Journal of Investigative Surgery

ISSN: 0894-1939 (Print) 1521-0553 (Online) Journal homepage: https://www.tandfonline.com/loi/iivs20

Effect of Enteral Immunonutrition on Immune, Inflammatory Markers and Nutritional Status in Gastric Cancer Patients Undergoing Gastrectomy: A Randomized Double-Blinded Controlled Trial

Ka Li, Yujie Xu, Yanjie Hu, Yuwei Liu, Xinrong Chen & Yong Zhou

To cite this article: Ka Li, Yujie Xu, Yanjie Hu, Yuwei Liu, Xinrong Chen & Yong Zhou (2019): Effect of Enteral Immunonutrition on Immune, Inflammatory Markers and Nutritional Status in Gastric Cancer Patients Undergoing Gastrectomy: A Randomized Double-Blinded Controlled Trial, Journal of Investigative Surgery, DOI: 10.1080/08941939.2019.1569736

To link to this article: https://doi.org/10.1080/08941939.2019.1569736

Published online: 19 Mar 2019.

Journal of Investigative Surgery, 0: 1–10, 2019

ISSN: 0894-1939 print / 1521-0553 online
DOI: 10.1080/08941939.2019.1569736

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Effect of Enteral Immunonutrition on Immune, Inflammatory Markers and Nutritional Status in Gastric Cancer Patients Undergoing Gastrectomy: A Randomized Double-Blinded Controlled Trial

Ka Li[1]*, Yujie Xu[1]*, Yanjie Hu[2], Yuwei Liu[3], Xinrong Chen[1], Yong Zhou[3]

[1]*West China School of Nursing and Department of Nursing, West China Hospital, Sichuan University, Chengdu, China;* [2]*Department of Liver Surgery, West China Hospital, Sichuan University, Chengdu, China;* [3]*Department of Gastrointestinal Surgery, West China Hospital, Sichuan University, Chengdu, China*

ABSTRACT

Backgrounds: Enteral immunonutrition (EIN) has received increasing attention, however, evidence on its immunomodulatory and anti-inflammatory function in gastric cancer patients undergoing gastrectomy is poorly investigated. Objectives of this study were to evaluate the effect of EIN on immune function, inflammation response and nutrition status when compared to standard enteral nutrition (SEN). *Methods*: Totally 124 gastric cancer patients after gastrectomy were randomized to receive early 5-days postoperative EIN (formula enriched with arginine, glutamine, omega-3 fatty acids and nucleotide), or SEN. The primary end-points were $CD4^+$ T-cells, $CD3^+$ T-cells as well as counts of $CD4^+/CD8^+$, IgG, IgM, and IgA levels. Second-points included white blood cell (WBC), C-reactive protein (CRP), procalcitonin (PCT), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) levels and nutritional index such as serum albumin, prealbumin, and transferrin concentration. *Results:* There existed significant difference in primary end-points between EIN group and SEN group. The proportion of $CD4^+$ T-cells, $CD3^+$ T-cells and the counts of $CD4^+/CD8^+$, IgG, IgM, and IgA were higher in EIN group eventually. Meanwhile, the level of WBC, CRP and TNF-α were significantly lower in EIN group finally. But there were no other significant differences in nutritional markers between two groups. *Conclusion:* Early postoperative EIN significantly improves immune function and inflammatory response in gastric cancer patients undergoing gastrectomy.

Keywords: Enteral immunonutrition; gastric cancer; immune function; inflammatory markers; nutritional status

INTRODUCTION

Globally, gastric cancer (GC) is the fifth most common malignancy [1, 2], and more than 60% GC patients develop into malnutrition due to poor absorption of nutrients, active metabolism of cancer cells, and elective surgery [3]. Malnutrition is an important negative factor, which can suppress immune function, exaggerate stress response and alter inflammation reaction. Thus, these patients often have a higher risk at suffering infectious complications, delayed recovery and longer hospital stay in the postoperative period [4, 5].

Existing evidence have shown that patients requiring gastrectomy can benefit from perioperative enteral or parenteral nutrition by improving postoperative outcome [6–8]. Enteral nutrition (EN) is recognized as a more optimal nutritional therapy [8] and recommended on ESPEN guideline [9] thanks to its compliance with gastrointestinal (GI) structure, fewer complications, and reduced expenses. In recent years, enteral immunonutrition (EIN) including at least two of arginine (Arg), omega-3 fatty

Received 27 October 2018; accepted 10 January 2019.
*These authors contribute equally to this article.
Address correspondence to Prof. Yong Zhou, Department of Gastrointestinal Surgery, West China Hospital, Sichuan University, 37 Guo Xue Rd., Chengdu 610041, Sichuan Province, China. Email: Nutritioner@hotmail.com

acids, glutamine (Gln), and nucleotide gets more attention from dietitian and surgeon, which known as a kind of nutrition formula not only to supply needed energy, but also to modify immune function.

