotheses were set as follows:

H_0: $P_T = 0.48$

H_1: $P_T \neq 0.48$

where P_T was the postprocedural true proportion of primary endpoint. Based on the Japanese special regulation for approvals for orphan diseases, the statistical significance level was set at 10% (two-sided). The number of patients required to maintain 80% power based on exact binomial test was 22. A sample size of 25 was planned to account for dropouts or withdrawals.

Statistical analysis

The analysis population was defined as all participants who were registered in the study and underwent the trial procedures (ITT analysis). Continuous variables are reported as medians with interquartile ranges (IQRs) or ranges and categorical variables as counts and percentages. Clopper–Pearson confidence intervals [CIs] were applied to the primary and secondary endpoints. Due to special regulations for orphan diseases in Japan, the statistical significance level was set at 10% (two-sided). However, for scientific publications in this paper, it was planned to be set at 5% (two-sided). Subsequently, the results were interpreted based on 95% CIs, and 90% CIs were described for reference purposes.

The primary analysis for the primary endpoint was planned to apply the exact binomial test with a null hypothesis based on historical results of surgical treatment (48%). Due to the insufficient statistical precision of the result based on only 23 cases, Fisher's exact test was applied as a post-hoc additional analysis for the primary endpoint. The component and secondary endpoints were analysed to support the clinical interpretation of the primary composite endpoint. The primary composite endpoint, component endpoints, and secondary endpoints were evaluated in cohorts 1 (tumour size, <10 mm) and 2 (10–15 mm). All statistical analyses were conducted by clinical statisticians (Y. N. and M. Y.) using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

Study population

Of the 28 eligible patients who provided informed consent, three were excluded (no definitive diagnosis of PNEN grade 1 using EUS-FNA [one patient], a <2 mm distance between the tumour and main pancreatic duct [one patient], and considered as an inappropriate candidate [one patient]). Overall, 25 patients with non-functional tumours (median tumour size, 10.1 [IQR: 7.0–11.0] mm) were analysed and treated with EUS-EI. After treatment, 24 patients completed the schedule, and 1 died because of cardiac infarction 5 months after treatment. The characteristics of the enrolled patients and each cohort are shown in Table 1 and Supplemental Table 2.

Primary composite and component endpoints

The proportion of patients who achieved the primary composite endpoint comprising efficacy and safety was 76.0% (19/25) (95% CI, 54.9%–90.6%), which was significantly higher than that the historical results (48%) of surgical treatment (exact binomial test, P=0.0083) (Table 2). Additionally, the difference in the achievement rate (EUS-EI minus surgical treatment) was 28.2% (95% CI: -2.4 to 58.8, Fisher's exact test, P=0.0729, post-hoc analysis), which showed a trend similar to the primary result (Supplemental Table 3). Supplemental Table 4 illustrates the primary composite endpoint evaluated in each cohort.

Secondary endpoints

The secondary endpoints are shown in Table 3. Regarding efficacy, the complete ablation rate on CE-CT at 1 and 6 months was 88.0% (22/25). The 6-month overall survival rates were 96% (24/25) and one patient died due to a cardiac infarction 5 months after treatment. For safety, the prevalence of total and severe AEs within 1 month were 68.0% (17/25) and 4.0% (1/25),

respectively. No severe pancreatic fistulas were observed at 1 month after treatment. The incidence of DM or its exacerbation at 6 months was 12.0% (3/25). Among these, one patient had DM and two had worsened pre-existing DM. Device failure needle obstruction due to blood clots occurred in one patient (4.0%); ethanol could not be injected after puncturing the tumour. Therefore, once the needle was removed, it was flushed with a saline solution. None of the patients required surgery. Supplemental Table 5 illustrates the secondary endpoints evaluated in each cohort.

Treatment results

Supplemental Table 6 illustrates the treatment results. Among the 25 patients who underwent EUS-EI, 32.0% (8/25) underwent additional sessions within the same hospitalisation period. The number of punctures in the initial session was 1 in 4 (16.0%), 2 in 12 (48.0%), and 3 in 9 (36.0%) patients. Notably, all patients with a tumour size of 10–15 mm were treated with multiple punctures for other parts of the tumour. The median (range) injected ethanol volume per tumour was 1.0 (0.3–3.6), and the median (range) total ethanol volume per initial and additional session was 0.9 (0.3–2.0) and 0.9 (0.3–1.6) mL, respectively. Furthermore, the median (IQR) procedure time was 21.0 (14.0–30.0) min, and hospitalised days were 6 (5–7) days.

