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Original Research

# Additional effect of perioperative, compared with preoperative, immunonutrition after pancreaticoduodenectomy: A randomized, controlled trial

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ABSTRACT

*Background:* We have reported that perioperative and preoperative immunonutrition reduced infectious complications in patients undergoing pancreaticoduodenectomy; however, it is unclear whether perioperative immunonutrition has additional effects compared with preoperative immunonutrition. The present study evaluated whether perioperative, compared with preoperative, immunonutrition has additional effects on cell-mediated immunity and the infection rate after pancreaticoduodenectomy.

*Materials and methods:* This was a prospective, randomized clinical trial conducted in our institution. Oral supplementation enriched with arginine, ω-3 fatty acids, and dietary nucleotides was given by enteral infusion to 30 patients before and after surgery (perioperative group); 30 patients received the same enriched formula before surgery and standard enteral nutrition following surgery (preoperative group). The primary endpoint was concanavalin (Con A)- or phytohemagglutinin (PHA)-stimulated lymphocyte proliferation on postoperative day (POD) 7, which is an index of cell-mediated immunity; the secondary endpoint was the postoperative infection rate.

*Results:* There were no significant differences in Con A- or PHA-stimulated lymphocyte proliferation on POD 7 between the groups. There was no significant difference in the postoperative infection rate between the two groups. In the *post hoc* subgroup analysis, with respect to the effect on the infection rate, a significant interaction was found only between a long operative time and perioperative immunonutrition.

*Conclusions:* There were no additional effects of perioperative, compared with preoperative, immunonutrition on postoperative immunity and infectious complications in patients undergoing pancreaticoduodenectomy.

## 1. Introduction

Pancreaticoduodenectomy is currently the only curative treatment for malignancy of the periampullary region. Improvements in surgical techniques and perioperative management have resulted in mortality rates of less than 5% after pancreaticoduodenectomy at high-volume centers [1,2]. However, morbidity rates have been high, ranging from 40 to 60% [3,4].

To reduce postoperative complications and shorten hospital stays, use of immunonutrition, which is supplementation with nutrients such as arginine, ω-3 fatty acids, and dietary nucleotides, has been attracting increasing attention, especially for elective gastrointestinal tract surgeries. Waitzberg et al. conducted a meta-analysis of patients who received immunonutrition as part of their pre-, peri- (before and after), and postoperative management. Preoperative immunonutrition contributes to improved morbidity outcomes in elective surgery patients, particularly those undergoing gastrointestinal surgical procedures [5].

Several previously reported trials suggest that preoperative administration of an immune-enhancing diet is necessary to improve clinical outcomes in patients undergoing pancreaticoduodenectomy [6,7], but whether prolongation of immunonutrition postoperatively has additional effects remains unclear.

The aim of this study was to investigate the additional effects of perioperative, compared with preoperative, immunonutrition on cell-mediated immunity and the postoperative infection rate in patients undergoing pancreaticoduodenectomy.

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## 2. Patients and methods

The work has been reported in line with Consolidated Standards of Reporting Trials (CONSORT) 2010 Guidelines.

### 2.1. Patients

This study was a prospective, randomized clinical trial conducted at a single institution (Department of General Surgery, Chiba University Hospital). The participants included consecutive patients undergoing pancreaticoduodenectomy or pylorus-preserving pancreaticoduodenectomy who were candidates for elective surgery from January 2012 to August 2014. The exclusion criteria were as follows: age younger than 18 years or older than 80 years; active preoperative infection; treated with adrenocorticosteroids; gastrointestinal obstruction; respiratory dysfunction ($PaO_2 < 70$ mmHg); cardiac dysfunction (New York Heart Association class > 3); hepatic dysfunction (Child Class C); renal failure (hemodialysis); history of recent immunosuppressive or immunologic diseases (including preoperative chemotherapy and/or radiation therapy); or preoperative evidence of widespread metastatic disease at the time of entry into the study. Because of concerns about the effects of chemoradiation therapy on immune function after surgery, patients who received neoadjuvant chemotherapy and/or radiation therapy were excluded from the study.

### 2.2. Protocol

The protocol was reviewed and approved by the institutional review board of the Chiba University Hospital, and all participants provided their written, informed consent. The study was conducted in accordance with the Declaration of Helsinki. This study was registered with ClinicalTrials.gov (NCT01969110).

