A randomized controlled trial comparing antioxidant-enriched enteral nutrition with immune-enhancing enteral nutrition after esophagectomy for cancer: A pilot study

Takeshi Nagano,[1] Hiromasa Fujita,[1] Toshiaki Tanaka,[1] Satoru Matono,[1] Kazutaka Murata, [1] Nobuya Ishibashi,[1] Kazuo Shirouzu,[1] Takashi Yanagawa[2]

[1]Department of Surgery, Kurume University School of Medicine, Kurume
[2]Biostatistics Center, Kurume University, Kurume
Asahi-machi 67, Kurume-city, Fukuoka 830-0011, Japan
Tel: +81-942-31-7566, Fax: +81-942-34-0709

Correspondence to: Takeshi Nagano, MD
Department of Surgery, Kurume University School of Medicine
67 Asahi Machi, Kurume City, Fukuoka 830-0011, Japan
Tel: +81-942-31-7566, Fax: +81-942-34-0709
e-mail: nagano_takeshi@kurume-u.ac.jp

Abstract

Purpose

The objective of this study was to compare the effects of two types of enteral supplements, an antioxidant-enriched enteral nutrition (AeEN) and an immune-enhancing enteral nutrition (IeEN), on the nutrition, immunoinflammatory response, antioxidant capacity and clinical outcomes in patients after esophagectomy for cancer.

Methods

Patients (n=20) undergoing esophagectomy for cancer were randomized in this single-center, open-label study. Two types of enteral supplements were used for five days before surgery and seven days after surgery. The circulating levels of nutritional markers, immunoinflammatory markers, oxidative stress markers, and the antioxidant capacity were compared throughout the perioperative period, and the patients' clinical outcomes were also compared.

Results

The circulating levels of nutritional markers decreased after surgery, but the changes were not significantly different between the AeEN group and the IeEN group throughout the perioperative period. Surgery increased the immunoinflammatory markers, and the levels were not significantly different between the groups after surgery. Surgery also increased the levels of oxidative stress markers, but there were no significant differences between the groups throughout the study period.

Conclusions

The results of this pilot study suggest that AeEN and IeEN have a similar effect on nutrition, the immunoinflammatory response, antioxidant capacity and clinical outcomes after esophagectomy for cancer. These findings therefore warrant further studies on a larger scale.

Introduction

In the past ten years, there have been many reports concerning the efficacy of immune-enhancing nutrients (IeNs) including arginine, omega-3-unsaturated fatty acids, nucleotides and other molecules used prior to gastrointestinal surgery (1-4). The immune-enhancing enteral nutrition (IeEN) is considered to increase the immune response and improve the surgical outcomes, such as mortality and morbidity, the length of the intensive care unit (ICU) stay, the length of the hospital stay, and other outcomes. Impact® includes supplemental arginine, omega-3-unsaturated fatty acids, nucleotides, and is specialized as IeEN. We have previously reported a retrospective study that showed that the Impact® IeEN reduced the duration of systemic inflammatory response syndrome (SIRS) and the morbidity after esophagectomy for cancer (5). According to the consensus recommendations of the American Society for Parenteral and Enteral Nutrition (ASPEN), the preoperative use of IeEN offers a benefit for patients undergoing elective gastrointestinal surgery, such as esophagectomy (6). In the guidelines on enteral nutrition of the European Society for Clinical Nutrition and Metabolism (ESPEN), the perioperative management is focused on the enhanced recovery of patients after surgery (ERAS). According to those guidelines, the preoperative use of enteral nutrition including immunomodulatory substances is recommended for cancer patients undergoing major upper abdominal surgery (7).

A variety of diseases are recognized to be related to oxidative stress resulting from an imbalance between oxidants produced by various stresses and the natural antioxidant capacity. The overproduction of reactive oxygen species (ROS) arising from inflammation due to stresses such as surgery, trauma, burns and other causes, as well as in life-style-related diseases, can cause damage to cells. Accordingly, it is hoped that antioxidants can reduce the oxidative stress and the subsequent organ damage (8). There was a report that indicated that antioxidant-enriched enteral nutrition (AeEN) increased the immune response in patients undergoing major gastrointestinal surgery (9). In contrast, there was another report that the AeEN including glutamine and an antioxidant solution did not improve the prognoses of burned and major trauma patients (10), and that AeEN including an antioxidant, and vitamins A, C, and E improved the blood level of antioxidants, but did not improve the clinical outcomes (11). The Anom® AeEN includes antioxidants such as catechin and proanthocyanidin. It also includes arginine, omega-3-polyunsaturated fatty acids and nucleotides.

The purpose of this prospective randomized trial was to compare the effects of AeEN (Anom®) and IeEN (Impact®) on the nutritional markers, immunoinflammatory

markers, antioxidant capacity and clinical outcomes in patients undergoing esophagectomy for cancer.

Methods

During the period from June 2007 to June 2009, 20 patients were enrolled in this trial who met the following conditions; 1) younger than 75 years of age, 2) potentially-resectable esophageal cancer of clinical stage II-III, 3) no pretreatment, 4) possible peroral intake, 5) proposed operation being right-sided transthoracic esophagectomy and esophageal reconstruction using a gastric conduit, and 6) the informed consent provided. They were randomly divided into two groups: an of AeEN group that received Anom® an IeEN group that received Impact®. The elements per 100 ml of Anom® and Impact® are described in Table 1. Both formulas included the basic nutrients such as protein, fat and carbohydrates. They both also contained immune-enhancing nutrients such as arginine, omega-3-polyunsatuilated fatty acids and nucleotides. However, the levels of such nutrients were much higher in the Impact® than in the Anom® supplement. On the other hand, glutamine and antioxidants, such as catechin and proanthocyanidin, were included in the Anom®, but not in the Impact® supplement.

Both nutrient supplements were administered according to the standard Clinical Pathway (Figure 1). In the AeEN group, 800 ml of Anom® were given perorally for five days before surgery at a total dose of 4,000 ml, while in the IeEN group, 750 -1,000 ml of Impact® were given perorally for five days before surgery for a total dose of 4000 ml. For seven days after surgery, Anom® (from 400 ml to 1,600 ml) was given using a gastrostomy at a total dose of 8,400 ml, while in the IeEN group, Impact® (from 500 ml to 1,500 ml) was given using a gastrostomy for a total dose of 8,500 ml. All the protocols were approved by the institutional review board of the Kurume University School of Medicine (#06058: 29th September, 2006), and all the patients provided informed consent for their participation in this trial.

The patients' demographic information, such as their age, gender, body weight and body mass index (BMI) on admission, diet on admission, co-morbidities, the clinical and pathological TNM stages (UICC 7th ed.)(12), surgical procedures, and the length of the operation and blood loss were not significantly different between the AeEN group and the IeEN group (Tables 2-3). All the patients underwent right-sided transthoracic esophagectomy with thoracoabdominal two-field or cervicothoracoabdominal three-field lymphadenectomy, followed by esophageal reconstruction using a gastric conduit. The

route of esophageal reconstruction was a retrosternal route in one patient and a subcutaneous route in the other nine patients in the AeEN group, while it was a subcutaneous route in all ten patients in the IeEN group. None of the patients underwent a perioperative blood transfusion, although all of the patients had autotransfusions of 800 ml prepared in case a transfusion was necessary. Enteral nutrition was not discontinued in any patient, although abdominal distension was observed in two patients in the IeEN group.