Procedure-related AEs

Procedure-related AEs occurred in 60.0% (15/25) of the patients: 14 (56.0%) and 1 (4.0%) had grade 1 or 2 and grade 3 AEs, respectively. Acute pancreatitis occurred in 20.0% (5/25): 4 showed mild pancreatitis and 1 had moderate pancreatitis, which improved with conservative treatment within 7 days. Hyperamylasaemia occurred in eight, all of whom had decreased serum amylase levels without treatment. Sedation-induced hypotension occurred in one (4.0%)

during the procedure; the patient experienced a rapid increase in blood pressure following rapid fluid replacement (Table 4).

Discussion

This is the first prospective multicentre study to evaluate EUS-EI for small grade 1 PNENs. This study set the maximum amount of ethanol required per session to ensure safety, and an additional session was planned to optimise the complete ablation rate. Complete ablation could not be achieved in all cases with EUS-EI; however, a high rate (88%) was observed. Severe AEs occurred in only one patient (4%).

Observation of stable, small, incidentally discovered PNENs is considered reasonable for selected patients; however, the 5-year survival of such patients and characteristics of small-sized tumours that may subsequently grow remain undetermined.[4] Regarding tumour size, previous reports on lymph node metastasis indicated an increased risk with tumours sized >15 mm.[26] A recent international study reported that an unfavourable prognosis of non-functional small PNETs was related to a tumour size of >15 mm, Ki-67 index of >3%, and nodal metastasis.[27] Therefore, a tumour size less than 15 mm was considered appropriate for local endoscopic treatments with curative intent.

EUS-EI and RFA have recently been performed for small PNENs. A recent meta-analysis including 181 (100 EUS-RFA, 81 EUS-EI) patients with PNETs (mean size 15.1±4.7 mm) reported no significant difference in the rates of technical success (94.4% vs 96.7%, *P*=0.42), clinical success (85.2 vs 82.2%, *P*=0.65), and AEs (14.1% vs 11.5%, *P*=0.7) between EUS-RFA and EUS-EI, respectively.[28] However, the included reports studied only non-functional PNENs, and the complete ablation rate for EUS-EI (60%–80%) was lower than that for EUS-RFA (86%–100%) [18]. While the complete ablation rate was 64.0% (16/25) in a single session in this study, consistent with that reported previously, we planned an additional

session for patients with insufficient response.[8] As a result, the complete ablation rate increased to 88.0% (22/25). Regarding additional sessions, CE-EUS was conducted for patients in whom identifying residual tumours with B-mode was challenging. [25] Notably, among eight patients receiving additional treatment, CE-EUS was performed in six (75%), and complete ablation was achieved in four. Previous reports assessed complete ablation on CE-CT at 3 months posttreatment and planned additional ethanol therapy for incomplete cases. [6,7] We have encountered cases where surgical resection was necessary after EUS-EI. Pathological findings of resected specimens revealed highly fibrotic changes in the ethanol-treated areas, with residual tumours surrounded by significant fibrosis.[8] This fibrosis probably prevented the spread of injected ethanol into the residual tumour. Higher treatment efficacy was achieved by performing additional treatment 3–5 days post-initial therapy compared with that reported previously. Based on the tumour size, the complete ablation rate was 91.7% (11/12) and 84.6% (11/13) for tumours sized <10 and 10–15 mm, respectively. Although the results were obtained in relatively small cases, EUS-EI may be sufficiently effective, particularly for tumours sized <10 mm (Supplemental Table 7).

Khoury et al's [22] meta-analysis of EUS-RFA including 292 patients with PNENs reported a technical success rate of 99.2% (95% CI 97.9%–99.9%), a complete radiological response of 87.1% (95% CI 80.1%–92.8%), and an AE incidence of 20.0% (95% CI 14.0%–26.7%), while the severe AE incidence was 0.9% (95% CI 0.2%–2.3%). The most common AEs were transient mild abdominal pain (19 patients, 6.5%), and mild-to-moderate pancreatitis (23 patients, 7.9%). In their report, complete ablation was associated with the power setting of RFA system. A power setting of <50 W achieved complete ablation in 92.4% of cases, while that of 50 W achieved complete ablation in 84.6%. In RFA treatment, using lower wattage for ablation results in longer ablation times and broader ablation areas compared with higher wattage.[29] Consequently, there is a possibility of spreading heat effect to the peripancreatic

area, potentially leading to complications. In RFA treatment, there have been reports of complications such as pancreatic necrosis, bleeding of the gastrointestinal wall, or death, [19-21] which aren't typically experienced with EUS-EI.

Regarding safety, only one patient had moderate pancreatitis, which improved with conservative treatment. Among 5 and 20 patients with or without pancreatitis after EUS-EI, the median total EI volume was 1.4 mL and 1.0 mL, respectively, indicating a higher ethanol volume in patients with pancreatitis. Moderate pancreatitis occurred in patients who were injected 2.0 mL of ethanol /session (Supplemental Table 8). The ethanol volume/session is associated with pancreatitis; thus, it should be minimized to the extent possible.