Current data in malnutrition patients undergoing upper GI surgery indicated that early EIN could result in decreased postoperative infectious or noninfectious complications, and reduced length of hospital stay [10–12]. For instance, Gln represents a necessary substance, which used for intestinal mucosal cellular metabolism to fight against inordinate intestinal immune system after surgery [13]; Arg plays multiply roles in cellular metabolism as a semi-essential amino acid [14]. But for omega-3 fatty acid, it replaces arachidonic acid in cell membranes and plays an important role in immunomodulation and anti-inflammation.

However, the evidence of direct index reflecting on immune function and inflammatory response with EIN formula in patients undergoing elective gastrectomy is still lacking. The indicators, took into several existent researches, are not comprehensive and the results are conflicting [15, 16]. Giger et al. [17] evaluated serum tumor necrosis factor-α (TNF-α) and C-reactive protein (CRP) levels of 46 postoperative patients with GI surgery and identified significance decrease in EIN group. While Klek et al. [10] drew the contradictory conclusion through assessing total lymphocyte count and CRP of 305 participants. A recent meta-analysis [18] which focused on the improvement on immune function markers refer to $CD4^+$, $CD8^+$, IgM, IgG, lymphocyte cell with EIN showed a wider view of this issue, but heterogeneity coming from different timing of nutrition and multiple type of operation between included studies would inevitably lead to bias. Therefore, trials with better methodology quality and going deep into overall improvements on immunization, inflammation and nutrition are needed to assess the effect of EIN in patients undergoing gastrectomy for gastric cancer.

We designed the study based on a hypothesis that EIN be able to reduce serum inflammatory markers and restore immune function indicators in postgastrectomy patients when compared to standard enteral nutrition (SEN). The primary objective of this randomized controlled trial was to investigate the effect of early postoperative EIN on inflammatory markers and immune indicator in a homogeneous group of gastrectomy patients. Meanwhile, to study the impact of EIN on nutritional status was the secondary objective.

MATERIALS AND METHODS

This study was conducted at Gastrointestinal Surgery Center in West China Hospital of Sichuan University between June 2017 and August 2018, as a prospective, double-blind, single-center, randomized, controlled clinical trial. Ethical approval was given by the Biomedical Research Ethical Committee of West China Hospital of Sichuan University (2014–94). Research protocol could be found in Clinicaltrial.gov with registration number NCT03730545. In addition, complete informed consent was obtained from each patient before surgery following the Declaration of Helsinki.

Patients Eligibility and Randomization

Patients aged 18–80 years with histologically diagnosed cancer of stomach called for subtotal or total gastrectomy were enrolled. No previous nutrition therapy and no severely malnourished was applied. Exclusion criteria included: pregnant or lactating woman, diagnoses of mental diseases, resent severe concomitant diseases (chronic cardiopulmonary disease, chronic renal failure, etc.), known immunodeficiency or autoimmune diseases, history of heart, lung or kidney failure, known allergies to nutrition formula or component, and drug intolerance. Patients who complied with one of the following conditions would have withdrawal of the trial: intolerance to the feeding formula (vomiting or diarrhea or abdominal distension or abdominal pain lasted for 12–24 h even in the minimum flow rate), unplanned removal of feeding tube, discontinuation enteral nutrition for any reason in the first three days of administration.

Eligible patients were randomly assigned in equal numbers to undergo 5 days SEN or EIN after surgery by a dietary nurse who had no knowledge with this trial and managed a computer-generated randomization (http://www.randomization.com). An EIN formula enriched with L-Arg (0.17 g/100 ml), Gln (0.4 g/100 ml), EPA (0.1 g/100 ml), DHA (0.07 g/100 ml), RNA (0.18 g/100 ml), and had an omega-3 to omega-6 ratio of 0.4. A SEN formula was balanced standard feeds (1.0 kcal/ml and 4 g protein per 100 ml) with an omega-3 to omega-6 ratio of 0.2, but without any other immunonutrients. The detailed characteristics and composition of the two formula was presented in Table 1.