The patients' body weight and the BMI were measured seven and one day before surgery, and on the first, third, seventh and thirteenth days after surgery. As nutritional markers, the levels of serum protein, albumin, transferrin and retinol binding protein (RBP) were measured seven and one day before surgery and on the seventh and thirteenth days after surgery. As immunoinflammatory markers, the WBC, C-reactive protein (CRP), interleukin-6 (IL-6) and interleukin-8 (IL-8) level were measured. The WBC and CRP level were measured seven and one day before surgery and days after surgery. IL-6 and IL-8 were measured immediately and on the first and second days after surgery. As oxidative stress markers, the urinary levels of 8-hydroxy-2'-deoxyguanosine (8OHdG), an oxidized nucleotide, and 8-Isoprostane, an oxidized lipid, were measured, and the potential antioxidant (PAO) level in the serum was measured as an indicator of the antioxidant capacity. These three parameters were measured seven and one days before surgery and on the second and thirteenth days after surgery.

The incidence of postoperative complications, the length of SIRS, the length of hospital stay, nutritional markers, immunoinflammatory markers and the antioxidant capacity were compared between the two groups. SIRS was defined as fulfilling at least two of the following four conditions; (1) body temperature > 38℃ or < 36℃, (2) pulse > 90/min, (3) respiration > 20/min, $PaCO_2$ < 32 mmHg or need for assisted ventilation, (4) WBC > 12,000/mm^3 or < 4,000/mm^3. The length of hospital stay was defined as the duration until the initiation of postoperative chemotherapy and/or radiotherapy in patients receiving adjuvant therapy.

The Wilcoxon-Mann-Whitney test was used to examine the differences between the AeEN group and the IeEN group at the initial time point of measurements with a 5% level of significance. The difference between the two groups after the initial time point were analyzed by examining the $\beta 2$ in the following model that was adjusted for the difference in the measurements at the previous time point.

$y = \beta 0 + \beta 1$ (the previous value) + $\beta 2$ (group) + γ (the previous value)(group)

The p-values at several time points after the initial measurement were summarized

using summary statistics which were constructed by adding $\beta 2$ at those time points. Since the absolute sizes of the two groups were fairly small for conventional statistical tests with a 5% level of significance, the Akaike Information Criterion (AIC) which chose the most reasonable model based on the Kullback-Libler Information (13) was employed except for the test at the initial point; this was determined by simple computation to indicate the equivalence of the two groups if the p-value was > 0.18 and no equivalence if the p-value was < 0.18.

Results

The changes in the body weight and the BMI are shown in Figure 2. Both values were increased for several days after surgery and decreased to the preoperative level within a week. No differences were observed in the perioperative changes in the body weight and BMI between the two groups, except for the thirteenth day after surgery when they were higher in the IeEN group.

The changes in the nutritional markers, such as protein, albumin, transferrin and RBP levels appear were in Figure 3. The levels of the nutritional markers were increased by the preoperative administration of the nutrients for five days in both groups, and they decreased after surgery, and then increased again by the postoperative administration of the nutrients for seven days in the both groups. The total protein level was higher on the seventh day after surgery in the IeEN group, while it was higher on the thirteenth day after surgery in the AeEN group. The transferrin level was higher on both the seventh and thirteenth days after surgery in the IeEN group. However, no significant differences were found overall in any of the four nutritional markers between the groups. In short, no differences were observed in the nutritional markers between the AeEN group and the IeEN group through the period from seven days before surgery to the thirteenth day after surgery.

Figure 4 shows the changes in immunoinflammatory markers such as the WBC and the CRP, IL-6 and IL-8 levels. All of these markers were increased after surgery; however, IL-6 and IL-8 rapidly decreased by the day after surgery, and the CRP level slowly decreased for one or two weeks. The WBC remained high for two weeks after surgery. The WBC was high in the AeEN group on the first and thirteenth days after surgery, while it was higher in the IeEN group on the seventh day after surgery. The CRP level was higher in the AeEN group on the seventh day after surgery. In addition, overall CRP level was significantly higher in the AeEN group. No significant differences were found in the levels of IL-6 and IL-8 between the groups at any point or overall. In

short, only the level of CRP was significantly different between the two groups, being no difference was observed in the inflammatory markers between the AeEN group and the IeEN group throughout the period after surgery, except for CRP which was more reduced in the IeEN.

Figure 5 shows the changes in the oxidative stress, as indicated by 8OHdG and 8-Isoprostane in the urine, and that of the antioxidant capacity, as determined by the PAO level in the serum. The levels of oxidative stress markers were increased after surgery, while the antioxidant capacity was decreased after surgery. The levels of 8OHdG in the urine were higher in the IeEN group on the thirteenth day after surgery, and the overall level was higher in the IeEN group. No differences were found in the 8-Isoprotane in urine or in the PAO in serum between the groups at any point after surgery or overall. In short, no difference was observed in the levels of the oxidative stress marker, 8-Isoprostane, or in the antioxidant capacity (based on the PAO) between the AeEN and IeEN groups, although the level of 8OHdG (another marker of oxidative stress) was found to have decreased to a greater extent by the AeEN.

As shown in Table 3, the mean and median the length of SIRS after surgery were 1.8±1.5 days and one day, respectively, in the AeEN group, while these were 3.4±3.5 days and one day, respectively, in the IeEN group. No significant difference was found in the length of SIRS between the groups. The mean and median length of hospital stay after surgery were 39.6±15.0 days and 35 days, respectively, in the AeEN group, and were 41.2±12.0 days and 38 days, respectively, in the IeEN group. These values were not significantly different between the groups. Postoperative complications were observed in four (40%) among the 10 patients in the AeEN group; anastomotic leaks developed in three patients (30%) and surgical site infections (SSI) developed in three patients (30%). Four (40%) of the 10 patients in the IeEN group also developed postoperative complications; anastomotic leaks in developed in three (30%), a SSI developed in one (10%) and a pulmonary complication occurred in one (10%) patient. No differences were observed in the incidences of postoperative complications between the groups.

Discussion

It is commonly considered that the preoperative use of IeNs including arginine, glutamine, omega-3-polyunsaturated fatty acids, nucleotides and other factors, increase the immune response, decrease the postoperative complications and improve the patient outcomes after elective gastrointestinal surgery (1-4). According to the

consensus recommendations of the ASPEN, the preoperative use of IeNs at a dose of 1,200~1,500 ml/day for five to seven days offers a benefit for patients undergoing an elective gastrointestinal surgery, such as esophagectomy (6). At the end of the recommendations, a list of the immune-enhancing formulas is provided. In the ESPEN guidelines on enteral nutrition, the perioperative management is focused on the ERAS. In those guidelines, the preoperative use of IeNs for five to seven days is recommended for cancer patients undergoing major upper abdominal surgery (7).

The Impact® formula includes arginine, omega-3-polyunsaturated fatty acids and nucleotides, and it is specialized as an immune-enhancing diet. There were two trials comparing Impact® with the conventional enteral nutrients contained in Osmolite®. In critically-ill patients in the ICU, the use of Impact® increased the immune response, however it did not shorten the length of hospital stay (14). In postoperative gastrointestinal cancer patients, the use of Impact® improved the immune function, and reduced the incidence of infections and wound complications by 70% and shortened the length of hospitalization by 22% (15).