A study on the surgical resection of benign pancreatic tumours revealed that the morbidity rates for pancreaticoduodenectomy, distal pancreatectomy (DP), and parenchyma-preserving resection were 52%, 47%, and 44%, respectively.[5] In a recent study comparing the treatment results of EUS-RFA and surgical treatment for pancreatic insulinoma, the surgical resection morbidity and severe AE rates were 61.8% (55/89) and 15.8%.[30] These data are similar to the historical results of surgical treatment referred to in this study. Furthermore, in pancreaticoduodenectomy and DP, which involve extensive resection of the pancreas, postoperative complications, such as DM and impaired nutrient absorption, occurred in 14%–18% and 17%–33% of cases, respectively. [5,31] The incidence of newly developed DM was 4.0% (1/25) in this study, and EUS-EI essentially preserved the pancreatic function. In EUS-EI, serious AEs and pancreatic fistulas occurred in 1/25 and 0/25 patients, respectively, within 1 month, whereas in surgical treatment, both occurred in 15/23 patients, which is a significant improvement in the primary composite endpoint.

This study has some limitations. First, this study was designed as a multicentre, single-arm prospective study rather than a randomised controlled trial (RCT). Considering the limited number of potentially eligible patients, it was difficult to conduct a larger RCT with adequate

statistical power. Second, we referred to a limited-sized historical surgical treatment study result for the primary endpoint analysis. Due to the insufficient statistical precision of the reference, we performed post-hoc additional analyses. The difference in the achievement rates between EUS-EI and surgical treatments was 28.2% (95% CI: -2.4 to 58.8, Fisher's exact test: *P*=0.0729). Although it did not indicate statistically significant, the 95% CI showed a trend similar to the primary result. Because the distribution of patient characteristics wasn't balanced between EUS-EI in this study and surgical resection, a logistic regression model was additionally applied to the primary endpoint, incorporating treatment procedures (surgical resection/ EUS-EI) as an independent variable and function/non-function, tumour size (<10/ 10–15 mm) as covariates. This revealed an odds ratio for the treatment procedures of 3.964 (95% CI: 0.925–16.980, *P*=0.0635), which was similar to the main results of this study. Third, since we planned the follow-up period after treatment as 6 months to assess the acute and sub-acute course of patients with PNEN after treatment with EUS-EI, the follow-up period was inadequate to evaluate tumour recurrence. So et al's study on long-term treatment outcomes of EUS-EI for small PNENs revealed that of the 97 patients treated with EUS-EI, 63 (65%) showed complete ablation (mean tumour size, 12.08±3.6 mm).[17] During follow-up, 29 (46.0%) patients showed local recurrence after complete ablation. The median duration from the first session to recurrence was 34.5 months. Therefore, a long-term follow-up of at least 5 years is required to prove the efficacy of EUS-EI. For future treatment outcomes, we have already initiated a long-term prospective observational study involving patients who participated in this study (UMIN000044094).

In conclusion, EUS-EI appears safe, effective, and minimally invasive for treating small PNENs. Therefore, in addition to surgical treatment or observation, it could be considered an optimal treatment option for small PNENs.

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Figure Legends

Figure 1.

Flowchart of the study.

CE-CT, contrast-enhanced computed tomography; EUS, endoscopic ultrasonography; ITT, intention-to-treat.

Figure 2.

Images of treatment using EUS-guided ethanol injection.

A: A well-enhanced tumour measuring 10 mm located in the body of the pancreas (arrow).

B: A 25-gauge needle is inserted into the far side of the tumour.

C: Ethanol being injected until the hyperechoic bubble extended to the tumour margin.

D: Once the needle was removed, we examined the low-echoic part of the tumour (arrow). The needle is inserted into the low-echoic area.

E: Ethanol being injected until the hyperechoic bubble extended to the tumour margin.

F: No enhanced areas were noted in the tumour 3 days after the procedure.

EUS: endoscopic ultrasonography

Video legend

Video text

A 25G needle was used to prevent ethanol leakage from the puncture points and for easy handling. First, we advanced the needle into the far side of the tumour. Subsequently, 0.5 mL of ethanol was injected into this area until a hyperechoic bubble extended to the tumour margins. The needle was kept inside the tumour for at least 1 min to avoid ethanol backflow. Once the needle was removed, low-echoic areas of the tumour (arrow) were examined. The needle was

inserted into a low-echoic area, and 0.4 mL of ethanol was injected into it. After this second injection, another small low-echoic area tumour was detected (arrow). A third injection was administered with 0.1 mL of ethanol. A hyperechoic blush extended to the tumour margin wall. There were no other low-echoic areas in the tumour.