The 71 enrolled patients were randomized into two groups, the perioperative group and the preoperative group, through the use of sealed envelopes containing computer-generated distribution numbers. According to our previous trials [6,7], both groups received oral supplementation (1000 kcal/day) containing arginine, ω-3 fatty acids, and dietary nucleotides (oral IMPACT; Nestle Health Science Co., Ltd, Kobe, Japan) for 5 days before surgery, in addition to a 50% (1000 kcal/day) reduction in the amount of regular food to reduce the effects of energetic and overfeeding reactions. A total of 1000 mL of oral IMPACT contains 12.8 g of arginine, 3.4 g of omega-3 fatty acids, and 1.29 g of dietary nucleotides. All patients were hospitalized by 6 days before surgery, and the amount of oral supplementation and food intake was monitored by primary nurses not involved in the study.

Postoperative enteral feeding with oral IMPACT in the perioperative group or standard formula in the preoperative group started about 12 hours after surgery. A gastrostomy catheter, the tip of which was placed in the jejunum during surgery, was used for enteral feeding in both groups. Enteral feeding of all patients was initiated on postoperative day (POD) 1 at 20 mL/h and was increased progressively by 20 mL/day until the full nutritional goal of 25 mL (25 kcal)/kg/day was reached. All patients were allowed to consume water as desired on POD 2, and oral food intake was allowed from POD 3–5 according to clinical conditions, with enteral feeding decreasing gradually as oral intake increased. The amount of oral intake was monitored by primary nurses not involved in the study, and the enteral feeding was continued until oral intake was approximately 800 kcal/day. No patient received total parenteral nutrition before or after the operation. After surgery, serum glucose levels were controlled at 180 mg/dL or less with intravenous insulin as needed. There were no differences in the insulin requirement to achieve the targeted glucose levels between the two groups.

As antibiotic prophylaxis, 1 g of flomoxef sodium (FMOX) (Flomox; Shionogi, Co., Ltd, Osaka, Japan) was administered intravenously via a drip infusion during induction of anesthesia in most of the patients; the antibiotic was changed when antimicrobial susceptibility tests of preoperative bile cultures indicated drug-resistant bacteria. Additional doses of antibiotic were given every 3 hours. There were no differences in the use of antibiotic prophylaxis between the two groups. After surgery, no patient received steroids or anti-inflammatory drugs.

### 2.3. Operative procedure

All patients with obstructive jaundice (serum bilirubin > 5 mg/dl) were treated preoperatively with percutaneous transhepatic biliary drainage, endoscopic nasobiliary drainage, or endoscopic biliary stenting. All patients underwent pancreaticoduodenectomy (Whipple procedure) or a pylorus-preserving pancreaticoduodenectomy. Reconstruction was performed by creation of a Roux-en-Y jejunal limb via the retrocolic-anteduodenal route. Passage was restored with an end-to-side pancreatojejunostomy, an end-to-side hepatojejunostomy, and an end-to-side gastrojejunostomy or pylorojejunostomy with Braun anastomosis according to the Child procedure. A feeding gastrostomy catheter was inserted through the anterior wall of the stomach using a modified Witzel technique before closing the wound. The tip of the feeding catheter was placed 10–15 cm below the last intestinal anastomosis. All operations in this study were performed by surgeons who specialized in hepatobiliary-pancreatic procedures. There was a balance of operative surgeons between the two groups.

### 2.4. Definition of infectious complications and other complications

Trained members of the surgery staff who were not involved in the study recorded postoperative complications. Detailed daily records of patients' postoperative courses were kept, and infectious complications were recorded for up to 30 days after surgery. Wound infection was defined as a purulent discharge with positive cultures, defined in accordance with the Centers for Disease Control and Prevention (CDC) definition of surgical site infection [8]. An infected pancreatic fistula was defined as purulent discharge and a positive culture from abdominal drains. The peripancreatic drains were maintained in place. and if they were not fully draining the fistula, the positions of the drains were adjusted. Other infectious complications were classified as enteritis, pneumonia, and urinary tract infection. Microbiological analysis and positive cultures confirmed all infectious complications.

In this study, the definition of pancreatic fistula was in accordance with the International Study Group on Pancreatic Fistula classification [9,10], and grades B and C were counted as complications. Delayed gastric emptying, as defined by the International Study Group of Pancreatic Surgery [10,11], was counted as a complication. The severity of postoperative complications was graded according to the Clavien-Dindo classification [12,13]. Systemic inflammatory response syndrome (SIRS) was defined using the definition of the Society of Critical Care Medicine Consensus Conference [14]. Other noninfectious complications were also counted. Complication rates and duration of SIRS were determined.

### 2.5. Laboratory analyses

Blood samples were obtained 6 days before surgery at the start of preoperative immunonutrition (preoperative day 6, POD -6), 1 day before surgery (preoperative day 1, POD -1), immediately after surgery (POD 0), and on PODs 1, 3, 7, 14, and 21. Blood samples were centrifuged at 3000 rpm for 20 min at 4 °C and stored at −20 °C until measurement.