However despite the many promising findings, there are also negative data concerning the IeNs. A meta-analysis indicated that the arginine included in the IeNs is useful for patients undergoing elective surgery, but is harmful for septic patients (16). In the Canadian Clinical Practice Guidelines, it is noted described that the arginine-containing enteral products should not be used for nutritional support for mechanically ventilated critically-ill adult patients (17). Moreover, it was reported that the IeNs do not offer a clinical benefit for adult ICU patients (18). In the ESPEN Guidelines on Enteral Nutrition, the postoperative use of IeNs after uncomplicated surgery was given a C with regard to the level of recommendation (7). In short, the preoperative use of IeNs seems to be useful for patients undergoing elective gastrointestinal surgery, but there is no evidence of efficacy regarding the postoperative use of IeNs for patients undergoing gastrointestinal surgery or in critically-ill ICU patients

It is thought considered that the use of antioxidants reduces the oxidative stress caused by surgery, trauma and other conditions, and thereby decreases the subsequent organ damage. AeEN was reported to increase the immune response in patients undergoing major gastrointestinal surgery (9). Anom®, an AeEN includes antioxidants such as catechin and proanthocyanidin, as well as arginine, omega-3-polyunsaturated fatty acids, and nucleotides. However, it does not include as much of these components as the Impact formula.

There are negative data concerning the use of AeEN. For example, it was reported

that the AeEN including glutamine and an antioxidant solution did not improve the prognosis of burned and major trauma patients (10), and that, while an AeEN including antioxidants, and vitamins A, C, and E improved the blood levels of antioxidants, it did not improve the clinical outcomes of patients (11). According to the Canadian Clinical Practice Guidelines for nutritional support, glutamine-enriched formula should be considered for patients with severe burns and trauma, while the use of antioxidants and probiotics has no evidence to support its recommendation (17). In short, the use of AeEN seems to increase the circulating levels of the immune response markers in patients undergoing major gastrointestinal surgery and those with severe burns and trauma, but there is no evidence that it improves the clinical outcomes after such surgery, or has any impact on critically-ill ICU patients.

There is controversy with regard to the method of administration and the dose of the nutrients. Some investigators have recommended that the supplements should be used only preoperatively and immediately after surgery. In the present randomized control trial, both the AeEN (Anom®) and the IeEN (Impact®) were administered at doses of 750 to 800 ml/day for five days before surgery, and for seven days after surgery according to the standard clinical pathway at our hospital. The administration according to the protocols was successfully completed in both groups.

This pilot study was designed as a small randomized trial to investigate the potential equivalence or advantages in terms of various clinical effects using two types of nutrients. We focused on the perioperative courses and surgical outcomes after esophagectomy for cancer. The number of cases was designed to be small to discover any potential statistical differences in nutritional markers, immunoinflammatory markers, oxidative stress markers and other markers, and in the clinical outcomes between the groups. Because we included only a small number of cases, the results from this trial could be carefully and accurately controlled. There is commonly an imbalance between two groups in studies involving a small number of patients, which can be attributed to the pre-study values. Therefore, we formulated the following regression model for our analyses:

$y=\beta 0+\beta 1(\text{the previous value})+\beta 2(\text{group})+\gamma(\text{the previous value})(\text{group})$

The p-values at several time points were summarized using statistics which were constructed by adding β2 at those time points. The AIC method was adopted for the statistical analysis, in which the model better corresponding to the obtained data was selected. If the model indicated no differences, then those data were assumed to be equivalent. The equivalence of the two groups was concluded if the p-value was <0.18 and no equivalence was considered to be present if the p-value was > 0.18, because the

number of patients in each group was 10. The probability of a false-positive finding in this AIC method was estimated to be less than 40%.

All 20 patients could take food perorally. However, such patients are generally in a poor nutritional state due to their disease. Nutritional markers such as the total protein, transferrin and RBP levels were low before nutritional therapy (on the sixth day before surgery), and increased after nutritional therapy (as indicated the day before surgery). The efficacy in terms of improving these markers was similar between Anom® and Impact®. After surgery, all four nutritional markers were decreased, and then the levels of transferrin and RBP (rapid turn-over proteins) increased on the thirteenth day after surgery. Such postoperative changes in nutritional markers were similar between the both groups. Accordingly, both diets seem to have similar effects on the nutrition of patients who undergo esophagectomy.

Concerning the immunoinflammatory markers, no differences were observed in the changes in the WBC, IL-6 and IL-8 levels throughout the perioperative period between the groups. However, the CRP level was more rapidly reduced after surgery in the IeEN group than in the AeEN group. With regard to the immunoinflammatory response, the IeEN (Impact®) seemed to be similar or slightly superior to the AeEN (Anom®) formula. Concerning the oxidative stress markers and the anti-oxidant capacity, no significant differences were observed in the changes in 8-Isoprotane and PAO throughout the perioperative period. However, the level of 8OHdG was suppressed throughout the perioperative course in the AeEN group compared to the IeEN group. Therefore, with regard to the anti-oxidant capacity, the AeEN (Anom®) seems to be similar or slightly superior to the IeEN (Impact®).

One patient in the IeEN group developed aspiration pneumonia on the second day after surgery, when he underwent mini-cricothyroidectomy. In this patient, the level of IL-6 decreased from 794 pg/ml immediately after surgery to 388 pg/ml on the first day after surgery, when a tracheal tube was removed, and increased to 1,070 pg/ml on the second day after surgery when he was suffering from aspiration pneumonia. The SIRS period continued for 11 days after surgery. If this patient was excluded from the trial, the inflammatory markers were more equivalent between the groups. However, all 20 cases were enrolled based on randomization and all were analyzed according to the intent-to-treat.

The Impact® formula is an immune-enhancing diet including arginine, omega-3-polyunsaturated fatty acids and nucleotides. Omega-3-polyunsaturated fatty acids are considered to suppress the inflammation by competing against omega-6-polyunsaturated fatty acids to inhibit the production of prostaglandin E2

(PGE2) and leukotriene B4 (LTB4). It is also considered to suppress the inflammation by inhibiting NF-κB (19). Arginine has many functions, such as inhibiting bacterial growth, regulating T-cells and enhancing the production of the cytokines. It seems to suppress inflammation by means of NO (16). Nucleotides, elements of DNA, are considered to enhance the movement of WBCs (20). On the other hand, Anom® is antioxidant-enriched enteral nutritional supplement that include glutamine and polyphenol, while the content of arginine, omega-3-polyunsaturated fatty acids and nucleotides is lower than those present in the Impact® formula. Accordingly, both formulas seem to have strong points that differ from each other. However, no definitive difference was found in the postoperative changes in immunoinflammatory markers, oxidative stress markers or the antioxidant capacity between the diets. In addition, no large difference in the postoperative outcomes, such as the duration of SIRS, the incidence of postoperative complications and the duration of hospital stay was found between the groups. This pilot trial therefore suggests that AeEN (Anom®) and IeEN (Impact®) showed a similar potential in terms of their perioperative effects for patients undergoing esophagectomy for cancer.