Video Image

EUS-guided small volume ethanol injections at multiple sites

Supplementary Material

Author Names

Kazuyuki Matsumoto, Hironari Kato, Takao Itoi, Masayuki Kitano, Kazuo Hara, Masaki Kuwatani, Mamoru Takenaka, Reiko Ashida, Shuntaro Mukai, Nozomi Okuno, Kazumichi Kawakubo, Tatsuhiro Yamazaki, Jun Sakurai, Yuki Nakatsuka, Michihiro Yoshida, Motoyuki Otsuka

Title of Paper

Efficacy and Safety of Endoscopic Ultrasonography-Guided Ethanol Injection of Small Pancreatic Neuroendocrine Neoplasms: a prospective, multicenter study

Supplemental Figure Legends

Supplemental Figure 1

A 25G needle was used to prevent ethanol leakage from the puncture points and to allow easy handling. The injection was started from the parts of the tumour, which were deeper on the EUS image, because a spread hyperechoic bush prevents recognition of tumour's low-echoic parts on the far side. Once the needle was removed, we looked for the low-echoic parts; if detected, ethanol injection was added into these low-echoic parts. For safety, the amount of ethanol per puncture, total number of punctures per session, and

maximum volume of ethanol injected per session were set to 1 mL, 3 times, and 2 mL, respectively.

Supplemental Figure 2

Protocol for EUS-guided ethanol injection therapy. CE-CT was performed 3–5 days after the initial procedure. If there was an enhanced tumour area, additional injections were administered during the same hospitalisation period. Otherwise, the patient was discharged. For safety, the total number of punctures and maximum volume per session were set to 3 and 2.0 mL, respectively.

CE-CT: contrast-enhanced computed tomography

Supplemental Figure 3

Images of additional treatment of EUS-guided ethanol injection.

A: A well-enhanced tumour measuring 14 mm located in the head of the pancreas (arrow).

B: Ethanol being injected until the hyperechoic bubble extended to the tumour margin.

C: CE-CT findings 3 days after the procedure. There was a small enhanced area at the periphery of the tumour (arrow).

D: The residual tumour is clearly enhanced with CE-EUS (arrow) (left image: B-mode, right image: CE-mode).

E: Pinpoint ethanol injection of the residual part with a 25-gauge needle.

F: No enhanced areas were found in the tumour 3 days after the procedure.

EUS: endoscopic ultrasonography

CE-CT: contrast-enhanced computed tomography

CE-EUS: contrast-enhanced endoscopic ultrasonography

Supplemental Table 1. Patient characteristics and outcomes of historical data

	Operation (n=23)	EUS-EI (n=8)
Age, median (IQR), years	65 (54–67)	58 (55–70)
Male/female, *n*	11/12	5/3
Tumour size, median (IQR), mm	11.0 (8.0–13.0)	9.5 (8.0–12.0)
Tumour location, *n* (%)		
Head	10 (43.5)	4 (50.0)
Body	7 (30.4)	2 (25.0)
Tail	6 (26.1)	2 (25.0)
Non-function/function, *n*	15/8	8/0
Insulinoma	6	0
Gastrinoma	2	0
Operation, *n*		
PD	10	-
DP	10	-
Central resection	3	-
Morbidity*, *n* (%)		
Overall	15 (65.2)	1 (12.5)
Severe	8 (34.8)	0
Pancreatic fistula**, *n* (%)		
Overall,	12 (52.2)	0
Grades B–C	9 (39.1)	0
Days hospitalised, median (IQR), days	24 (18–35)	4 (3–4)
Achievement of composite endpoints, % (*n*)	47.8 (11)	75.0 (6)
Local control rate at 1 and 6 months	100.0 (23)	75.0 (6)
Incidence of severe AEs within 1 month	34.8 (8)	0
Rate of pancreatic fistula at 1 month	34.8 (8)	0
Incidence of DM and/or exacerbation of DM at 6 months	13.0 (3)	0

EUS-EI: endoscopic ultrasonography-guided ethanol injection, IQR: interquartile range, PD: pancreaticoduodenectomy, DP: distal pancreatectomy, AE: adverse event, DM: diabetes mellitus. *Morbidity was evaluated using the Clavien–Dindo classification for operation and the American Society for Gastrointestinal Endoscopy (ASGE) and Common Terminology Criteria for Adverse Events (CTCAE) for EUS-EI. Severe was defined as grade ≥III for operation, moderate or higher in ASGE, and grade ≥III in CTCAE for EUS-EI.

