**Title/Cover page**

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**Title:** The efficacy and safety of scheduled early endoscopic ultrasonography-guided ethanol reinjection for patients with pancreatic neuroendocrine tumors: A prospective pilot study

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**Conflict of interest**

All authors declare no conflicts of interest.

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**Authors' Contributions**

KM, HK and SH: conception and design of the research and writing the paper. SK and HF: evaluation of radiographic findings. KN: evaluation of pathological findings. RH, MF and RY: establishment of the safety evaluation committee and the analysis and interpretation of

data. YM and TY: collection of the surgical specimens. HO: final approval of the article. All authors read and approved the final manuscript.

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**ABSTRACT**

Endoscopic ultrasonography (EUS)-guided ethanol injection was recently proposed for the treatment of patients with small pancreatic neuroendocrine tumors (p-NETs); however, tips on how to perform safe and effective procedures are unclear. We launched a pilot study for scheduled early EUS-guided ethanol reinjection for small p-NETs. The major eligibility criteria were the presence of a pathologically diagnosed grade (G) 1 or G2, a tumor size of ≤2 cm and being a poor or rejected candidate for surgery. For the treatment, we used a 25-gauge needle and pure ethanol. Contrast-enhanced computed tomography (CE-CT) was performed on postoperative day 3, and if enhanced areas of the tumor were still apparent, an additional session was scheduled during the same hospitalization period. The primary endpoint was the complete ablation rate at one month after treatment, and the secondary endpoint was the procedure-related adverse events. A total of five patients were treated. The median size of the tumor was 10 (range: 7-14) mm. Of the five patients, three underwent an additional session. The median volume of ethanol injection per session was 0.8 (range: 0.3-1.0) mL, and the total was 1.0 (0.9-1.8) mL. Complete ablation was achieved in 4 of the 5 tumors (80%) with no adverse events. During one year of follow-up, none of the patients reported any procedure-related adverse events, and no recurrence of tumor. Scheduled early

EUS-guided ethanol reinjection appears to be safe and effective for treating small p-NETs (UMIN number: 000018834).

**Key words:** pancreatic neuroendocrine tumor, ethanol injection, EUS-guided therapy

## INTRODUCTION

The optimal approach for treating patients with nonfunctional, small (≤2 cm in diameter) pancreatic neuroendocrine tumors (p-NETs) is controversial[1,2]. Recently, endoscopic ultrasonography (EUS)-guided ablation has been proposed for the treatment of patients with small p-NETs[3-8]. Using a pure ethanol or ethanol-lipiodol emulsion, the complete ablation rate has been reported to be about 50% with a single session and up to 60% with an additional session[3,4]. As for complication, mild pancreatitis occurred in few patients with the smaller tumor than 2 cm which required the injection with large amount of ethanol of > 2 mL in one session[3-7].

When an additional session is scheduled before the first injection, the volume of ethanol can be adjusted to be within the safe range. Therefore, we launched the current trial to assess whether or not scheduled early EUS-guided ethanol reinjection is effective and safe for treating small p-NETs.

## CASE REPORT

*Patients*

This was a single-center, prospective pilot study conducted between October 2015 and March 2019. The major eligibility criteria were an age ≥20 years, the presence of a pathologically diagnosed grade (G) 1 or G2 p-NET, a tumor size of ≤2 cm on contrast-enhanced computed tomography (CE-CT) and being a poor candidate or rejected for surgery. The details have been described in a previous protocol article[9].

Written informed consent was obtained. This study was conducted in compliance with the principles of the Declaration of Helsinki. The study's protocol has been approved by the institutional review board of our hospital (approval number. 1510-003) and registered in UMIN (number. 000018834).

*Study flow and EUS-guided procedures*

Figure 1 presents the flow chart of the study. For the treatment, a 25-G fine-needle aspiration needle (Expect; Boston Scientific Corporation, Marlborough, MA, USA) filled with ethanol was advanced into the tumor under EUS. Then pure ethanol (Mylan Seiyaku Ltd., Tokyo, Japan) was injected until a hyperechoic blush extended to the tumor's whole margin and kept the needle inside the tumor at least one minute to avoid the back flow of ethanol. Once

the needle was removed, we checked for low-echoic areas of the tumor. If such areas were detected, ethanol was added to the site. We set the amount of ethanol per puncture at 1 mL and the total number of punctures per session at 3 for the sake of safety. To evaluate the tumor's viable regions, contrast-enhanced EUS (CE-EUS) imaging with perflubutane (Daiichi-Sankyo Co., Ltd., Tokyo, Japan) was used as the contrast agent.