Conclusions

This pilot study suggested that the antioxidant-enriched enteral nutrition (Anom®) and the immune-enhancing enteral nutrition (Impact®)showed similar effects on nutrition, the immunoinflammatory reaction, the oxidative stress and on clinical outcomes after esophagectomy for cancer when used perioperatively. Further, large-scale studies are now warranted to determine whether these findings can be generalized to wider populations.

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Conflict of Interest Statement

Takeshi Nagano has no conflict of interest to declare.

References

1) Heys SD, Walker LG, Smith I, Eremin O: Enteral nutritional supplementation with key nutrients in patients with critical illness and cancer: a meta-analysis of randominzed controlled clinical trials. Ann Surg 1999; 4: 467-477.
2) Beale RJ, Bryg DJ, Bihari DJ. Immunonutrition in the critically ill: a systemic review of clinical outcome. Crit Care Med 1999; 27: 2799-2805.
3) Heyland DK, Novak F, Drover JW, Jain M, Su X, Suchner U. Should immunonutrition become routine in critically ill patients?: a systemic review of the evidence. JAMA 2001; 286: 944-953.
4) Montejo JC, Zarazaga A, Lopez-Martinez J, Urrutia G, Roque M, Blesa AL, Celaya S, Conejero R, Galban C, Garcia de Lorenzo A, Grau T, Mesejo A, Ortiz-Leyba C, Planas M, Ordonez J, Jimenez FJ, for the nutritional and metabolic working group of the Spanish society of intensive care medicine and coronary units (SEMICYUC). Immunonutrition in the intensive care unit. A systematic review and consensus statement. Clin Nutr 2003; 22: 221-233.
5) Tsubuku T, Tanaka T, Sueyoshi S, Tanaka Y, Matono S, Mori N, Fujita H, Shirouzu K. Clinical effect of immunonutrition for esophageal surgery (Japanese with an English Abstract). Nippon Geka Kansensho Gakkai Zasshi 2007; 4： 513-517,
6) ASPEN committee: Consensus recommendations from the U.S. summit on immune-enhanceing enteral therapy. JPEN 2001; 25 (suppl) : S61-63
7) Weimann A, Braga M, Harsanyi L, Laviano A, Ljungqvist O, Soeters P. ESPEN Guidelines on enteral nutrition: Surgery including organ transplantation. Clin Nutr 2006; 25: 224-244.
8) Goodyear-Bruch C, Pierce JD. Oxidative Stress in Critically Ill Patients. Am J Crit Care 2002; 11: 543-551.
9) van Stijn MF, Ligthart-Melis GC, Boelens PG, Scheffer PG, Teerlink T, Twisk JW, Houdijk AP, van Leeuwen PA. Antioxidant enriched enteral nutrition and oxidative stress after major gastrointestinal tract surgery. World J Gastroenterol 2008; 14: 6960-6969.
10) Soguel L, Chiolero RL, Ruffieux C, Berger MM. Monitoring the clinical introduction of a glutamine and antioxidant solution in critically ill trauma and burn patients. Nutrition 2008; 24: 1123-1132.
11) Preiser JC, van Gossum A, Berre J, Vincent JL, Carpentier Y. Enteral feeding with a solution enriched with antioxidant vitamins A, C, and E enhances the resistance to oxidative stress. Crit Care Med 2000; 28: 3828-3832.
12) International Union Against Cancer (UICC). TNM classification of malignant tumours. 7th ed. Sobin LH, Gospodarowicz MK, Wittekind Ch, editor. Oxford, Wiley-Blackwell, 2009.
13) Konishi S and Kitagawa G. Information Criteria and Statistical Modeling. New York, Springer-Verlag, 2008.
14) Cerra FB, Lehman S, Konstantinides N, Konstantinides F, Shronts EP, Holman R. Effect of

enteral nutrition on in vitro tests of immune function in ICU patients: a preliminary report. Nutrition 1990; 6: 84-87.

15) Daly JM, Lieberman MD, Goldfine J, Shou J, Weintraub F, Rosato EF, Lavin P. Enteral nutrition with supplemental arginine, RNA, and omega-3 fatty acids in patients after operation: Immunologic, metabolic, and clinical outcome. Surgery 1992; 112: 56-67.
16) Ochoa JB, Makarenakova V, Bansal V: A rationale use of immune-enhancing diets: when should we use dietary arginine supplementation? Nutr Clin Pract 2004; 19: 216-225.
17) Heyland DK, Dhaliwal R, Drover JW, Gramlich L, Dodek P, Canadian Critical Care Practice Guidelines Committee. Canadian clinical practice guidelines for nutrition support in mechanically ventilated, critically ill adult patients. J Parenter Enteral Nutr 2003; 27: 355-373.
18) Kieft H, Roos AN, van Drunen JD, Bindels AJ, Bindel JG, Hofman Z. Clinical outcome of immunonutrition in a heterogeneous intensive care population. Intensive Care Med 2005; 31: 524-532.
19) Weaver KL, Ivester P, Seeds M, Case LD, Arm JP, Chilton FH. Effect of dietary fatty acids on inflammatory gene expression in healthy humans. J Biol Chem 2009; 284: 15400-15407.
20) Kulkarni AD, Fanslow WC, Drath DB, Rudolph FB, van Buren CT. Influence of dietary nucleotide restriction on bacterial sepsis and phagocytic cell function in mice. Arch Surg 1986; 121: 169-172

Figure legends

Figure 1. The dosing protocol for the enteral supplements

The AeEN (Anom®) group: 4,000 ml (800 ml/day×5days) before surgery + 8,400 ml (400~1,600 ml/day) after surgery

The IeEN (Impact®) group: 4,000 ml (750 ml/day×4days + 1,000 ml/day×1day) + 8,500 ml (500~1,500 ml/day) after surgery

Figure 2. The perioperative changes in the body weight and body mass index

The initial p-value was calculated using the Wilcoxon-Mann-Whitney test, and other p-values, including the summary p-value, were calculated according to the Akaike Information Criterion.

*A statistically significant difference was defined as being less than 0.18 according to the statistical methods employed.

Figure 3. The perioperative changes in nutritional markers

Figure 4. The perioperative changes in inflammatory markers

Figure 5. The perioperative changes in oxidative stress markers and the antioxidant capacity

Table 1 Contents/100ml

	AeEN (Anom®)	IeEN (Impact®)
Calorie (Kcal)	100	100
Protein (g)	5.0	5.6
Arginine (g)	0.46	1.28
Glutamine (g)	0.75	-
Fat (g)	2.8	2.8
ω3/ω6*	0.5	1.3
Carbohydrate (g)	14.0	13.4
Vitamins		
C (mg)	100	9.5
E (mg)	5.0	0.67
Others	A, B_1, B_2, B_6, B_{12}, D, K_2, niacin, pantotheniac acid, folic acid	
Minerals		
Zn (mg)	1.5	0.67
Cu (mg)	0.15	0.12
Se (μg)	5.0	3.3
Cr (μg)	6.0	2.0
Others	Na, Cl, K, Ca, Mg, P, Fe, I, Mn	
Nucleotide (g)	0.013	0.129
Antioxidant (mg) (Polyphenol)	Catechin 35 Proanthocyanidin 20	-