Supplemental Table 2. Patients' characteristics in each cohort

	Tumour size <10 mm (*n*=12)	**Tumour size 10–15 mm (*n*=13)**
Age, median (IQR), years	66 (59–72)	54 (46–62)
Male/female, *n*	6/6	9/4
Tumour size, median (IQR), mm	7.0 (7.0–8.5)	11.0 (10.3–12.0)
Tumour location, *n* (%)		
Head	5 (41.7)	6 (46.2)
Body	3 (25.0)	5 (38.5)
Tail	4 (33.3)	2 (15.4)
Non-function/function, *n*	12/0	13/0
Performance status, *n* (%)		
0	11 (91.7)	11 (84.6)
1	1 (8.3)	2 (15.4)
Presence of DM, *n* (%)	3 (25.0)	5 (38.5)

DM: diabetes mellitus, IQR: interquartile range

Supplemental Table 3. Difference in the proportion of achievement (%) between the EUS-guided EI and surgical treatment (post-hoc additional analysis)

Endpoints	EUS-guided EI (N=25)	Surgical treatment (historical data) (N=23)	Difference in the proportion of achievement (%) (EUS-EI minus surgical)			Fisher's exact test
	Number of achievements (%)	Number of achievements (%)	Estimate	90% CI*	95% CI	*P* value**
Composite endpoint (ITT analysis)	19 (76.0)	11 (47.8)	28.2	1.8-54.5	-2.4 to 58.8	0.0729
1. Efficacy						
Complete ablation at 1 month	22 (88.0)	23 (100.0)	-12.0	-26.9 to 2.9	-28.9 to 4.9	-
Complete ablation at 6 months	22 (88.0)	23 (100.0)	-12.0	-26.9 to 2.9	-28.9 to 4.9	-
2. Safety						
Avoidance of severe AEs within 1 month	24 (96.0)	15 (65.2)	30.8	9.1-52.5	5.7-55.9	-
Avoidance of pancreatic fistula at 1 month	25 (100.0)	15 (65.2)	34.8	14.3-55.3	11.1-58.4	-
Avoidance of incidence and/or exacerbation of DM	21 (84.0)	20 (87.0)	-3.0	-17.9 to 23.8	-21.1 to 27.0	-

at 6 months

ITT: intention-to-treat, AE: adverse event, DM: diabetes mellitus, CI: confidence interval

*For scientific publications, statistical significance level was set at 5% (two-sided). The corresponding two-sided 90% CI was shown purely as a reference because the sample size was calculated with a two-sided significance level of 10%.

** Compared with historical control of surgical treatment.

Supplemental Table 4. Details of achievements of primary composite and the component endpoints in each cohort

ITT: intention-to-treat, AE: adverse event, DM: diabetes mellitus, CI: confidence interval

Endpoints	Tumour size, mm	Number of patients	Number of achievements	Number of non-achievements	Number of unevaluable patients*	Percentage of achievement (%)			Exact binomial test
						Point estimation	90% CI	95% CI	*P* value**
Primary composite endpoint (ITT analysis)[a)]	＜10	12	10	2	0	83.3	56.2–97.0	51.6-97.9	.3751
	10-15	13	9	4	0	69.2	42.7-88.7	38.6-90.9	.0758
Component endpoints									
1. Efficacy									
Complete ablation at 1 month	＜10	12	11	1	0	91.7	66.1-99.6	61.5-99.8	-
	10-15	13	11	2	0	84.6	59.0-97.2	54.6-98.1	
Complete ablation at 6 months	＜10	12	11	1	0	91.7	66.1-99.6	61.5-99.8	-
	10-15	13	11	1	1	84.6	59.0-97.2	54.6-98.1	
2. Safety									
Avoidance of severe AEs within 1 month	＜10	12	12	0	0	100.0	77.9-100.0	73.5-100.0	-
	10-15	13	12	1	0	92.3	68.4-99.6	64.0-99.8	
Avoidance of pancreatic fistula at 1 month	＜10	12	12	0	0	100.0	77.9-100.0	73.5-100.0	-
	10-15	13	13	0	0	100.0	79.4-100.0	75.3-100.0	
Avoidance of incidence and/or exacerbation of DM at 6 months	＜10	12	11	1	0	91.7	66.1-99.6	61.5-99.8	-
	10-15	13	10	2	1	76.9	50.5-93.4	46.2-95.0	

a) Achievement proportional to the composite endpoint in surgical treatment was 67% (4/6) in tumour size less 10mm and 41% (7/17) in tumour size 10-15mm.

* One patient died due to cardiac infarction 5 months after the procedure.