The study was double-blind to minimize bias. The SEN and EIN feeds were similar, not only in color but also in container, and a dietary nurse who was not involved in the trial labeled the feeds with study number. Owing to different caloric concentrations in two formulas, dietary nurses must calculate each formula volume and ensure the same caloric intake between groups by different rates of flow. Otherwise, two groups were allocated to different

TABLE 1 Compositions (per 1000 ml) and characteristics of the two formulas

	EIN group	SEN group
Proteins (g)	58.6	40
L-Arginine (g)	1.7	0
Glutamine (g)	4	0
RNA (g)	1.8	0
Fat (g)	72	38.9
Polyunsaturated fatty acid (g)	11	12.3
n-3: n-6	1:2.5	1:5
EPA (g)	1	0
DHA (g)	0.7	0
Carbohydrates (g)	104	123
Maltodextrin (g)	91	103
Dietary fiber (g)	13	15
Minerals and trace elements		
Sodium (mg)	1600	1000
Potassium (mg)	2400	1500
Chloride (mg)	1600	1250
Calcium (mg)	670	800
Phosphorous (mg)	630	720
Magnesium (mg)	270	230
Iron (mg)	13	16
Zinc (mg)	10	12
Copper (mg)	1.3	1.8
Manganesium (mg)	2.7	2.2
Fluoride (μg)	1300	1000
Molybdenum (μg)	100	100
Selenium (μg)	67	57
Chromium (μg)	66	66.8
Iodine (μg)	133	130
Vitamins		
A (μg)	2000	820
Carotenoids (mg)	0	2
D3 (μg)	4.6	0
E (μg)	27	18.7
K1 (μg)	66	52
B1 (μg)	1300	1400
B2 (μg)	1700	1600
B6 (μg)	1600	1700
B12 (μg)	2.6	2.1
Niacin (mg)	12	8.7
Pantothenic ac. (mg)	4.6	5.2
Folic ac. (μg)	130	266
Biotin (μg)	130	40
Vit C (mg)	80	100
Choline (mg)	266	360
Water (ml)	800	850
Osmolarity (Osm/L)	390	250
Calorie (kcal)	1300	1000
Calorie concentration (kcal/ml)	1.3	1

wards, and staff member and patients had no idea about the flow rates and volume.−

Clinical Managements and Formula Administration

All patients received standard preoperative intervention on the basis of enhanced recovery after surgery (ERAS) protocol published by ERAS society in 2014 [19], for example, fasting solid foods for 6 h and water for 2 h before surgery. But any preoperative oral immunonutrition was not added. All of them underwent a standard nutritional assessment before surgery, and daily caloric requirements were calculated by dietary nurse with an equation: 25 kcal/kg*weight, if body mass index (BMI)>25, ideal body weight (IBW, males: IBW = 50 + [0.91*(height cm − 152.4)], females: IBW = 45.5 + [0.91*(height cm − 152.4)]) would be used. Moreover, demographics, primary diagnosis, BMI, and the presence of comorbidity were recorded in all patients.

During procedure, the surgeon and assistant would deliver the enteral feeding tube into 20–30 cm below the anastomosis. Preceding surgery, operating time, intraoperative blood loss, and transfusion volume were assessed.

At 6 h after operation, feeding tubes were first flushed with water. EN was started in 12 h after surgery. Since the difference of concentration in kcal and protein between SEN and EIN formulas (1.0kcal/ml vs. 1.3kcal/ml) might lead to difference in tolerance to nutrition, the flow rate needed to be adjusted to ensure consistent energy intake for hours. Therefore, there were an infusion rate of 20 ml per hour for SEN group and 16 ml per hour for EIN group in the first 24 h. The rates of flow were gradually increasing with 50 ml/h in SEN versus 40 ml/h in EIN on day 2, 70 ml/h versus 56 ml/h on day 3. Feeding was continued at maximum 100 ml/h versus 80 ml/h on day 4 and 5 after surgery depending on the feeding tolerance. A continuous infusion pump was used to administer the standard or immunonutrition diet for 24 h and ensure tube flushing every 4 h, along with beginning and end of feeding using 20 ml of 5% glucose in both groups. The amount of enteral nutrition administrated was recorded in the medical record. Moreover, routine blood investigations on immune, inflammation, and nutrition were measured 1 day before surgery and 1, 3, and 5 days after surgery. Both serum and plasm fractions were obtained from blood samples and stored at −80 °C until analysis.

The length of treatment lasted for 5 days after surgery, and in this period the patients were only fed the enteral formula, or water. After 5 days, the diet transformed to oral feeding gradually. Administration of immunosuppressors and any protein supplements was not allowed.

Primary and Secondary Objectives

The primary end-points of this trial were to test the impact of EIN formula on immune function in gastrectomy patients. Immunological assessments including the T-lymphocyte cells proportion and serum immunoglobulin concentration on postoperative day

(POD) 1 and 5. The proportion of $CD4^+$ T-cells, $CD3^+$ T-cells as well as counts of $CD4^+/CD8^+$ were determined by immunofluorescence quantitative analysis. And electrophoresis method was used to analyze the amount of IgG, IgM, and IgA.