*: ratio of the omega-3-polyunsaturated fatty acid to the omega-6-polyunsaturated fatty acid

Table2 Patients' Background

	AeEN (Anom®) n = 10	IeEN (Impact®) n = 10	*p*- value
Age	67±5	65±7	ns
Sex (Male : Female)	8:2	10:0	ns
Body weight (kg)#	55±11	58±8	ns
Body mass index#	21.1±3.3	21.1±3.3	ns
Diet			
Normal/gruel/liquid	9/0/1	7/1/2	ns
Co-morbidities			
Pulmonary diseases*1	1	0	ns
Liver dysfunction*2	3	4	ns
Diabetes mellitus*3	1	2	ns
Cardiac diseases	0	1 *4	ns
Clinical T-stage			
cT1/T2/T3/T4	3/0/7/0	2/0/8/0	ns
Clinical N-stage			
cN0/N1/N2/N3	2/3/4/1	3/2/4/1	ns
Clinical M-stage			
cM0/M1-Lym	10/0	7/3	ns
Clinical stage*5			
cStage I/II/III/IV	2/1/7/0	2/1/4/3	ns
Pathological T-stage			
pT1/T2/T3/T4a/T4b	3/1/5/1/0	2/1/4/2/1	ns
Pathological N-stage			
pN0/N1/N2/N3	3/2/3/2	6/0/2/2	ns
Pathological M-stage			
pM0/M1-Lym*5	10/0	8/2	ns
Pathological stage*5			
pStage I/II/III/IV	3/1/6/0	2/2/4/2	ns
Residual tumor*5			
R0/R1	10/0	8/2	ns

ns: no significant difference, #: on admission, *1: Chronic obstructive pulmonary diseases ($FEV_{1.0}$<2,000 ml), *2: chronic viral hepatitis or alcoholic liver disease assessed as more than grade I with ^{99m}Tc-GSA scintigraphy,
*3: Diabetes requiring medication, *4: Atrial fibrillation, *5: UICC (2009)

Table 3 Surgical Procedures and Perioperative Outcomes

	AeEN (Anom®) n = 10	IeEN (Impact®) n = 10	*p*- value
Approach[*1]			
Thoracoscopic/open	2/8	2/8	ns
Lymphadenectomy			
2-field/3-field	3/7	0/10	ns
Route of esophageal reconstruction[*2]			
Subcutaneous/retrosternal	9/1	10/0	ns
Operating duration (min)	612±90	585±80	ns
Bleeding amount (g)[*3]	466±353	405±167	ns
SIRS (days)			
mean	1.8±1.5	3.4±3.5	ns
median	1	1	ns
Hospital stay			
mean	39.6±15.0	41.2±12.0	ns
median	35	38	ns
Postoperative complications	4 (40%)	4 (40%)	ns
Anastomotic leak[*4]	3 (30%)	3 (30%)	ns
SSI	3 (30%)	1 (10%)	ns
Pulmonary complication	0	1 (10%)[*5]	ns

ns: no significant difference, SIRS: systemic inflammatory response syndrome, SSI: :surgical site infection, *1: Transthoracic esophagectomy (TTE), *2: Gastric conduit, *3: No patient received blood transfusion perioperatively, *4: minor leak spontaneously healed, *5: aspiration pneumonia requiring mini-cricothyroidotomy

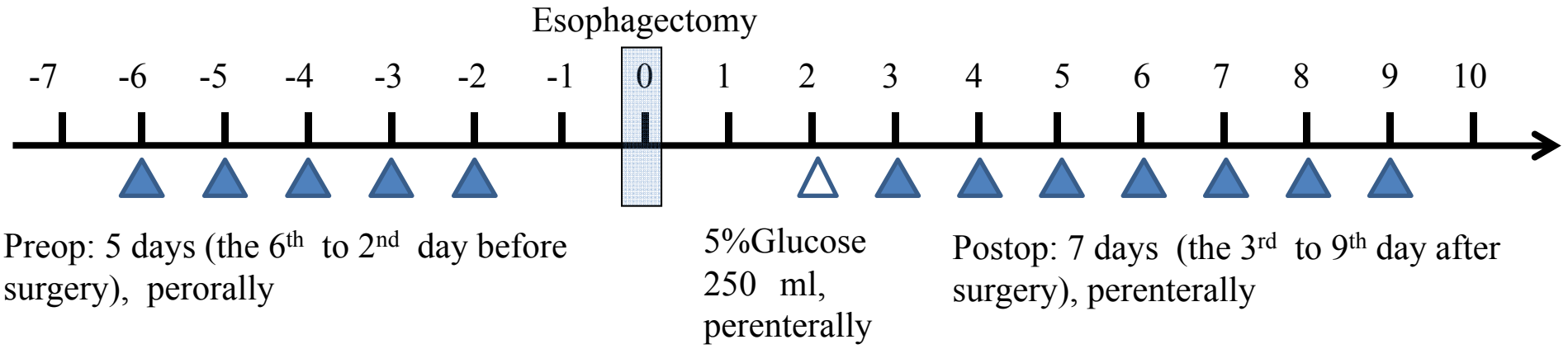
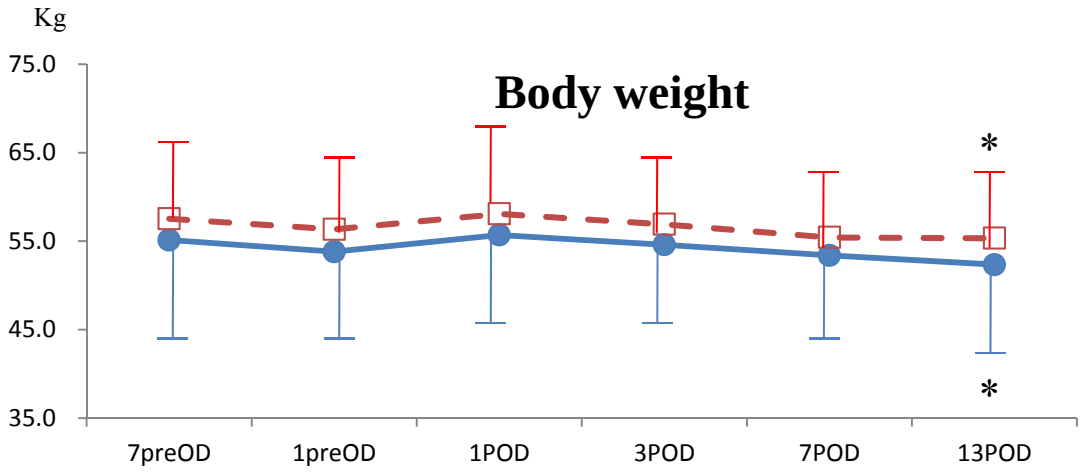
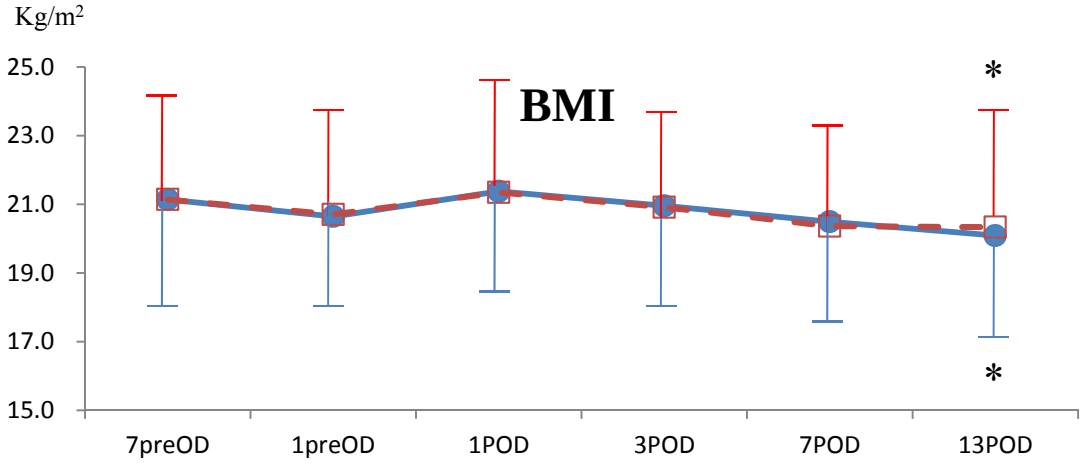