** The null hypothesis was set with the historical result of a study that included surgical treatment.

Supplemental Table 5. Details of secondary endpoints in each cohort

	Tumour size <10 mm (n=12)	Tumour size 10–15 mm (n=13)
Efficacy		
Complete ablation at 1 month, % (*n*)	91.7 (11)	84.6 (11)
Complete ablation at 6 months, % (*n*)	91.7 (11)	84.6 (11)
Six-month overall survival, % (*n*)	100 (12)	92.3 (12*)
Safety		
Prevalence of total adverse events, % (*n*)	66.7 (8)	69.2 (9)
Prevalence of severe AEs within 1 month, % (*n*)	0.0 (0)	7.7 (1)
Prevalence of severe pancreatic fistula at 1 month, % (*n*)	0.0 (0)	0.0 (0)
Prevalence of DM incidence and/or exacerbation at 6 months, % (*n*)	8.3(1)	15.4 (2)
Prevalence of device failures, % (*n*)	0.0 (0)	7.7 (1)
Prevalence of conversion to surgery, % (*n*)	0.0 (0)	0.0 (0)

AE: adverse event, DM: diabetes mellitus, IQR: interquartile range

* One patient died due to a cardiac infarction 5 months after treatment

Supplemental Table 6. Treatment results

IQR: interquartile range

	All patients (n=25)	Tumour size <10 mm (n=12)	Tumour size 10–15 mm (n=13)
Number of therapies during hospitalisation, *n* (%)			
One time	17 (68.0)	10 (83.3)	7 (53.8)
Two times	8 (32.0)	2 (16.7)	6 (46.2)
Number of punctures per session, *n* (%)			
Initial session (n=25)			
One puncture	4 (16.0)	4 (33.3)	0 (0.0)
Two punctures	12 (48.0)	5 (41.7)	7 (53.8)
Three punctures	9 (36.0)	3 (25.0)	6 (46.2)
Additional session (n=8)			
One puncture	1 (12.5)	0 (0.0)	1 (16.7)
Two punctures	5 (62.5)	2 (100.0)	3 (50.0)
Three punctures	2 (25.0)	0 (0.0)	2 (33.3)
Injected ethanol volume per tumour, median (range), mL	1.0 (0.3–3.6)	0.8 (0.3–1.9)	1.1 (0.7–3.6)
Initial session (n=25)			
First puncture	0.5 (0.1–1.0)	0.3 (0.1–0.9)	0.7 (0.2–1.0)
Second puncture	0.3 (0.1–1.0)	0.3 (0.1–0.5)	0.3 (0.1–1.0)
Third puncture	0.4 (0.1–0.8)	0.5 (0.2–0.6)	0.4 (0.1–0.8)
Total ethanol volume per session	0.9 (0.3–2.0)	0.8 (0.3–1.4)	1.0 (0.4–2.0)
Additional session (n=8)			
First puncture	0.4 (0.2–1.0)	0.5 (0.3–0.6)	0.4 (0.2–1.0)
Second puncture	0.3 (0.1–0.5)	0.3 (0.2–0.4)	0.3 (0.1–0.5)
Third puncture	0.6 (0.2–0.9)	-	0.6 (0.2–0.9)
Total ethanol volume per session	0.9 (0.3–1.6)	0.8 (0.5–1.0)	0.9 (0.3–1.6)
Total procedure time, median (IQR), min	21.0 (14.0–30.0)	20.5 (12.5–31.0)	21.0 (18.0–29.0)
Days hospitalised, median (IQR), days	6 (5–7)	6 (6–7)	6 (5–7)

Supplemental Table 7. Details of incomplete ablation cases

Case	Age	Sex	Tumour size, mm	Location	Number of therapies	Total ethanol volume/ initial session, mL	Total ethanol volume/ additional session, mL	Total ethanol volume/ session, mL	Contrast medium assisted ethanol injection
1	62	F	11	Head	2	1.4	0.9	2.3	Yes
2*	61	M	9	Tail	1	1.0	-	1.0	No
3	59	M	15	Body	2	2.0	1.6	3.6	Yes