To assess cell-mediated immunity, concanavalin A (Con A)- and phytohemagglutinin (PHA)-stimulated lymphocyte proliferation rates were determined. Stimulation indices were calculated by dividing the counts per minute of $^3$H-thymidine in mitogen-stimulated cells by the counts per minute in cells cultured without mitogens. The plasma level of interleukin (IL)-6 was determined as a measure of the inflammatory response. Plasma IL-6 assays were performed with a commercially

available enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN, USA). The serum fatty acid composition was analyzed by gas chromatography (ModelGC-9A; Shimadzu Co., Kyoto, Japan) programmed to separate methyl esters ranging from 12:0 to 22:6 ω-6. The split ratio was 1/20 and the flow was 4.7 mL/min. Identification was made by comparisons with known methyl ester standards [15].

### 2.6. Sample size calculation and statistical analyses

Using statistical power analyses, the required sample size was calculated. Con A- or PHA-stimulated lymphocyte proliferation on POD 7 was selected as the primary endpoint. We have reported that, on POD 7, Con A- and PHA-stimulated lymphocyte proliferation, indices of cell-mediated immunity, are most suppressed, and they are higher in patients who received preoperative and perioperative immunonutrition than in patients who received no immunonutrition after pancreaticoduodenectomy [6,7]. On the basis of experience and previous publications [6,7], and assuming that the difference in each arm of Con A- or PHA-stimulated lymphocyte proliferation (stimulation index) on POD 7 was 50 with a standard deviation of 60, a type I error of 0.05, and a power of 0.8 required, the target sample size was determined to be 60 patients (30 patients in each arm). All values are expressed as means ± standard of error of the mean (SEM) or numbers and percentage of patients. Categorical data were analyzed using the chi-squared test and Fisher's exact test. The Mann-Whitney *U* test was used to compare ordinal variables. $P < 0.05$ was considered to indicate significance.

Con A- or PHA-stimulated lymphocyte proliferation on POD 7 was used as the primary endpoint. Postoperative complications, duration of SIRS, plasma IL-6, and nutritional parameters (albumin, prealbumin, retinol binding protein, transferrin) were considered secondary endpoints.

## 3. Results

### 3.1. Patient characteristics

Between January 2012 and August 2014, a total of 94 patients were screened for entry. Applying the exclusion criteria, 23 of these patients were excluded. The remaining 71 patients were allocated to 2 groups: perioperative group (n = 36) and preoperative group (n = 35). Subsequently, 11 of these patients were excluded from the analysis: 5 patients were excluded intraoperatively because of metastatic disease or unresectable primary tumor, and 6 patients were excluded postoperatively because of discontinuation of the enteral nutrition due to early postoperative complications such as chylous ascites. Of the remaining 60 patients available for analysis, 30 were in the perioperative group and 30 were in the preoperative group (Fig. 1).

Table 1 shows the patients' characteristics, baseline data, and surgical variables. No significant differences were found between the two groups in sex, age, baseline nutrition, preoperative cell-mediated immunity, biliary drainage methods, diabetes, diagnosis, and pTNM classification. Also, there were no significant differences between the two groups in risk factors for postoperative complications after pancreaticoduodenectomy [1,16–18] (American Society of Anesthesiologists score, body mass index, undernourishment, operative procedure, operative time, blood loss, rate of blood transfusion, main pancreatic duct dilatation, and remnant pancreatic texture).

No significant differences were found between the two groups in compliance and the tolerability of enteral nutrition. The patients of the two groups complied fully with more than 80% of the supplementation before surgery. After surgery, there were no substantial differences in the mean period required to reach the full nutrition goal.

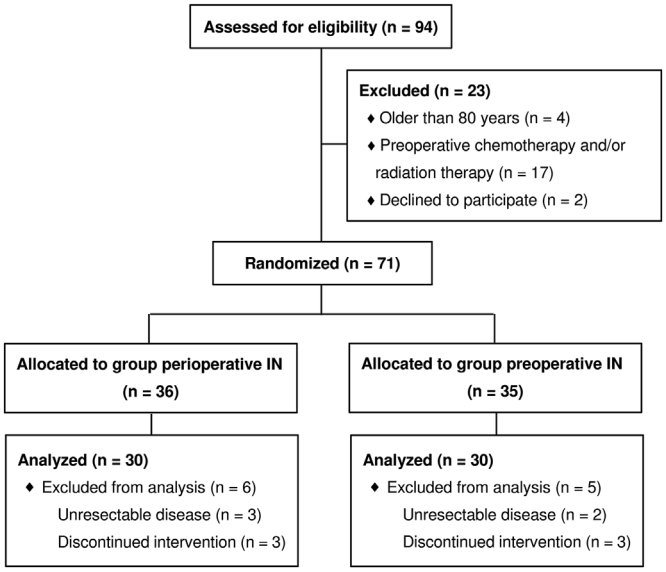


**Fig. 1.** Diagram of patient enrollment in the study (CONSORT statement). IN, immunonutrition; PD, pancreaticoduodenectomy.