Figure 1 Regimen of enteral supplements



AeEN	55.1±11.0	53.8±10.3	55.7±9.9	54.6±10.1	53.4±9.8	52.3±9.6
IeEN	57.5±8.6	56.3±8.2	58.1±9.0	56.9±8.1	55.4±8.3	55.3±7.9
P-value	**0.623**	**0.523**	**0.761**	**0.983**	**0.531**	**0.039**

Summary
P=0.804



AeEN	21.1±3.3	20.6±3.1	21.4±2.9	21.0±3.1	20.5±3.0	20.1±2.8
IeEN	21.1±3.3	20.7±3.2	21.3±3.4	20.9±3.1	20.4±3.1	20.3±3.0
P-value	**0.910**	**0.692**	**0.615**	**0.921**	**0.518**	**0.065**

AeEN (Anom®)

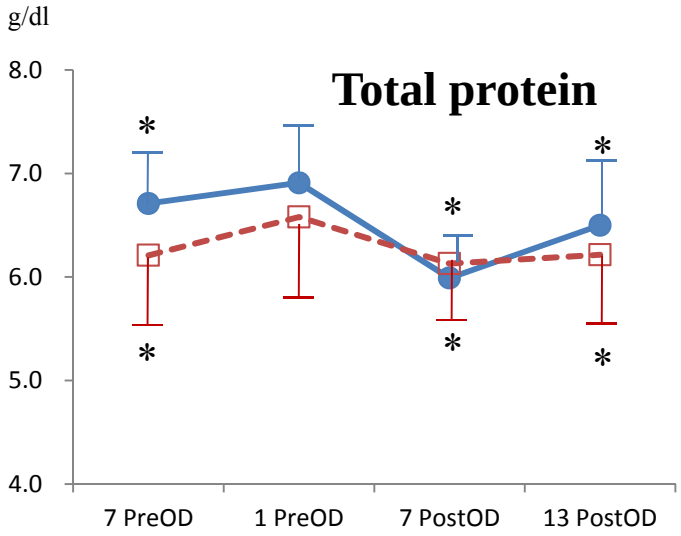
IeEN (Impact®)

*: p<0.18

Summary
P=0.721

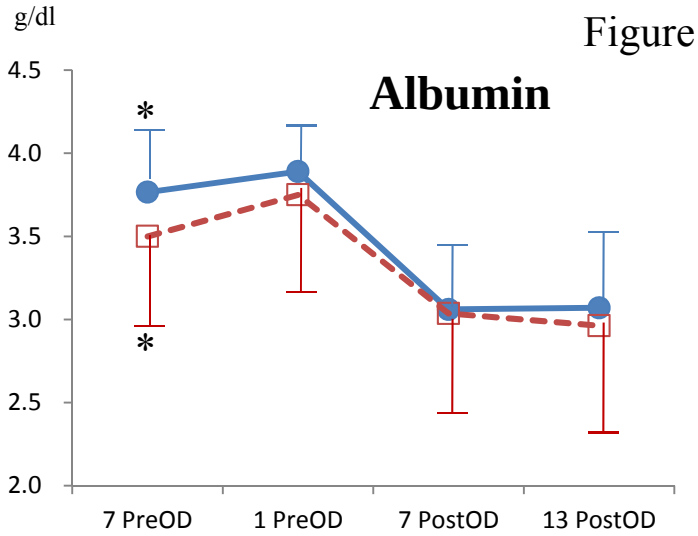
Figure 2 Body Weight and Body Mass Index (BMI)

Figure 3 Nutritional markers



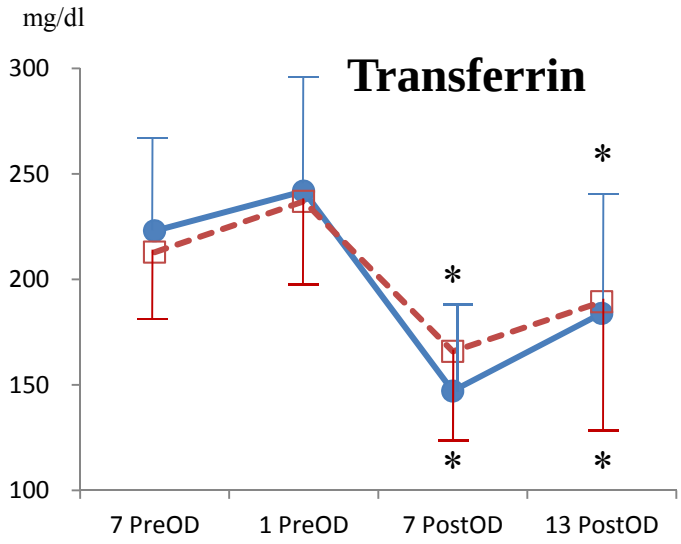
Summary
p=0.371

AeEN	6.71±0.44	6.91±0.44	5.99±0.22	6.50±0.46
IeEN	6.21±0.56	6.58±0.61	6.13±0.50	6.22±0.57
P-value	**0.085**	**0.938**	**0.165**	**0.103**



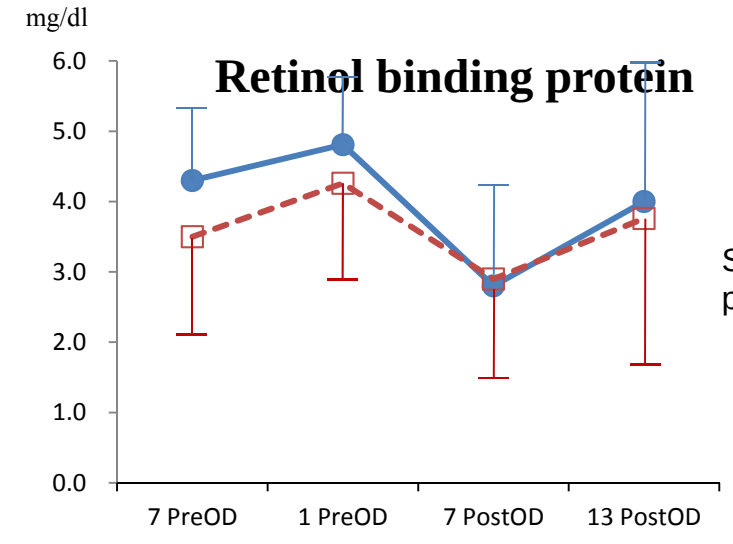
Summary
p=0.446

AeEN	3.77±0.25	3.89±0.20	3.06±0.32	3.07±0.36
IeEN	3.50±0.47	3.75±0.54	3.04±0.49	2.96±0.57
P-value	**0.149**	**0.933**	**0.840**	**0.609**