F: female, M: male

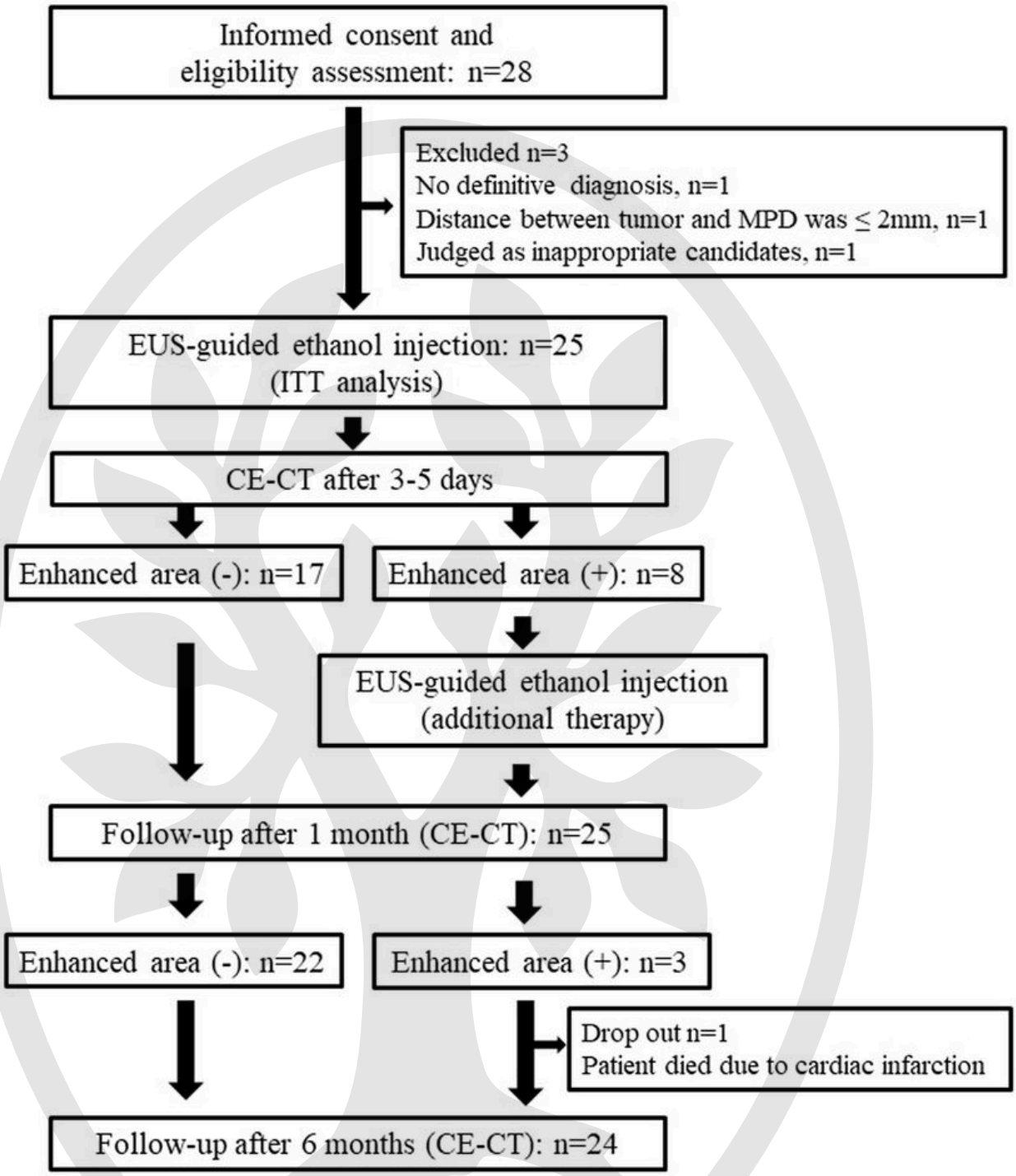
*Mild pancreatitis occurred after the initial session