Three days after the treatment, CE-CT was performed to evaluate the tumor viability and procedure-related adverse events. If enhanced areas of the tumor were noted on CE-CT, an additional session was scheduled during the same hospitalization period. Blood testing at 2 h postoperatively and on postoperative day (POD) 1 and POD 3 was performed.

*Endpoints and follow-up*

The primary endpoint was the complete ablation rate at one month after the first ethanol treatment, and the complete ablation was defined as the absence of enhanced areas within the tumor with 1-mm thickness on CE-CT. The CE-CT images were reviewed by an expert radiologist and an independent expert gastroenterologist. The secondary endpoints measured the adverse events associated with the procedure, volume of ethanol injected, number of sessions, number of days spent in the hospital, incidence of diabetes mellitus after treatment, and recurrence of the tumor.

Follow-up CE-CT was performed every three months. Recurrence was defined as the detection of enhanced areas in the complete ablated tumor during one-year follow-up on CE-CT. If incomplete ablation occurred after the second treatment, we abandoned ethanol injection therapy and started considering other treatments.

*Results*

Table 1 shows the characteristics and treatment outcomes of five patients. All patients had non-functional tumors. Of the five patients, three underwent an additional session during the same hospitalization period. For the treatment, the median (range) total volume of ethanol injection was 1.0 (0.9-1.8) mL, the volume of ethanol injected per session was 0.8 (0.3-1.0) mL, and the number of punctures per session was 2 (2-3).

Complete ablation was achieved in 80% (4/5) of patients. There were no adverse events during or after the procedures (median [range] serum AMY levels before the procedure and at POD 1 and POD 3: 66 (49-88), 125 (71-266) and 78 (58-118) U/L, respectively). Furthermore, in the patients who achieved complete ablation, there were no cases of deterioration in the hemoglobin A1c (HbA1c) before the operation or every 3 months after the procedure (median values of HbA1c with National Glycohemoglobin Standardization Program before the procedure and at 6 and 12 months after the procedure:

6.0, 5.9 and 5.9, respectively). During one year of follow-up, there were no cases of recurrence in the patients with complete ablation.

The images of the patients successfully treated with and without an additional session are shown (no. 3; supplemental Figure 1 and Video 1, no. 5; Figure 2 and supplemental Video 2). For patient no. 2, although an additional 1.2 mL of ethanol was injected at the second treatment (total 3.0 mL for tumor), enhanced parts of the tumor still remained. Although the patient had initially refused surgery, we continued to suggest the need of surgery, and the patient ultimately decided to undergo surgery 15 months after the second procedure. The pathological findings revealed viable tumors at the periphery of the ablation area, and the center of the tumor showed fibrotic tissue changes (Figure 3).

## DISCUSSION

This is the first prospective study concerning scheduled early ethanol reinjection therapy for treating small p-NETs during the same hospitalization period.

Previous reports on treatment with EUS-guided ethanol injection have described the amount of ethanol as ranging from 0.3-8.0 mL[3-7]. Park et al[3]. reported the outcomes of 11 patients with 14 tumors, and a median 1.6 (range: 0.5-3.8) mL of ethanol per session was used for tumors of a median 12.2 (range 8-19) mm in size. The median total amount of ethanol for each tumor was thus 2.5 (range 0.5-7) mL. The tumor size that could be treated with the least amount of ethanol (0.5 mL) was 11 mm in the pancreatic body, while the size that needed the most amount of ethanol (7 mL) was 12 mm in the pancreatic head. It is therefore difficult to define the proper amount of ethanol needed to ablate a tumor. However, 3 out of 11 treated patients developed pancreatitis, all of whom were injected with >2.0 mL of ethanol per session (2.3, 2.4 and 7.0 mL). Therefore, considering the adverse events, ≤2.0 mL of ethanol per session seems to be better for avoiding adverse events. Choi et al.[4] reported that the presence of a capsule around the tumor was a significant factor influencing the success of complete ablation ($p$=0.008).

Performing an evaluation at three days after the procedure allowed us to not only the tumor viability but also the early adverse events. However, the animal experiments for

ethanol injection using porcine pancreas suggested that the effect of ethanol continued and spread after the injection[10]. A further examination is therefore necessary to determine the optimal timing for evaluating tumors after initial ethanol injection.