**Table 1**
Patient characteristics, baseline data and intraoperative variables.

| | Perioperative group | Preoperative group | P value |
|---|---|---|---|
| Number of patients (n) | 30 | 30 | |
| Sex (male/female) | 16/14 | 18/12 | 0.602 |
| Age (years) | 67.8 ± 9.3 | 67.6 ± 7.5 | 0.952 |
| ASA-PS score (1/2/3/4/5/6) | 6/22/2/0/0/0 | 9/20/1/0/0/0 | 0.425 |
| Body Mass Index (kg/m$^2$) | 22.0 ± 2.6 | 21.3 ± 2.9 | 0.347 |
| Preoperative weight loss > 10% (n) | 2 | 3 | 0.640 |
| Smoking (n) | 5 | 4 | 0.718 |
| Albumin (g/dL) | 4.0 ± 0.4 | 4.0 ± 0.4 | 0.732 |
| Prealbumin (mg/dL) | 23.1 ± 6.2 | 23.5 ± 8.0 | 0.818 |
| RBP (mg/dL) | 3.0 ± 1.2 | 3.0 ± 1.2 | 0.905 |
| Transferrin (mg/dL) | 233 ± 42 | 234 ± 51 | 0.884 |
| Con A (SI) | 181 ± 68 | 156 ± 96 | 0.277 |
| PHA (SI) | 210 ± 77 | 172 ± 98 | 0.116 |
| Jaundice (n) | 14 | 16 | 0.606 |
| Biliary drainage (n) | | | |
| PTBD/ENBD/EBS | 1/11/2 | 2/10/4 | 0.454 |
| Diabetes mellitus (n) | 9 | 4 | 0.117 |
| Diagnosis (n) | | | |
| Pancreatic carcinoma | 9 | 10 | 0.781 |
| Bile duct carcinoma | 5 | 5 | 1.000 |
| Ampullary carcinoma | 3 | 3 | 1.000 |
| Others (IPMN, p-NET) | 14 | 13 | 0.795 |
| pTNM classification (UICC) | | | |
| Stage (0/I/II/III/IV) | 3/8/4/6/5 | 4/4/5/8/6 | 0.583 |
| Surgical procedure (n) | | | |
| PD/PpPD | 24/6 | 26/4 | 0.488 |
| Operative time (min) | 444 ± 110 | 463 ± 86.4 | 0.486 |
| Blood loss (g) | 784 ± 838 | 697 ± 420 | 0.617 |
| Blood transfusion (n) | 5 | 7 | 0.519 |
| MPD dilatation > 3 mm (n) | 12 | 12 | 1.000 |
| Remnant pancreatic texture (n) | | | |
| soft/firm | 18/12 | 19/11 | 0.791 |

Data are expressed as mean ± SEM. ASA-PS, American Society of Anethesiolosists Physical Status classification; RBP, retinol binding protein; Con A, Concanavalin A-stimulated lymphocyte proliferetion; PHA, phytohemagglutinin-stimulated lymphocyte proliferation; SI, stimulation index; PTBD, percutaneous transhepatic biliary drainage; ENBD, endoscopic nasobiliary drainage; EBS, endoscopic retrograde biliary stenting; IPMN, intraductal papillary mucinous neoplasm; p-NET, pancreatic neuroendocrine tumor; PD, pancreaticoduodenectomy; PpPD, pylorus preserving pancreaticoduodenectomy; MPD, main pancreatic duct.

**Table 2**
Changes in the nutritional index, fatty acids composition, immune function, and inflammatory response in both groups during the perioperative period of nutritional supplementation.

