

Original Article



# Pancreatic Compression during Lymph Node Dissection in Laparoscopic Gastrectomy: Possible Cause of Pancreatic Leakage

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**Author Contributions**

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**Conflict of Interest**

No potential conflict of interest relevant to this article was reported.

## ABSTRACT

**Purpose:** Postoperative pancreatic fistula is a serious and fatal complication of gastrectomy for gastric cancer. Blunt trauma to the parenchyma of the pancreas can result from an assistant's forceps compressing and retracting the pancreas, which in turn may result in pancreatic juice leakage. However, no published studies have focused on blunt trauma to the pancreas during laparoscopic surgery. Our aim was to investigate the relationship between compression of the pancreas and pancreatic juice leakage in a swine model.

**Materials and Methods:** Three female pigs were used in this study. The pancreas was gently compressed dorsally for 15 minutes laparoscopically with gauze grasped with forceps. Pancreatic juice leakage was visualized by fluorescence imaging after topical administration of chymotrypsin-activatable fluorophore in real time. Amylase concentrations in ascites collected at specified times was measured. In addition, pancreatic tissue was fixed with formalin, and the histology of the compressed sites was evaluated.

**Results:** Fluorescence imaging enabled visualization of pancreatic juice leaking into ascites around the pancreas. Median concentrations of pancreatic amylase in ascites increased from 46 U/L preoperatively to 12,509 U/L 4 hours after compression. Histological examination of tissues obtained 4 hours after compression revealed necrotic pancreatic acinar cells extending from the surface to deep within the pancreas and infiltration of inflammatory cells.

**Conclusions:** Pancreatic compression by the assistant's forceps can contribute to pancreatic juice leakage. These findings will help to improve the procedure for lymph node dissection around the pancreas during laparoscopic gastrectomy.

**Keywords:** Blunt injury; Laparoscopy; Optical imaging; Pancreatic juice; Swine

## INTRODUCTION

In Japan, laparoscopic gastrectomy with suprapancreatic lymph node dissection is widely accepted as a standard treatment for gastric cancer with risk of lymph node metastases. Several recent studies have reported that laparoscopic gastrectomy has better short-term outcomes than open gastrectomy [1-4]. However, laparoscopic gastrectomy has specific limitations in that the axis of the device is restricted and organs cannot be palpated [5]. These limitations may result in postoperative complications, including those related to unexpected organ injury.

Postoperative pancreatic fistula is a potential serious and fatal complication of radical gastrectomy for gastric cancer. Pancreatic juice leakage followed by a postoperative pancreatic fistula may result in bleeding, anastomotic leakage, and intra-abdominal abscess formation. Since the publication of the results of a multi-institutional phase II trial (Japan Clinical Oncology Study Group [JCOG]; JCOG0703) that examined the safety of laparoscopic distal gastrectomy for clinical stage I gastric cancer, the incidence of postoperative pancreatic fistula after the procedure performed by credentialed surgeons has been as low as 1.1% [6]. However, the results of clinical trials conducted in centers of excellence may not be applicable to surgeons in general. On the other hand, some recent retrospective studies have reported that postoperative pancreatic fistula occurs more frequently after laparoscopic gastrectomy than after open gastrectomy [7,8]. In a prospective cohort study using a National Clinical Database, Hiki et al. [9] found a higher incidence of postoperative pancreatic fistula after laparoscopic distal gastrectomy than after open distal gastrectomy (2.2% vs. 1.0%;  $P=0.04$ ). Thus, the issue of pancreatic fistula after laparoscopic gastrectomy in the clinical setting needs to be addressed, and this requires determination of the underlying mechanism(s).

Pancreatic juice leakage after laparoscopic gastrectomy may be attributable to either operator- or assistant-related causes. The operator can injure pancreatic tissue by direct cutting or causing thermal injury from energy devices used during dissection of the suprapancreatic lymph nodes [5]. During suprapancreatic lymph node dissection, an assistant must compress and retract the pancreas to achieve a good view of the suprapancreatic area; thereby, the pancreas can be injured. Both penetrating injury and blunt trauma are major causes of pancreatic trauma [10]. We recently reported that the anatomical position of the pancreas is an independent predictor of postoperative pancreatic fistula after laparoscopic distal gastrectomy [11].

We therefore hypothesized that excessive pancreatic compression may cause blunt trauma that results in pancreatic juice leakage. The aim of this study was to use intraoperative fluorescence imaging and measurements of amylase concentrations in ascitic fluid to evaluate the effect of compression on the development of pancreatic juice leakage in an animal model.