Several limitations associated with the present study warrant mention. First, it was conducted in a small number of patients. Second, the decision to perform complete ablation was carried out CE-CT without histology. Third, the follow-up period was not sufficient to evaluate tumor recurrence.

In conclusion, scheduled early ethanol reinjection to minimize the amount of total ethanol injection per session is effective and feasible. Future multi-center studies with increased numbers of patients will be required.

**Conflicts of Interest**

Authors declare no conflicts of interest.

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**Figure legends**

**Figure 1.** The flow chart of this study. EUS: endoscopic ultrasonography. CE-CT: contrast-enhanced computed tomography.

**Figure 2.** A: Contrast-enhanced computed tomography (CE-CT) image showing a hypervascular tumor 10 mm in diameter in the pancreatic body (arrow). B: Endoscopic ultrasonography (EUS)-guided puncture of the tumor (arrows) with a 25-G needle (arrowhead) and injections of pure ethanol into the tumor. C: CE-CT image three days after the procedure. There were enhanced areas in the periphery of the tumor (arrows), so an additional session was planned. D: EUS image of an additional session. There were low-echoic areas in the periphery of the tumor (arrows). E: CE-CT image one month after the additional session. The previously enhanced areas of the tumor could not be detected on CE-CT (arrow), and the tumor was treated completely with ethanol ablation. F: CE-CT image one year after the procedure. There were no enhanced areas in the ablated area (arrow).

**Figure 3.** A: Contrast-enhanced computed tomography (CE-CT) image showing a hypervascular tumor 14 mm in diameter in the pancreatic head (arrow). B: CE-CT image one month after the first treatment. There were enhanced areas in the periphery of the tumor

(arrows), indicating that the tumor had been treated incompletely. C: Contrast-enhanced endoscopic ultrasonography (CE-EUS) image showing enhanced areas in the periphery of the tumor (arrows). Left image: B mode. Right image: CE mode. D: Macroscopic view of the surgically resected specimen. The ethanol-injected area shows up as white in the pancreatic parenchyma (arrow). E: There are viable tumors in the periphery of the treated area (arrows). The center part of the tumor shows fibrosis, which was suspected to be due to tumor necrosis (H&E staining). F: Masson trichrome staining shows fibrotic changes at the center of the tumor. Viable tumors are clearly visualized. G: The viable tumors show a rosette like structure. H: The Ki-67 index was <2%. These results were compatible with NET G1 findings.

**Supplemental files**

**Figure 1.** A: Contrast-enhanced computed tomography (CE-CT) image showing a hypervascular tumor 12 mm in diameter in the pancreatic tail (arrow). B: Endoscopic ultrasonography image showing a low-echoic tumor with cystic changes. C: CE-CT image one month after the procedure. There were no enhanced areas in the tumor (arrow), and the tumor was treated completely with ethanol ablation. D: CE-CT image one year after the procedure. The tumor could not be detected (arrow).

**Video legend**

**Video 1.** EUS-guided ethanol injection at the first session. The tumor was located in the tail of the pancreas and was 12 mm in diameter. We were able to ablate the tumor using 1.0 ml of pure ethanol in a single session.

**Video text**

1. A low-echoic tumor with cystic changes was located in the tail of the pancreas. The tumor showed a hypervascular presence on color Doppler imaging.

2. A 25-G needle was inserted into the tumor.

3. Pure ethanol was injected until a hyperechoic blush extended to the tumor's margins.

4. The blood flow of the tumor could not be detected on color Doppler imaging.

**Video 2.** EUS-guided ethanol injection at an additional session. The tumor was located in the body of the pancreas and was 10 mm in diameter. We injected 0.6 ml of pure ethanol into the tumor for the initial treatment; however, some residual parts (not-ablated areas) were still observed in the tumor. For additional sessions, we added a total of 0.3 ml of ethanol to the residual parts of the previously ablated area. As a result, no enhanced parts in

the tumor were observed on CE-CT at one month after the procedure (after the completion of successful ablation).

**Video text**

1. A low-echoic tumor was located in the body of the pancreas. We detected low-echoic parts in the periphery of the tumor (arrows) after the first ethanol treatment.

2. First, we advanced a 25-G needle into the far side of the residual portion and injected pure ethanol.

3. We then treated the near side of the residual portion.

4. Contrast-enhanced harmonic-EUS imaging with perflubutane was performed to evaluate the tumor's viable regions. Based on our findings, we considered the tumor to be completely treated.