| Parameters | Group | POD -6 | POD -1 | POD 0 | POD 1 | POD 3 | POD 7 | POD 14 | POD 21 |
|---|---|---|---|---|---|---|---|---|---|
| Alb, g/dL | peri | 4.0 ± 0.1 | 4.0 ± 0.1 | - | 2.8 ± 0.1 | 2.8 ± 0.1 | 2.8 ± 0.1 | 3.2 ± 0.1 | 3.1 ± 0.1 |
| | pre | 4.0 ± 0.1 | 4.0 ± 0.1 | - | 2.8 ± 0.1 | 2.9 ± 0.1 | 2.8 ± 0.1 | 3.1 ± 0.1 | 3.5 ± 0.1 |
| Prealbumin, mg/dL | peri | 23.1 ± 1.1 | 23.8 ± 0.8 | - | 14.2 ± 0.6 | 9.8 ± 0.4 | 14.1 ± 1.0 | 14.6 ± 1.0 | 17.4 ± 5.0 |
| | pre | 22.9 ± 1.4 | 24.4 ± 1.3 | - | 14.3 ± 0.9 | 9.5 ± 0.5 | 12.1 ± 0.7 | 12.8 ± 0.8 | 13.8 ± 1.2 |
| RBP, mg/dL | peri | 2.8 ± 0.2 | 3.4 ± 0.2 | - | 1.5 ± 0.1 | 1.2 ± 0.1 | 1.9 ± 0.1 | 2.0 ± 0.1 | 1.5 ± 0.1 |
| | pre | 2.8 ± 0.2 | 3.3 ± 0.2 | - | 1.5 ± 0.1 | 1.1 ± 0.1 | 1.6 ± 0.1 | 1.6 ± 0.1 | 1.7 ± 0.2 |
| Transferrin, mg/dL | peri | 229 ± 7 | 237 ± 6 | - | 132 ± 6 | 117 ± 6 | 157 ± 6 | 197 ± 7 | 193 ± 9 |
| | pre | 230 ± 8 | 235 ± 6 | - | 134 ± 7 | 108 ± 4 | 146 ± 6 | 190 ± 8 | 208 ± 10 |
| EPA, μg/ml | peri | 63.9 ± 4.4 | 183.7 ± 9.2 | - | - | 68.5 ± 4.0** | 89.4 ± 5.8** | 56.6 ± 3.9** | 44.8 ± 3.2 |
| | pre | 57.9 ± 3.8 | 163.3 ± 9.5 | - | - | 48.3 ± 3.2 | 38.9 ± 2.3 | 40.1 ± 2.3 | 38.9 ± 1.9 |
| EPA/AA | peri | 0.43 ± 0.04 | 1.01 ± 0.06 | - | - | 0.66 ± 0.04* | 0.79 ± 0.06** | 0.42 ± 0.03** | 0.36 ± 0.04 |
| | pre | 0.34 ± 0.02 | 0.94 ± 0.04 | - | - | 0.50 ± 0.03 | 0.39 ± 0.02 | 0.34 ± 0.03 | 0.29 ± 0.02 |
| Con A, SI | peri | 190 ± 16 | 175 ± 14 | - | - | 135 ± 13 | 77 ± 9 | 127 ± 12* | 133 ± 13 |
| | pre | 156 ± 18 | 180 ± 15 | - | - | 107 ± 13 | 97 ± 13 | 92 ± 11 | 135 ± 15 |
| PHA, SI | peri | 196 ± 13 | 195 ± 13 | - | - | 156 ± 15 | 94 ± 12 | 162 ± 16** | 194 ± 20 |
| | pre | 172 ± 18 | 215 ± 20 | - | - | 121 ± 16 | 116 ± 16 | 102 ± 12 | 188 ± 20 |
| Plasma IL-6, pg/ml | peri | 5 ± 1 | 4 ± 1 | 841 ± 142 | 309 ± 40 | 53 ± 6 | 29 ± 4 | 16 ± 2 | 40 ± 8 |
| | pre | 4 ± 1 | 5 ± 1 | 895 ± 153 | 347 ± 47 | 54 ± 7 | 30 ± 3 | 18 ± 3 | 17 ± 3 |

Data are expressed as mean ± SEM. Perioperative group (n = 30), preoperative group (n = 30); POD, postoperative day; RBP, retinol binding protein; EPA, eicosapentaenoic acid; AA, arachidonic acid; Con A, concanavalin A-stimulated lymphocyte proliferation; PHA, phytohemoglutinin-stimulated lymphocyte proliferation; SI, Stimulation index; IL-6, interleukin-6. *$P < 0.05$ indicates significance versus preoperative group, **$P < 0.01$ indicates significance vs preoperative group.

### 3.2. Cell-mediated immunity

Table 2 shows the perioperative kinetics of Con A- or PHA-stimulated lymphocyte proliferation (stimulation index), the primary endpoint of this study. On POD 7, there was no significant difference in Con A- or PHA-stimulated lymphocyte proliferation between the two groups, but on POD 14, it was significantly greater in the perioperative group than in the preoperative group ($P < 0.05$).

### 3.3. Plasma IL-6 and CRP

There were no significant differences in plasma IL-6 and CRP levels between the two groups during the perioperative period. Postoperatively, the IL-6 concentration increased and peaked on POD 0 in both groups (Table 2).