As for the secondary objectives, they were to compare the impact on inflammatory response and nutritional status between different formulas. On 1 day before surgery, and POD 1, 3, and 5, white blood cell (WBC), CRP, interleukin-6 (IL-6), and TNF-α, procalcitonin (PCT) in plasma were analyzed as markers of inflammation. Measurement of CRP and PCT was performed by immunofluorescence quantitative analysis method. IL-6 and TNF-α were analyzed by an ELISA kit. Meanwhile, nutritional status was evaluated by analyzing serum albumin, prealbumin, and transferrin on preoperative baseline, POD 3, and 5. Prealbumin and transferrin were analyzed by immunodiffusion assay, while albumin was measured by chromogenic reaction for protein.

Sample Size

The sample size was determined to detect difference in serum IgG concentration by 2-sample noninferiority or superiority model admitting a type I error of 0.025 and a power of 0.80. According to the results published, SEN could increase IgG concentration by (0.10 ± 1.33) g/L. We assumed a noninferiority or superiority Margin for 0.70 and calculated that the inclusion of at least 58 participants in each group was required.

Statistical Analysis

All analyses were performed on an intent-to-treat basis with the software SPSS v.20 package. Mean ± Standard Deviation (SD) was used to describe continuous data. Difference of variables over time within one factor were tested by Tukey post hoc tests, while variables between two groups by 2-factor ANOVA. Results were deemed statistically significant at a $P < .05$.

RESULTS

Demographic and Preoperative Clinical Factors

Finally, we figured 126 patients but 2 patients refused informed consent, so 124 patients were enrolled, then were assigned to the two feeding groups by random. Six participants, two from EIN group and four from SEN group, did not complete the feeding protocol. There were some reasons for the incompliance: not able to tolerance enteral formula ($n = 2$), self-removal of feeding tube ($n = 3$), and protocol violation ($n = 1$). Figure 1 showed the CONSORT diagram.

Table 2 summarized the main demographic characteristics, clinical and surgical baselines in two groups. No significant differences were found in baseline characteristics. Mean age of the total group under study was 56.17 ± 9.74 yr. (57.32 ± 10.19 for EIN group and 55.02 ± 9.61 for SEN group). Among them, 70 were males and 54 females. The main clinical features in comorbidity included hypertension (10.5% of the cases) and diabetes (13.7%). These baseline information, including mean age, BMI, and composition of sex, presentation of existing condition, surgical methods, operation duration, operative blood loss, and transfusion volume were comparable between two groups, which reflect the patients' homogeneity.

Protocol Compliance

There were no significant differences between SEN group and EIN group as far as everyday intake of energy, protein and nitrogen. Postoperative intolerance to two feeding formula was excellent. Only 39 patients (31.5%) experienced some type of adverse GI symptoms. The most common symptom was abdominal distension in 23 cases (18.5%), followed by abdominal pain in 11 (8.9%) and diarrhea in 5 (4.0%) patients. But except for three cases exited the study because of intolerance, all patients can tolerant the goal of 25 kcal/kg/day on POD 3. Meanwhile, no significant difference related to the intolerance symptom frequency between EIN group and SEN group was observed.

Effects on Serum Immune Levels

Table 3 showed the baseline and postoperative immune parameter in two groups. Baseline of $CD4^+$ T-cells, $CD3^+$ T-cells as well as counts of $CD4^+/CD8^+$, IgG, IgM, and IgA were similar between groups. Changes between baseline and POD 5 showed a significant decrease of all the immune markers in SEN group, but no significant changes were identified in EIN group, except the decreased IgG counts on POD 5. Moreover, the proportion, not the number of $CD4^+$ T-cells, $CD3^+$ T-cells, and the counts of $CD4^+/CD8^+$, IgG, IgM, and IgA were significantly higher in EIN group on POD 5.

Effects on Serum Inflammatory Markers

The levels of WBC, CRP, IL-6, TNF-α, and PCT were the main cytokines to assess postoperative inflammation. The evolution of these inflammatory