## MATERIALS AND METHODS

Three female Yorkshire-Landrace swine (4 months old; weight, 60 kg) were used. After induction of general anesthesia, an incision was made on the inner right thigh and a catheter was inserted in the right femoral artery for blood sampling. This study was conducted in accordance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines [12]. All the procedures were performed at a facility approved by the Japan Health Sciences Foundation.

### **Surgical procedures**

Five ports (each 5 to 12 mm) were inserted in the umbilicus, left upper, left flank, right upper, and right flank quadrants, respectively. The omental bursa was opened using the tip of Thunderbeat (Olympus Medical Systems, Tokyo, Japan) to expose the pancreas. Gauze was grasped with forceps, and the pancreatic body was compressed dorsally for 15 minutes, as commonly done during laparoscopic gastrectomy in humans.

### **Visualization of pancreatic leaks using fluorescence imaging**

Fluorescence imaging techniques for intraoperative visualization of pancreatic leaks have been reported elsewhere [13,14]. Briefly, prior to each fluorescence imaging, 0.2 mL of a chymotrypsin probe (glutaryl-phenylalanine hydroxymethyl rhodamine green with added trypsin), which is specifically hydrolyzed by pancreatic chymotrypsinogen and emits fluorescence signals with a peak wavelength of approximately 520 nm [13-15], was sprayed onto the part of the pancreas that was being compressed with forceps. Fluorescence images were obtained under blue light illumination with a prototype laparoscopic imaging system before, immediately after, and 1, 2, and 4 hours after compression of the pancreatic body.

### **Pancreatic blood perfusion assessment with indocyanine green (ICG) fluorescence imaging**

Immediately after compression of the pancreas, 2.5-mg ICG (Diagnogreen; Daiichi Sankyo, Tokyo, Japan) was injected intravenously. Fluorescence images of the pancreas were obtained with a prototype laparoscopic near-infrared imaging system to assess blood perfusion [16,17].

### **Evaluation of amylase concentrations in ascitic fluid**

Ascites were collected by inserting 20 mL of saline solution into the suprapancreatic area via a soft catheter. Washing solution was collected before, immediately after, and 1, 2, and 4 hours after compression. Pancreatic amylase concentrations were measured using an enzymatic method (L-type amylase; Wako, Tokyo, Japan). During ascites collection, blood was sampled for determining the white blood cell count and serum pancreatic amylase concentration.

### **Histopathological evaluation of the pancreas**

The pancreas was rapidly extracted 4 hours after compression, and the pancreatic tissues were placed in 10% buffered formalin for 24 hours, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