### 3.4. Fatty acid composition

Table 2 shows the serum level of eicosapentaenoic acid (EPA) and the EPA/arachidonic acid (AA) ratio in the two groups: both values increased after preoperative supplementation (POD –1) compared with the baseline values ($P < 0.01$). On PODs 3, 7, and 14, both values were greater in the perioperative group than in the preoperative group ($P < 0.05$).

### 3.5. Correlations between serum EPA and Con A- or PHA-stimulated lymphocyte proliferation

Fig. 2 shows the correlations between the serum EPA level and the amount of Con A- or PHA-stimulated lymphocyte proliferation. The serum EPA level on POD 7 correlated with Con A- or PHA-stimulated lymphocyte proliferation on POD 14 ($r = 0.533$, $r = 0.506$, respectively).

### 3.6. Clinical outcomes

Table 3 shows the postoperative outcomes. Infectious complications occurred in 4 patients in the perioperative group and 8 patients in the preoperative group. There was no difference in the postoperative infection rate between the two groups. Postoperative infections that occurred included wound infection, intra-abdominal abscess, enteritis, pneumonia, and urinary tract infection. There was no difference in the rate of noninfectious complications between the two groups. Postoperative complications were graded according to the Clavien-Dindo classification, as shown in Table 3. There was no difference between the two groups in the severity level of postoperative complications. The duration of SIRS was not significantly different between the groups. No patients died during the postoperative course.

### 3.7. Subgroup analysis of the risk of development of infectious complications

As shown in Table 4, for analysis, the patients were divided into subgroups based on body weight loss (< 5% vs. ≥5%), prognostic nutritional index (< 50 vs. ≥50), Con A on preoperative day 1 (SI < 109 vs. ≥109, 109 = median), PHA on preoperative day 1 (SI < 139 vs. ≥139, 139 = median value), EPA on preoperative day 1 (< 175 μg/mL vs. ≥175 μg/mL, 175 = median), operative time (< 437 min vs. ≥437 min, 437 = median), blood loss (< 570 mL vs. ≥570 mL, 570 = median), texture (soft vs. firm), ASA-PS (ASA- I vs. ASA- II + III), UICC Stage (Stage I + II vs. III + IV), and biliary drainage (drainage + vs. drainage -). A significant interaction with respect to the effect on the postoperative infection rate was only found between perioperative immunonutrition and a long operative time. Among 29 patients with operative time > 437 min, infectious complications occurred in 0 of 13 patients in the perioperative group and 5 of 16 in the preoperative group ($P = 0.027$).

## 4. Discussion

This prospective, randomized controlled trial investigated the additional effects of perioperative, compared with preoperative, immunonutrition on cell-mediated immunity and infectious complications after pancreaticoduodenectomy. While the study was not powered for clinical outcome variables such as infectious complications, additional effects of perioperative immunonutrition on the primary endpoint and clinical outcomes were not found.

We have previously reported that perioperative (before and after) or preoperative immunonutrition, compared with no artificial nutrition, reduces the postoperative infection rate in patients undergoing

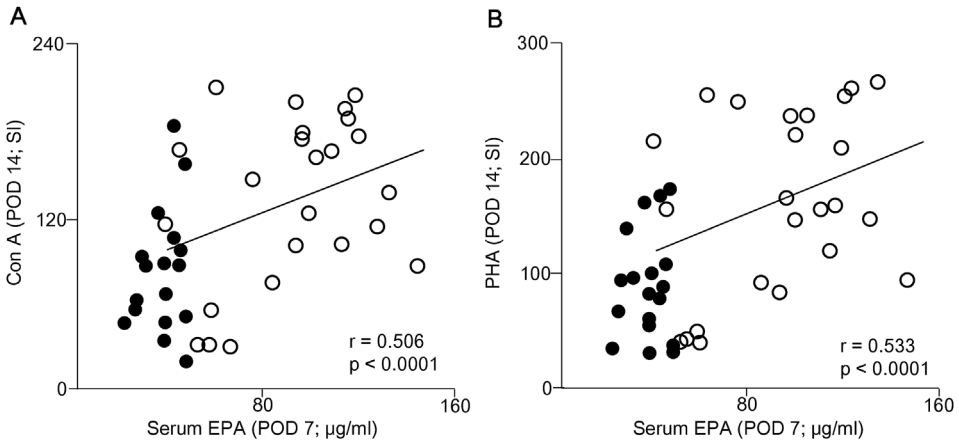


**Fig. 2.** (A) Correlation between EPA levels on POD 7 and Con A-stimulated lymphocyte proliferation on POD 14. (B) Correlation between EPA levels on POD 7 and PHA-stimulated lymphocyte proliferation on POD 14.
Open circles, perioperative immunonutrition group. Closed circles, preoperative immunonutrition group. EPA, plasma eicosapentaenoic acid; Con-A, concanavalin A-stimulated lymphocyte proliferation; PHA, phytohemagglutinin-stimulated lymphocyte proliferation; POD, postoperative day. *P* values determined using Pearson's correlation analysis.