Summary
p=0.988

AeEN	223±38	242±44	147±36	184±46
IeEN	213±29	237±36	166±38	189±54
P-value	**0.393**	**0.409**	**0.053**	**0.072**



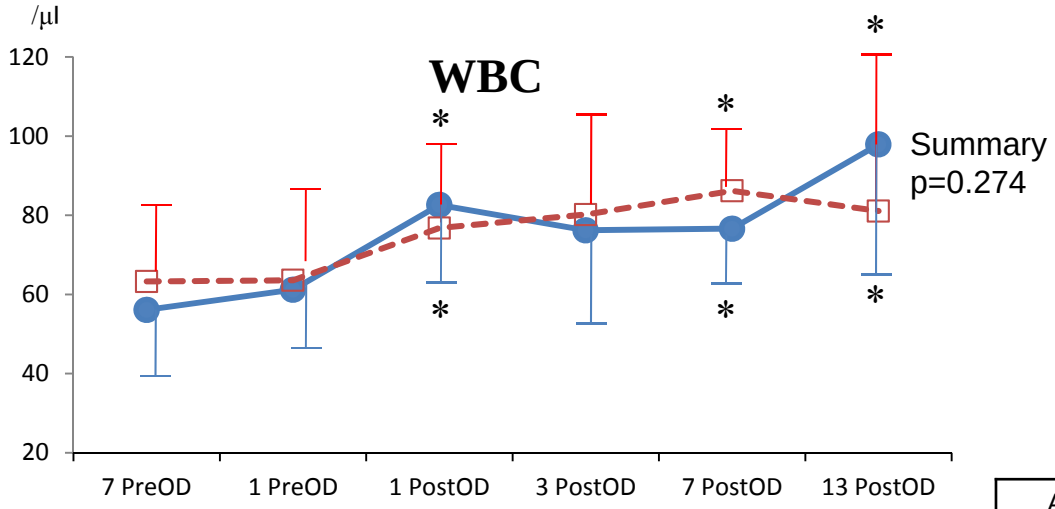
Summary
p=0.697

AeEN	4.3±0.9	4.8±0.7	2.8±1.1	4.0±1.7
IeEN	3.5±1.2	4.3±1.2	2.9±1.1	3.8±1.8
P-value	**0.480**	**0.746**	**0.193**	**0.356**

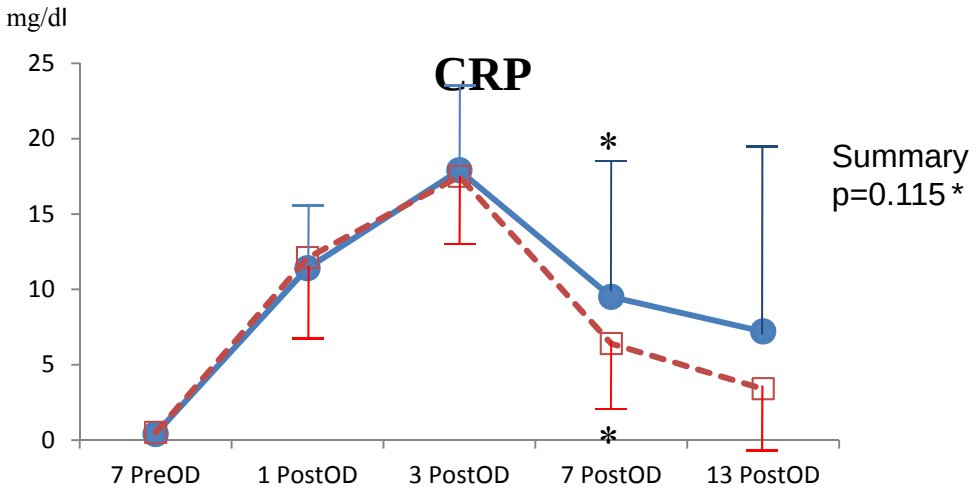
AeEN (Anom®)

IeEN (Impact®)

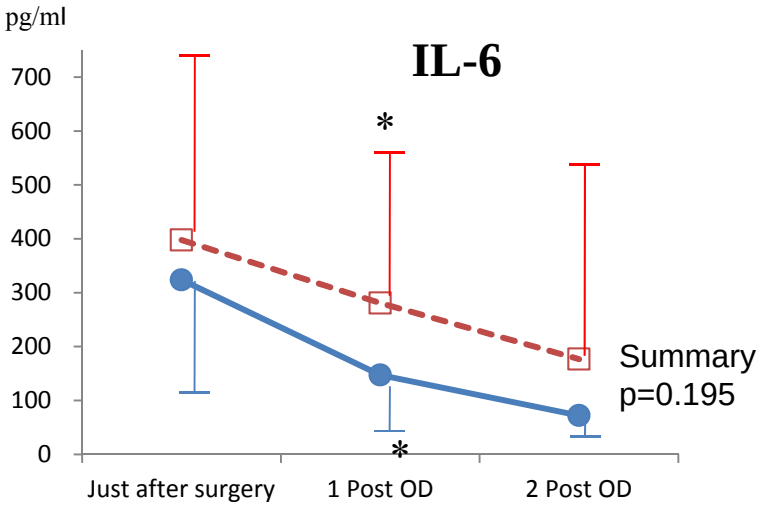
*: p<0.18



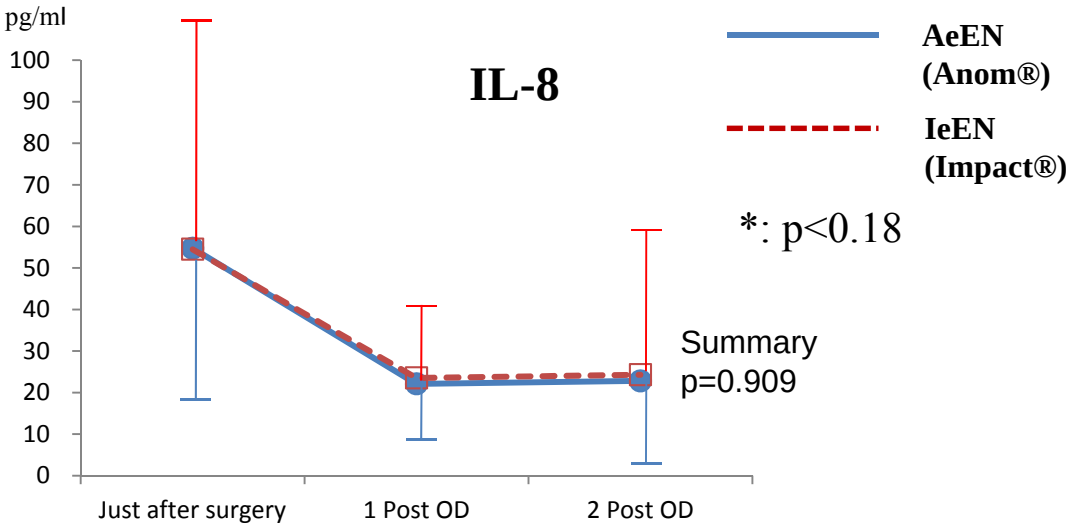
AeEN	56±11	61±15	83±21	77±21	77±11	98±28
IeEN	63±15	64±15	77±17	80±20	86±13	81±35
P-value	**0.424**	**0.399**	**0.110**	**0.228**	**0.107**	**0.179**