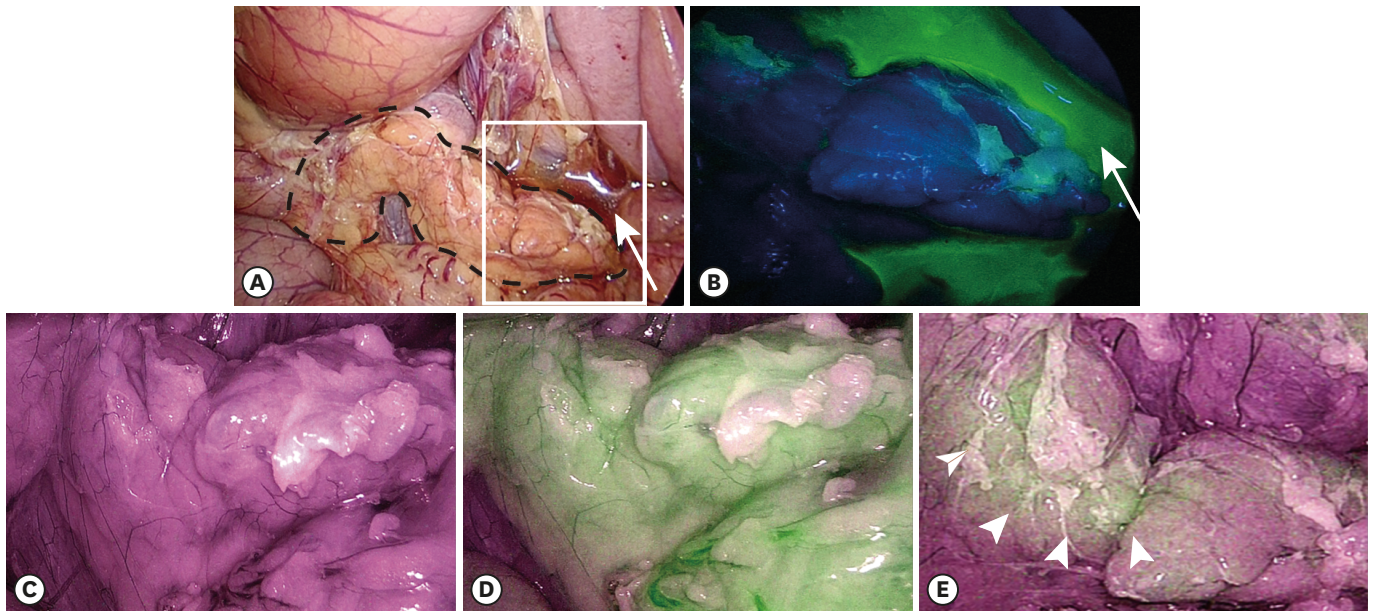
### **Statistical methods**

Continuous data are expressed as median (range) unless indicated otherwise.

## **RESULTS**

### **Visualization of pancreatic leaks and blood perfusion using fluorescence imaging**

Macroscopic examination revealed wine-colored ascites around the pancreas (**Fig. 1A**). The intensities of the fluorescence signal emitted from the ascitic fluid collections around the pancreas increased after topical administration of a chymotrypsin probe, indicating activation by leaking pancreatic juice; however, no leakage points were clearly identified on fluorescence imaging (**Fig. 1B** and **Supplementary Video 1**).



**Fig. 1.** Fluorescent imaging of the pancreas. The upper panels show a chymotrypsin probe (A, B), and the lower panels show the administration of ICG (C-E). Gross appearance in natural color (A). Image obtained through light-blocking glasses 2 minutes after administration of the chymotrypsin probe (B). Before (C), immediately after (D), and 2 hours after administration of ICG. The black dotted lines (A) indicate the borders of the pancreas. The white arrows (B) indicate ascites containing pancreatic juice. The white arrowheads (E) indicate pooled ICG fluorescence. ICG = indocyanine green.

According to ICG-fluorescence imaging, the compressed regions of the pancreas and surrounding regions received sufficient blood in the early phase (<3 minutes; **Fig. 1C and D**). By 2 hours after administration of ICG, fluorescence signals were detected only in the pancreatic regions that had been compressed with forceps, indicating retention of ICG in these specific areas of the pancreas (**Fig. 1E**).

#### **Amylase concentrations in ascitic fluid and blood test findings**

Before compression, the pancreatic amylase concentration in ascites was 46 U/L (28–706 U/L). They increased over time as follows: 502 U/L (163–2,610 U/L) immediately after compression, 2,268 U/L (391–11,608 U/L) 1 hour later, 1,916 U/L (1,358–31,385 U/L) 2 hours later, and 12,509 U/L (2,399–59,640 U/L) 4 hours later (**Fig. 2**).

White blood cell counts and serum pancreas amylase concentrations also tended to be higher 4 hours after than before compression. The white blood cell count before compression was  $15.1 \times 10^3/\mu\text{L}$  ( $4.1\text{--}21.5 \times 10^3/\mu\text{L}$ ), which increased to  $17.4 \times 10^3/\mu\text{L}$  ( $11.6\text{--}26.1 \times 10^3/\mu\text{L}$ ) after 4 hours. In addition, the serum pancreatic amylase concentrations before and 4 hours after compression were 3,520 mg/dL (2,760–4,265 mg/dL) and 3,780 mg/dL (2,980–8,478 mg/dL), respectively.

#### **Histopathological examinations of the pancreas**

Macroscopic examination revealed that the pancreatic capsules showed minimal evidence of injury after compression, without hematomas or lacerations (**Fig. 3A-C**). Histological examination revealed that compared with the normal pancreas (**Fig. 3D**), the pancreatic parenchyma 4 hours after compression showed marked necrotic changes in acinar cells with inflammatory infiltrates. These changes were most noticeable on the surface, being less evident in deeper layers of the pancreas (**Fig. 3E**).