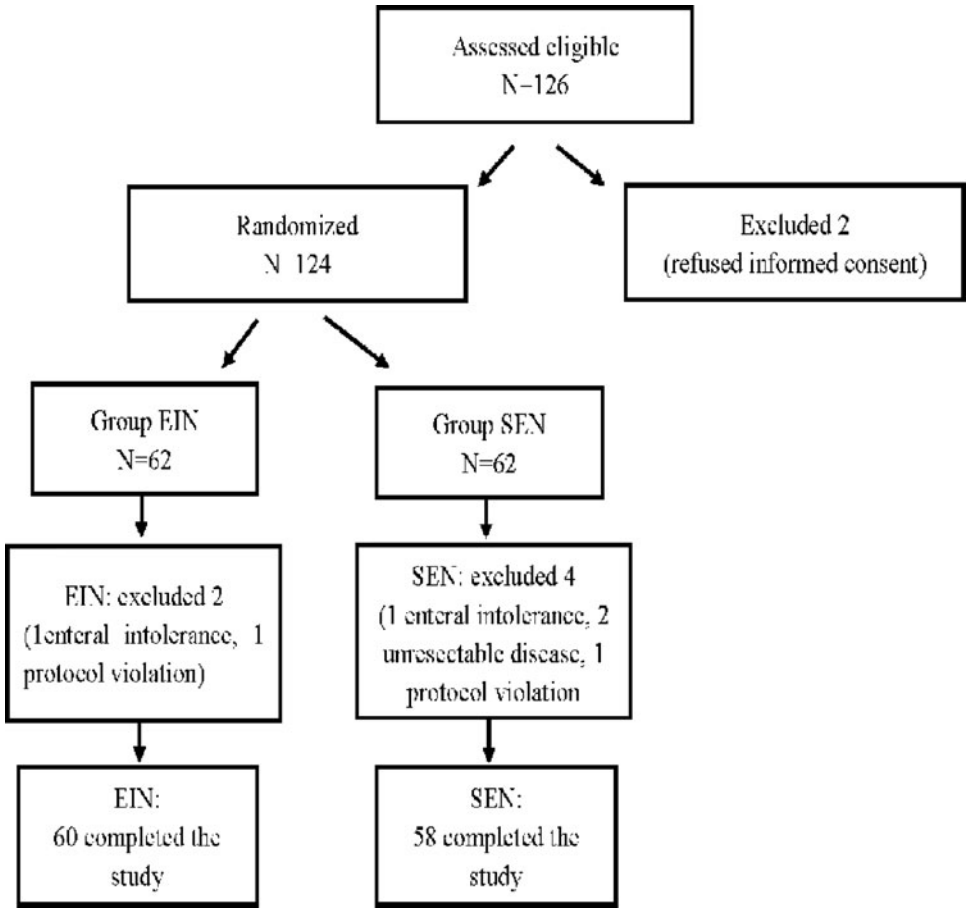

FIGURE 1 CONSORT diagram showing the flow of participants enrollment.

TABLE 2 Baseline characteristics and surgical parameters of patients

	EIN group ($n = 62$)	SEN group ($n = 62$)	P-value
Age, year	57.32 ± 10.19	55.02 ± 9.61	0.20
Male: Female	40:22	30:32	0.10
BMI			0.74
Less than 18.5	5	4	
18.5–24	38	35	
More than 24	19	23	
Comorbidity			
Hypertension	9	4	0.24
Diabetes	11	6	0.30
Coronary disease	4	5	0.50
COPD	2	6	0.27
Surgical procedure (n)			0.12
Proximal gastrectomy	4	8	
Distal gastrectomy	26	16	
Total gastrectomy	32	38	
Operative time mean (min)	229.35 ± 2.59	228.87 ± 2.21	0.93
Operative blood loss (ml)	99.19 ± 55.44	102.10 ± 56.02	0.76
Operative transfusion volume (ml)	1725.81 ± 455.18	1675.81 ± 351.44	0.53

BMI, body mass index; COPD, chronic obstructive pulmonary disease; EIN, enteral immunonutrition; SEN, standard enteral nutrition.

markers of SEN and EIN groups after surgery was summarized in Table 4. As expected, serum WBC, CRP, IL-6, PCT, and TNF-α all changed over time, which rose in groups after operation then gradually went down at a tendency to normalize; serum WBC, IL-6, PCT reached the peak on POD 1, while another two on POD 3; but only the levels of WBC and TNF-α approached preoperative values on POD 5.

TABLE 3 Immunization variables[a]

	EIN group ($n=62$)			SEN group ($n=62$)			
	Baseline	POD 5	P	Baseline	POD5	P	Normal range of the index
$CD4^+$ T cell (%)	41.15 ± 1.02	41.99 ± 0.58	0.95	40.84 ± 0.94	34.87 ± 0.87[b]	<0.001	33.19 to 47.85
$CD3^+$ T cell (%)	62.53 ± 1.39	60.82 ± 1.17	0.19	60.94 ± 1.21	50.59 ± 0.95[b]	<0.001	66.90 to 83.10
$CD4^+$/ $CD8^+$	2.20 ± 0.10	2.11 ± 0.09	0.64	2.17 ± 0.10	1.37 ± 0.07[b]	<0.001	0.97 to 2.31
IgG (g/L)	10.06 ± 0.31	8.10 ± 0.23	<0.001	9.89 ± 0.33	6.02 ± 0.33[b]	<0.001	8.00 to 15.50
IgM (g/L)	2.40 ± 0.18	1.90 ± 0.10	0.22	2.28 ± 0.15	1.43 ± 0.08[b]	<0.001	0.70 to 2.20
IgA (g/L)	2.01 ± 0.11	1.74 ± 0.08	0.17	1.92 ± 0.11	1.36 ± 0.07[b]	<0.001	0.84 to 2.90

[a]All values are means ± SEM. There was no difference between groups at baseline. EIN, enteral immunonutrition, SEN, standard enteral nutrition, POD, postoperative day.
[b]$P < 0.05$ compared with EIN group (Mann–Whitney U test).