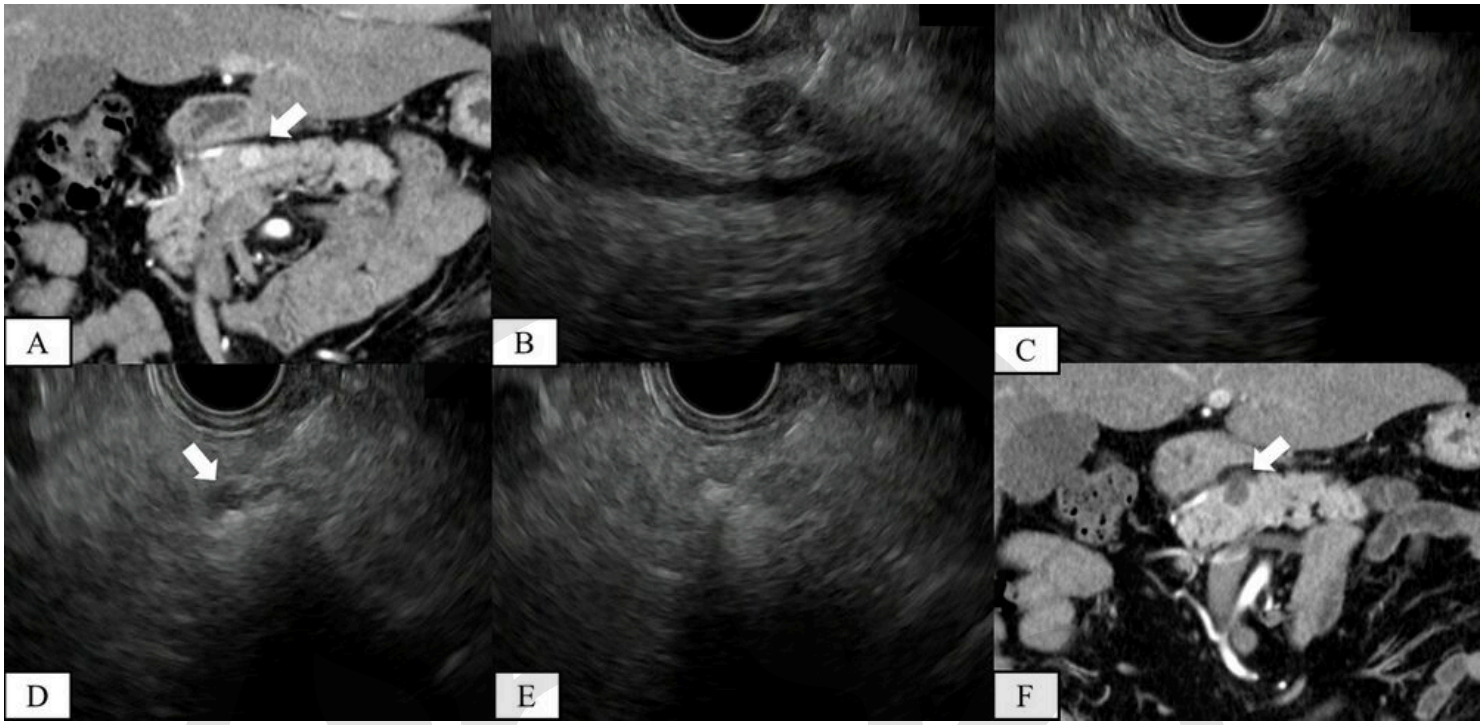
Supplemental Table 8. Details of pancreatitis cases

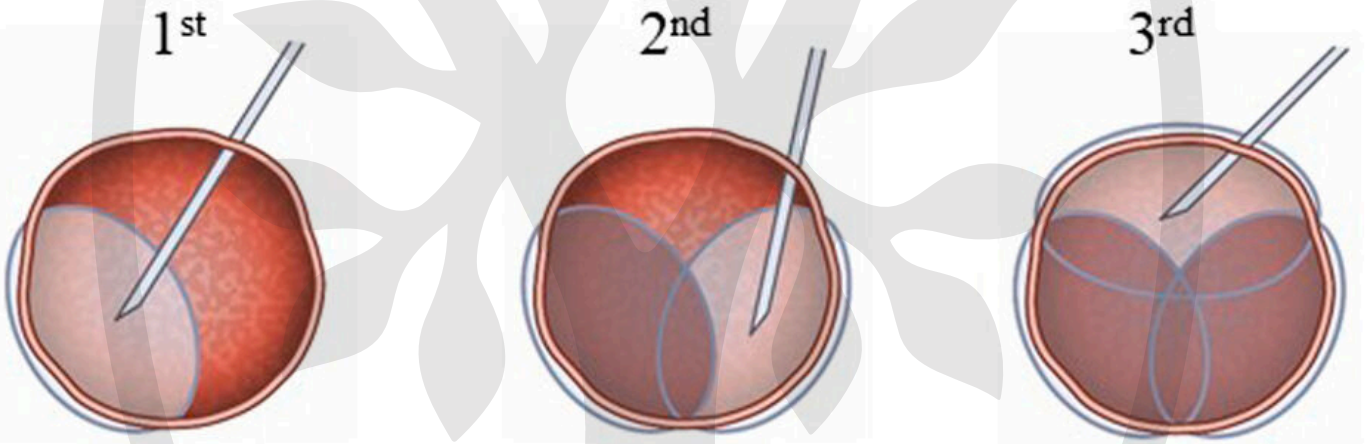
Case	Age	Sex	Tumour size, mm	Location	Pancreatitis grade*	Number of therapies	Total ethanol volume/ initial session, mL	Total ethanol volume/ session, mL
1	41	M	14	Head	Mild	2	2.0	3.0
2	56	F	7	Head	Mild	1	1.4	1.4
3	70	M	12	Tail	Moderate	1	2.0	2.0
4	61	M	9	Tail	Mild	1	1.0	1.0
5	75	M	9.7	Body	Mild	1	0.8	0.8

F: female, M: male

*According to the American Society for Gastrointestinal Endoscopy (ASGE) criteria







- ✓ Use 25 gauge needle
- ✓ Start injection from the deeper side
- ✓ Additional ethanol injections into low-echoic parts