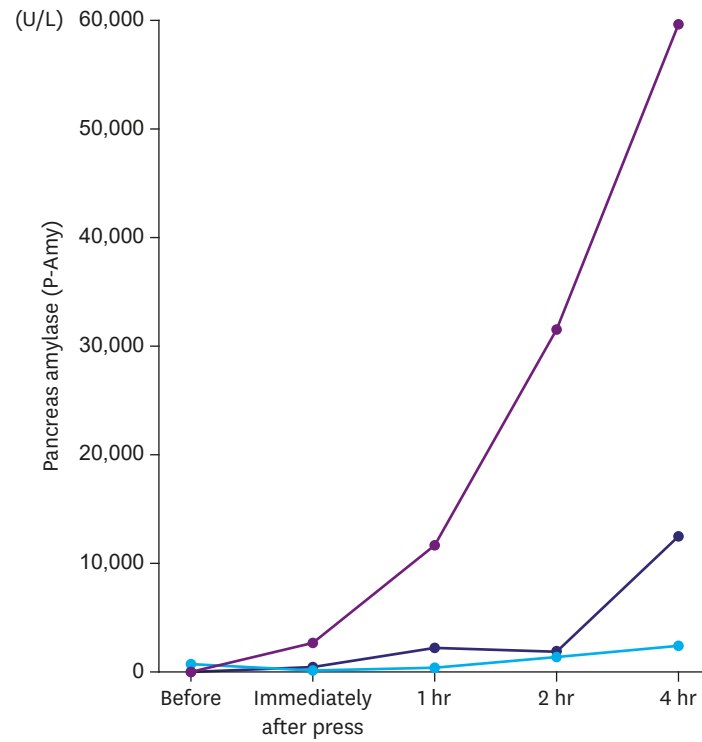


Fig. 2. Pancreatic amylase concentrations in ascites at specified times (n=3).

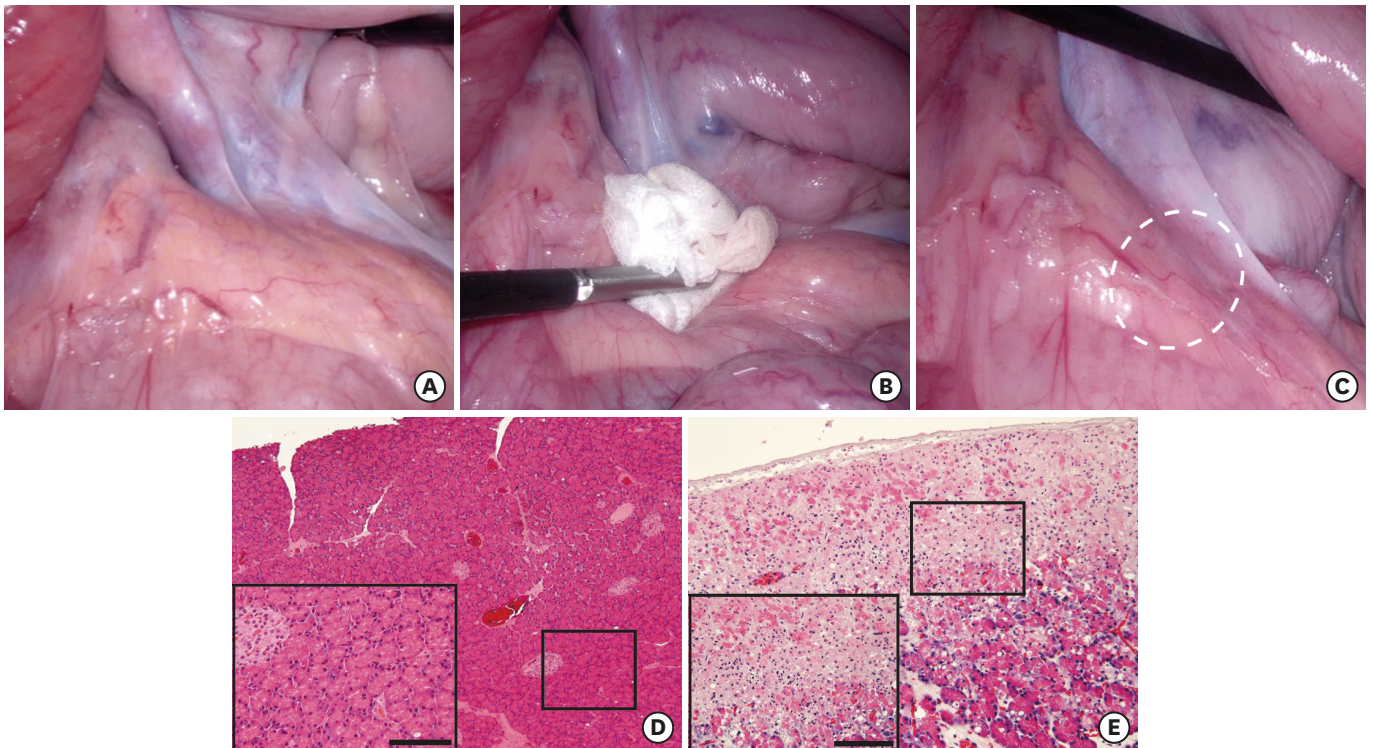


Fig. 3. Macroscopic and histopathological images of pancreatic tissues. Before (A), during (B), and immediately after compression (C). Normal pancreas (D) and injured pancreas (E). The injured pancreas (E) shows necrotic changes in acinar cells with inflammatory infiltrates in the pancreatic parenchyma. The white dotted circle (C) indicates the compressed area. Scale bar, 50  $\mu$ m.