**Table 3**
Postoperative outcomes.

| | Perioperative group | Preoperative group | P value |
|---|---|---|---|
| Number of patients (n) | 30 | 30 | |
| Infectious complications, n (%) | 4 (13.3) | 8 (26.7) | 0.166 |
| Wound infection | 3 | 5 | 0.448 |
| Infected pancreatic fistula | 3 | 5 | 0.448 |
| Enteritis | 0 | 1 | 0.313 |
| Pneumonia | 1 | 1 | 1.000 |
| Urinary tract infection | 0 | 1 | 0.313 |
| Noninfectious complications, n (%) | 13 (43.3) | 17 (56.7) | 0.302 |
| Pancreatic fistula (Grade B, Grade C) | 6 | 10 | 0.371 |
| Delayed gastric emptying | 2 | 4 | 0.389 |
| Chylous ascites | 5 | 6 | 0.739 |
| Others | 3 | 0 | 0.076 |
| Clavien-Dindo classification Grade (I/II/III/IV/V) | 3/8/8/0/0 | 4/10/11/0/0 | 0.731 |
| Duration of SIRS (days) | 1.9 ± 1.7 | 1.7 ± 1.1 | 0.722 |
| Mortality (n) | 0 | 0 | 1.000 |

Data are expressed as mean ± SEM.
SIRS, systemic inflammatory response syndrome.

**Table 4**
Effect of immunonutrition on risk of development of infectious complications, in relation to risk factors for pancreaticoduodenectomy.

| Factor | Median value | No. of patients | Perioperative group | Preoperative group | P value |
|---|---|---|---|---|---|
| BW loss (%) | ≥5 | 4 | 0/2 | 2/3 | 0.136 |
| | < 5 | 56 | 4/28 | 6/27 | 0.446 |
| PNI | ≥50 | 18 | 2/11 | 1/7 | 0.829 |
| | < 50 | 42 | 2/19 | 7/23 | 0.118 |
| Con A; POD -1 (SI) | ≥109 | 30 | 2/15 | 4/15 | 0.361 |
| | < 109 | 30 | 2/15 | 4/15 | 0.361 |
| PHA; POD -1 (SI) | ≥139 | 30 | 1/15 | 5/15 | 0.068 |
| | < 139 | 30 | 3/15 | 3/15 | 1.000 |
| EPA; POD -1 (μg/ml) | ≥175 | 30 | 2/16 | 4/14 | 0.272 |
| | < 175 | 30 | 2/14 | 4/16 | 0.464 |
| Operative time (min) | ≥437 | 29 | 0/13 | 5/16 | 0.027 |
| | < 437 | 31 | 4/17 | 3/14 | 0.889 |
| Blood loss (ml) | ≥570 | 30 | 3/14 | 8/16 | 0.105 |
| | < 570 | 30 | 1/16 | 0/14 | 0.341 |
| Pancreatic texture | Soft | 37 | 2/18 | 7/19 | 0.068 |
| | firm | 23 | 2/12 | 1/11 | 0.589 |
| ASA-PS | I | 15 | 0/6 | 2/9 | 0.215 |
| | II, III | 45 | 4/24 | 6/21 | 0.338 |
| Stage (UICC) | I, II | 34 | 2/19 | 5/15 | 0.102 |
| | III, IV | 25 | 2/11 | 3/14 | 0.840 |
| Biliary drainage | (+) | 29 | 2/14 | 4/15 | 0.419 |
| | (-) | 31 | 2/16 | 4/15 | 0.318 |

Data are expressed as number of patients. BW, body weight; PNI, prognostic nutritional index; POD -1, preoperative day 1; Con A, concanavalin A-stimulated lymphocyte proliferation; PHA, phytohemoglutinin-stimulated lymphocyte proliferation; SI, Stimulation index; EPA, eicosapentaenoic acid; ASA-PS, American Society of Anesthesiologists Physical Status classification.

pancreaticoduodenectomy [6,7]. However, reports of the effects of immunonutrition have been conflicting. In their meta-analysis, Hegazi et al. reported no significant difference in the effect of preoperative immunonutrition as compared with standard oral nutrition supplements on clinical outcomes in patients undergoing gastrointestinal tract surgeries [19]. One drawback of meta-analysis studies has been that they included a heterogeneous group of patients undergoing different surgical procedures for different types of cancer. The pathogenesis of postoperative infections depends partly on the magnitude of surgical stress.