Repeated measures ANOVA indicated that there were no significant differences in IL-6 levels ($F = 0.05$, $P = 0.82$) or PCT levels ($F = 0.04$, $P = 0.83$) between groups. When compared to the SEN group, patients in EIN group showed significantly smaller concentration of WBC on POD 3 ($P = 0.03$) and POD 5 ($P < 0.001$), reflecting a faster recovery of WBC in EIN group. While there were significantly lower decrease and faster recovery of CRP and TNF-α in EIN group, owing to the levels of serum CRP and TNF-α revealed significant differences on POD 3 and POD 5, with their higher levels in SEN than EIN group.

Effects on Nutritional Status

The secondary end-points were to assess the effects on nutritional status with each type of nutrition by analyzing serum albumin, prealbumin, and transferrin concentration. After surgery, all the nutrition parameters significantly declined and did not came back to the baseline values, except the level of albumin on the POD 5 ($P < 0.05$) in SEN group. However, there were no significant difference among above parameters by comparing the SEN and EIN group. The summary results are shown in Table 5.

Effects on Complications

Postoperative complications observed included abdominal abscess, anastomotic leakage, wound infection or dehiscence, drain infection, pneumonia, postoperative bleeding, and ileus. There was no complication in both groups.

TABLE 4 Inflammation variables[a]

	EIN group($n=62$)	SEN group($n=62$)	P[b]
WBC (10^9/L, normal range: 4–10*10^9/L)			
Baseline	6.15 ± 0.25	6.08 ± 0.20	0.87
POD1	10.71 ± 0.37[c]	11.33 ± 0.48[c]	0.25
POD3	7.66 ± 0.29[c,d]	9.04 ± 0.45[c,d]	0.03
POD5	5.91 ± 0.14[d,e]	7.99 ± 0.34[d,e]	<0.001
IL-6 (pg/ml, normal range: 0.00-7.00pg/L)			
Baseline	4.66 ± 0.43	4.25 ± 0.40	–
POD1	183.76 ± 9.87[c]	185.52 ± 6.87[c]	–
POD3	71.964 ± 5.31[c,d]	73.96 ± 7.12[c,d]	–
POD5	32.99 ± 2.01[c,d,e]	26.90 ± 1.79[c,d,e]	–
F	0.05	P[f]	0.82
CRP (mg/L, normal range: <5mg/L)			
Baseline	3.02 ± 0.23	3.05 ± 0.23	0.93
POD1	99.24 ± 4.64[c]	104.82 ± 4.20[c]	0.38
POD3	118.34 ± 6.78[c]	140.67 ± 9.43[c,d]	0.06
POD5	53.94 ± 3.08[c,d,e]	75.37 ± 4.05[c,d,e]	<0.001
TNF-α (pg/ml, normal range: <8.1pg/ml)			
Baseline	8.46 ± 0.30	8.68 ± 0.28	0.63
POD1	9.48 ± 0.40[c]	10.28 ± 0.70	0.38
POD3	9.56 ± 0.32[c]	11.79 ± 0.66[c]	0.01
POD5	8.09 ± 0.18[d,e]	9.30 ± 0.43[e]	0.02
PCT (ng/ml, normal range: <0.5 ng/ml)			
baseline	0.05 ± 0.01	0.09 ± 0.03	–
POD1	1.08 ± 0.09[c]	1.20 ± 0.11[c]	–
POD3	0.58 ± 0.04[c,d]	0.55 ± 0.04[c,d]	–
POD5	0.22 ± 0.02[c,d,e]	0.22 ± 0.02[c,d,e]	–
F	0.04	P[f]	0.83

[a]All values are means ± SD. There was no difference between groups at baseline. EIN, Enteral immunonutrition, SEN, standard enteral nutrition, POD, postoperative day.
[b]P values for the difference between EIN and SEN with respect to the time point were calculated when a significant treatment × time interaction was found.
[c]$P < 0.05$ compared with baseline (ANOVA and Turkey post hoc test).
[d]$P < 0.05$ compared with POD1 (ANOVA and Turkey post hoc test).
[e]$P < 0.05$ compared with POD3(ANOVA and Turkey post hoc test).
[f]P-value for the difference of treatment between EIN and SEN when there were not a significant treatment × time interaction.