## DISCUSSION

In this study, pancreatic compression by an assistant's forceps was associated with high amylase concentrations in ascites in a swine laparoscopic gastrectomy model of suprapancreatic lymph node dissection. In addition, marked necrotic changes in acinar cells and inflammatory infiltrates were observed on pathological examination.

Pancreatic injuries infrequently occur after blunt or penetrating abdominal damage. The American Association for the Surgery of Trauma states that localized blunt force to the upper abdomen can result in traumatic compression of the pancreas against the vertebral body; mild contusion and laceration without pancreatic duct injury is classified as grade 1 pancreatic injury [18]. We hypothesized that pressure from an assistant's forceps could cause pancreatic injury and accordingly created this swine model. During open gastrectomy, an assistant gently exposes the upper border of the pancreas by manually compressing the pancreas dorsally and caudally while protecting the pancreatic surface with folded gauze. A similar practice is common with laparoscopic gastrectomy. However, some retrospective studies have found that the incidence of pancreatic fistula after laparoscopic distal gastrectomy is as high as 5.3% to 11.8% [7,8,19]. Kumagai et al. [11] reported that depending on its anatomical position, the pancreas may have to be compressed during laparoscopic gastrectomy to enable suprapancreatic lymph node dissection. They also reported that such compression of the pancreas is a risk factor of postoperative pancreatic fistula. In the present study, although pathological examination showed an intact pancreatic capsule, necrosis extended into the deep layers of the pancreas. Although protecting the pancreas with gauze may be helpful in minimizing superficial lacerations, it does not prevent pancreatic injury. Although the procedure for pancreatic compression is superficially similar to that used in open gastrectomy, it differs in that the surgeon performing laparoscopic gastrectomy must manipulate the gauze with forceps and cannot feel the pancreas; in addition, the axis of the device is restricted [5]. This combination of factors can result in excessive compression. Accordingly, compression of the pancreas during laparoscopic gastrectomy should be avoided. Given that the indications for laparoscopic gastrectomy will likely soon be expanded to include procedures for advanced gastric cancer, it is even more imperative to avoid manipulating the pancreas during suprapancreatic lymph node dissection. Tsujiura et al. [19] reported a significant decrease in the incidence of postoperative pancreatic fistula when direct compression of the pancreas during suprapancreatic lymph node dissection and laparoscopic gastrectomy is avoided.

In the present study, we identified pancreatic juice leakage after compression of the pancreatic parenchyma by fluorescence imaging using a chymotrypsin probe [16]. Furthermore, pancreatic amylase concentrations in ascites increased over time from immediately after compression, reaching their highest values after 4 hours. We used the pancreatic amylase concentration in ascites as an objective marker of pancreatic juice leakage in the present study. Sano et al. [20] reported that amylase concentration in drainage fluid indicates that the incidence of postoperative pancreatic fistula increases with increasing surgical manipulation of the pancreas. Although we only observed the animals for 4 hours after compression in the present study, pancreatic leakage has the potential to increase abdominal inflammation, which results in intra-abdominal fluid retention and infection.

In the present study, we identified pancreatic juice leaking into ascitic fluid around the pancreas in real time after compression of the pancreas, as has been reported for a swine distal pancreatectomy model [15]. Although diffuse leakage of pancreatic juice caused by

pancreatic compression may be difficult to repair, intraoperative assessment of pancreatic leakage by fluorescence imaging may enable surgeons to determine the need for prophylactic abdominal drains. Our findings also suggest that retention of ICG in compressed regions of the pancreas is attributable to inflammatory/necrotic changes in the pancreatic parenchyma rather than to hematoma formation. Although postoperative pancreatic leakage was not evaluated in the present study, such histological changes in pancreatic tissue may be associated with late-onset postoperative pancreatic leakage.

The present study had some limitations. First, compression pressure was not quantified. In addition, the number of animals used was minimal for ethical reasons; thus, various compression times and strengths were not considered. However, pancreatic compression was performed by an experienced laparoscopic surgeon and in the usual manner. Therefore, our procedure closely resembled routine practice.

In conclusion, pancreatic compression contributes to pancreatic juice leakage. Surgeons, particularly assistants using forceps, should take care to minimize the incidence of pancreatic juice leakage. The results of this study will help improve the surgical procedure for lymph node dissection of the suprapancreatic area during laparoscopic gastrectomy.

## SUPPLEMENTARY MATERIAL

### Supplementary Video 1

Visualization of pancreatic leaks.

[Click here to view](#)

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