AeEN	0.4±0.6	11.4±2.8	17.9±7.5	9.5±7.2	7.2±10.4
IeEN	0.5±0.8	12.1±4.0	17.5±4.9	6.4±3.8	3.4±3.6
P-value	**0.469**	**0.879**	**0.446**	**0.102**	**0.719**

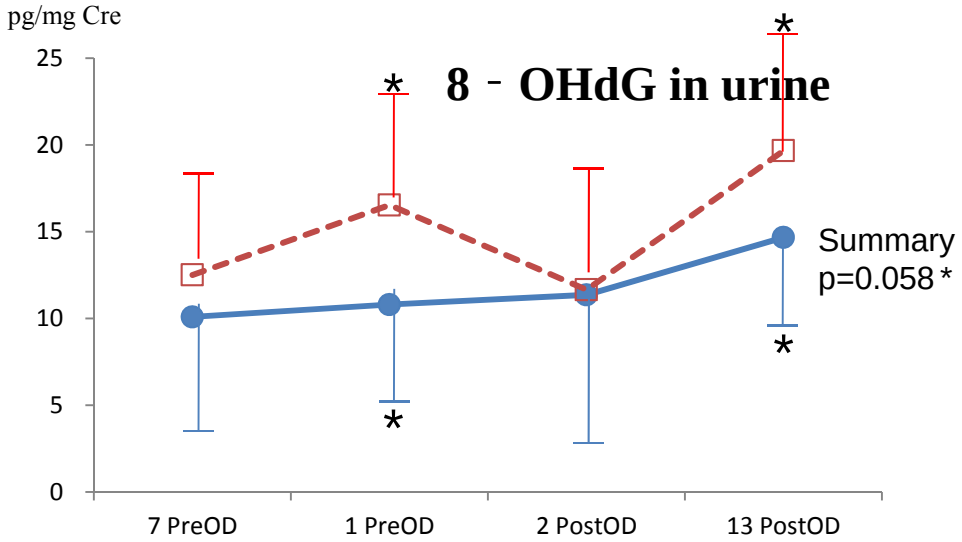


AeEN	324±185	147±75	72±34
IeEN	398±292	281±237	177±316
P-value	**0.529**	**0.150**	**0.588**

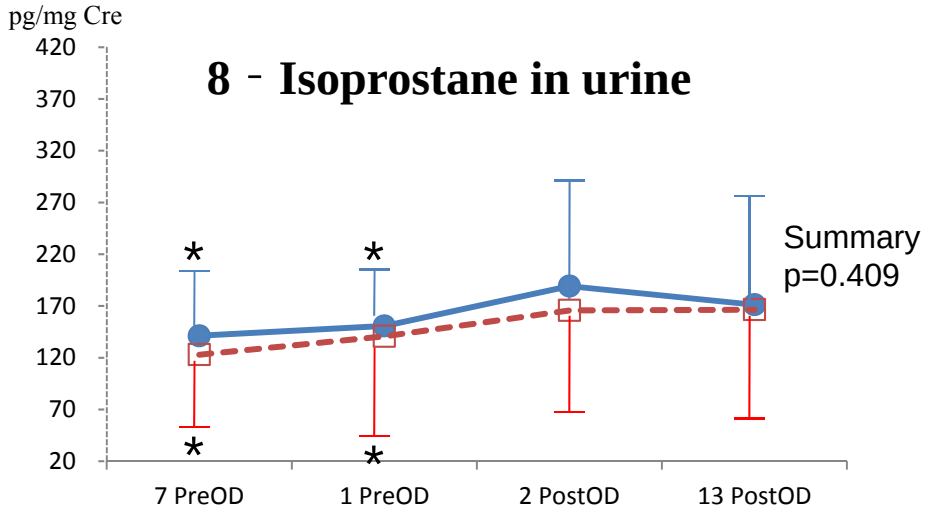


AeEN	54.7±30.9	22.1±13.2	22.8±18.3
IeEN	54.5±48.9	23.5±16.7	24.3±31.1
P-value	**0.738**	**0.787**	**0.967**

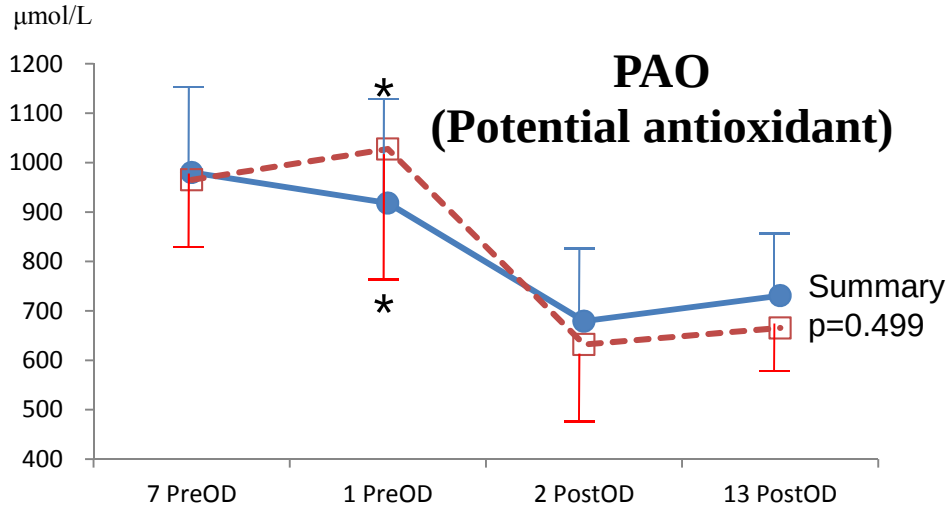
Figure 4 Inflammatory markers



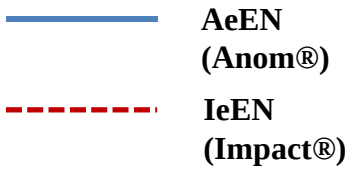
AeEN	10.1±7.0	10.8±6.2	11.4±8.5	14.7±5.4
IeEN	12.5±4.7	16.5±5.7	11.7±5.7	19.7±6.5
p-value	**0.203**	**0.022**	**0.780**	**0.078**



AeEN	130±64	137±41	184±104	183±97
IeEN	174±59	205±78	219±85	207±91
P-value	**0.041**	**0.051**	**0.983**	**0.981**



AeEN	980±154	918±177	679±135	731±117
IeEN	965±131	1028±223	632±120	666±82
P-value	**0.954**	**0.108**	**0.319**	**0.209**



*: p<0.18

Figure 5 Oxidative stress and antioxidant capacity