DISCUSSION

Gastric cancer was known as a common malignant tumor, and most patients were diagnosed in advanced stages, while surgery was the mainstay of curative treatment. However, surgical intervention often resulted in impairment of immune defense mechanisms and altered inflammatory responses,

TABLE 5 Nutritional variables[a]

	EIN group ($n = 62$)			SEN group ($n = 62$)			EIN compared with SEN, P[b]		
	POD 0	POD 3	POD5	POD 0	POD 3	POD5	POD0	POD3	POD5
Albumin (g/L)	40.19 ± 0.41	34.25 ± 0.66[c]	37.86 ± 0.44[c]	39.10 ± 0.56	35.07 ± 0.44[c]	38.19 ± 0.48[d]	0.17	0.30	0.55
Prealbumin (mg/L)	212.24 ± 6.57	127.14 ± 4.92[c]	152.89 ± 5.80[c,d]	211.30 ± 4.02	119.93 ± 4.56[c]	147.98 ± 4.85[c]	–	–	–
Transferrin (g/L)	2.33 ± 0.06	1.60 ± 0.05[c]	1.82 ± 0.05[c]	2.21 ± 0.06	1.58 ± 0.04[3]	1.78 ± 0.05[c],[d]	0.17	0.80	0.61

[a]All values are means ± SEM. There was no difference between groups at baseline. EIN, enteral immunonutrition, SEN, standard enteral nutrition, POD, postoperative day.
[b]P values for the difference between EIN and SEN with respect to the time point were calculated when a significant treatment × time interaction was found.
[c]$P < 0.05$ compared with baseline (ANOVA and Turkey post hoc test).
[d]$P < 0.05$ compared with POD3 (ANOVA and Turkey post hoc test).
[e]Normal range of these indexes: Albumin, 40–55g/L; Prealbumin, 280–360mg/L; Transferrin, 1.8–3.8g/L.

making these patients highly susceptible to infections, leading to poor clinical outcomes after surgery. Recent trials have concluded that immunonutrition substrates played a role in the modulating immune system. A meta-analysis [20] published in 2018 concluded that adding omega-3 fatty acid emulsion in parenteral nutrition formula could improve the postoperative indicators of immune function, reduce inflammatory reaction, and improve the postoperative curative effect. As for EIN, some published original studies and meta-analyses have evaluated its positive impact on the outcome of surgery [11, 21], including reduction of postoperative complications and shortening hospital stay [22, 23]. Recent studies extended to focus on longer term benefit of EIN. Klek et al. [24] investigated its positive effect on short-term survival in stage IV gastric cancer patients. Positive impact were found at the direct index for immunity and inflammation in some of the trials as well: EIN helped inflammation modulation and systemic immune enhancement due to significant reductions in CRP, TNF-α, and IL-6 [25] and an increase in $CD4^+$ levels [26], IgA levels [27]. In addition, not only clinical effectiveness, but also cost-effectiveness was been proved when EIN compared to SEN support [28].

Nevertheless, criticism aroused due to some studies on EIN which failed to find reduced complication [29], and prove its effect on immune function and inflammation response after surgery [30, 31]. Some researchers demonstrated a decrease only in infectious complication but lacking of cost-effectiveness [32, 33]. Likewise, other studies concluded that immunonutrition formula could increase immune cytokines, such as the level of $CD4^+$, $CD4^+/CD8^+$, IgG, and IgM, only when EIN support lasted longer than 7 days [18]. Increased risk of death in the critically ill receiving EIN was even been found in a few trials [34].

The following reasons can explain the inconsistencies: study group, various immunonutrition components, different timing and route of administration. First, complications were usually seen in malnourished patients, obviously, not mention EIN, any surgical intervention would be beneficial. Yet in these trials suggesting EIN, the proportion of malnourished patients reached close to 60 [26, 35], which leading to possibly exaggeration. Second, Song et al. [36] conducted a meta-analysis and draw the conclusion that formula added Arg, omega-3 fatty acid and Gln or RNA were the optimal regimes, but only Arg and RNA were not preferentially recommended compared to SEN. Luis et al [37] and De Luis et al. [38] also found higher dose of Arg-enriched enteral formula, more clinical benefit would reach. Third, the timing of EIN represented another issue and did not reach consensus. Evidence-based guidelines recommended perioperative immunonutritional intervention for 7–14 days in moderately or severely malnourished patients who were candidates for major gastric surgery [9]. Some other proved that pre- and post-EIN did not bring double benefits [39], but Song et al. [40] believed that perioperative EIN was the optimum timing. In our study, we designed a 5-day EIN support after surgery with study groups that homogenous in terms of characteristics and nutritional status.

The present randomized controlled trial indicated that patients receiving the immunonutrition formula had a quick recovery in immune response, reaching statistical significance for $CD4^+$ T-cells, $CD3^+$ T-cells as well as counts of $CD4^+/CD8^+$, IgG, IgM, and IgA. The decrease trends on inflammation markers were more gently in EIN group, and significantly lower were shown in the concentration of WBC, CRP, and TNF-α. Moreover, nutritional status and the incidence of intolerance complications in general were similar between two groups.