Braga et al. demonstrated that preoperative and perioperative, compared to no, immunonutrition reduced the postoperative infection rate in patients undergoing colorectal surgery, but no significant difference was observed between the preoperative and perioperative immunonutrition groups. Therefore, they suggested that postoperative prolongation of immunonutrition had no additional benefit after colorectal surgery [20].

On the other hand, pancreaticoduodenectomy is more stressful surgery than colorectal surgery: we previously demonstrated that the peak level of plasma IL-6 after pancreaticoduodenectomy was higher than that after gastric or colorectal surgery [6,7,21]. Furthermore, stress-induced immunosuppression was greater after pancreaticoduodenectomy than after gastric or colorectal surgery [6,7,21]. Because immunonutrition modulates the stress response and stress-induced immunosuppression, we hypothesized that prolonged, compared with preoperative, administration of immunonutrients would have additional effects after pancreaticoduodenectomy.

Braga et al. also reported that perioperative immunonutrition seemed to be a better approach than preoperative immunonutrition to support malnourished patients for major elective surgery [22]. In contrast, the current study targeted well-nourished patients, so 90% or more of enrolled patients were well nourished (body weight loss < 10% within the three months before surgery).

Although IPMN and pNET patients accounted for almost 50% of the patient population in the present study, there were no differences in the proportion of undernourished patients compared with our past RCTs for pancreaticoduodenectomy. A subgroup analysis with patients with adenocarcinoma only, excluding pNET and IPMN, was performed, and the results were similar (data not shown).

It was noted that Con A- or PHA-stimulated lymphocyte proliferation was higher in the perioperative group than in the preoperative

group on POD 14, and that plasma EPA levels on POD 7 were correlated with Con A- or PHA-stimulated lymphocyte proliferation on POD 14. It has been reported that cell-mediated immune dysfunction predisposes the patients to opportunistic infection, multiple organ dysfunction syndrome, and death [23,24]. We have demonstrated that an immune-enhancing diet enriched with EPA modulates cell-mediated immunity through T-cell differentiation [6,7]. These results may suggest that surgical patients with high levels of EPA are at an advantage regarding the host defense mechanisms.

Subgroup analysis was performed to explore the additional effects of perioperative immunonutrition on infectious complications according to the risk factors for pancreaticoduodenectomy. In the subgroup of patients who underwent operations requiring a long time period (> 437 min), perioperative immunonutrition had an additional effect on the postoperative infection rate. Plasma IL-6 levels were significantly higher in the long operative time group than in the comparison group (data not shown). These results may suggest that, even for well-nourished patients who undergo severely stressful, high-risk surgery, such as long operative time procedures, immunonutrition should be prolonged postoperatively.

There are several study limitations that need to be considered. First, the primary endpoint of this trial was Con A- or PHA-stimulated lymphocyte proliferation. This was because the focus was on the mechanisms of the effect of immunonutrition on immunological responses. Second, there was a risk of observer bias. However, this bias was reduced, as much as feasibly possible, by the use of validated definitions for outcomes and standardized protocols by assessors with relevant qualifications and experience.

## 5. Conclusion

The present study did not demonstrate additional effects of perioperative immunonutrition on postoperative immunity and infectious complications after pancreaticoduodenectomy. Preoperative immunonutrition is necessary and sufficient to achieve its immunological effects in patients undergoing severely stressful surgery. In case of high-risk surgery, such as long operative time procedures, immunonutrition may be prolonged postoperatively. The present study was not powered to answer questions regarding clinical endpoints; a larger randomized trial is required to address such questions and to confirm the current findings.

## Ethical approval

The protocol was reviewed and approved by the institutional review board of the Chiba University Hospital. The reference number is G24050.

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## Author contribution

Youhei Miyauchi: laboratory studies, data collection, data analysis, writing.
Katsunori Furukawa: study design, data analysis, review.
Daisuke Suzuki: assisting in laboratory studies, data collection.
Hideyuki Yoshitomi: data analysis, review.
Tsukasa Takayashiki: assisting in laboratory studies, data analysis.
Satoshi Kuboki: assisting in laboratory studies, data analysis.
Masaru Miyazaki: study design, review.
Masayuki Ohtsuka: manuscript editing, review.

## Conflicts of interest

All authors confirm that there are no relevant conflicts of interests.

## Reasearch registration number

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## Guarantor

Masayuki Ohtsuka, Katsunori Furukawa.

## Provenance and peer review

Not commissioned, externally peer-reviewed.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijsu.2018.11.028.

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