T lymphocyte-mediated cell-mediated immunity played an important role in antitumor immune

response [41, 42]. The ratio of $CD4^+$ T-cells, $CD3^+$ T-cells, and $CD4^+/CD8^+$ was a sensitive index to reflect the cellular immune function of an organism. Like those results reported, our trial confirmed that the increase in EIN group indicating the enhancement of body's cellular immune function. Meanwhile, immunoglobulins were important index to examine the humoral immune function of an organism, thus higher concentrations of IgA, IgM, and IgG may indicate reduced inflammation and enhanced immunity [39, 43].

CRP, IL-6, PCT, and TNF-α played an important role in response to tissue injury and inflammation in early trauma [42, 44]. TNF-α was a promoter of multidirectional inflammation, and IL-6 was an index to reflect the severity of inflammation and tissue damage. In present study, we found that there was a significant difference between EIN group and SEN group regarding TNF-α on the third and fifth postoperative day, while no significant on IL-6 levels. CRP levels after operation was a sensitive index to determine the degree of postoperative stress response and infectious complications. Our studies showed that an increase in CRP levels on POD 1, which reached a peak on POD 3, and the SEN group showing significantly higher levels on POD3 and POD 5. This indicated that EIN can reduce the inflammatory reaction in these patients.

All these data suggested that postoperative EIN had positive effect on the immune function and inflammatory response for gastric cancer patients. There were some explanations: (1) Gln was avidly consumed by rapidly immune cells, intestinal mucosal cells, and tumor cells, and had a strong association with the functional activity of these cells [45], including cell proliferation, antigen presentation cytokine, synthesis, superoxide production and phagocytosis [46]. (2) Arg, a semi-essential amino acid, served to enhance T-cells and could multiply in response to stimulation by mitogens or cytokines [47]. (3) Omega-3 fatty acids can increase production of leukotrienes, and reduce cytotoxicity of lymphocytes and macrophages by competing with omega-6 fatty acids for cyco-oxygenase metabolism [48].

A major strength of this trial was broad investigation into immune and inflammation markers of GC patients undergoing gastrectomy who receiving postoperative 5 days EIN, giving a more comprehensive understanding of EIN effects. Simultaneously, comparable demographic characteristics, and clinical baseline variables which related to nutrition and cancer guaranteed the homogeneity of these trial. Even so, there were some limitations need to demonstrate. The sample of our trial was limited based on the aim of present study, which was to evaluate impact on inflammation and immunity markers rather than postoperative outcomes of EIN. Meanwhile, there were some difference in fat content between the two types of feed formula, but only omega-3 fatty acids in EIN might be benefit for the observed improvement in systemic inflammatory response.

In conclusion, this present study finds that EIN support an improved immune function, and reduced inflammation after operation when compared to SEN. However, we did not demonstrate that EIN support increased nutritional status more than SEN support in well-nourished patients. Well-designed clinical trials are needed to explore deep into the most effective dose, timing and duration of EIN necessary to improve immune and an anti-inflammation function in gastric cancer patients.

ACKNOWLEDGMENTS

The study was supported by the Sichuan Provincial Science and Technology Support Project (no. 2018SZ0226).

DECLARATION OF INTEREST

The authors declare that they do not have any conflict of interest.

REFERENCES

[1] Fock KM. Review article: the epidemiology and prevention of gastric cancer. *Aliment Pharmacol Ther*. 2014;40(3):250–260.
[2] Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012. *Cancer J Clinic*. 2015;65(2):87–108.
[3] Schattner M. Enteral nutritional support of the patient with cancer: route and role. *J Clin Gastroenterol*. 2003;36(4):297–302.
[4] Bistrian BR, Blackburn GL, Hallowell E, et al. Protein status of general surgical patients. *JAMA*. 1974;230(6):858–860.
[5] Decker D, Schondorf M, Bidlingmaier F, et al. Surgical stress induces a shift in the type-1/type-2 T-helper cell balance, suggesting down-regulation of cell-mediated and up-regulation of antibody-mediated immunity commensurate to the trauma. *Surgery* 1996;119(3):316–325.
[6] Braga M, Gianotti L, Vignali A, et al. Artificial nutrition after major abdominal surgery: impact of route of administration and composition of the diet. *Crit Care Med*. 1998;26(1):24–30.
[7] Fujitani K, Tsujinaka T, Fujita J, et al. Prospective randomized trial of preoperative enteral immunonutrition followed by elective total gastrectomy for gastric cancer. *Br J Surg*. 2012;99(5):621–629.
[8] Gianotti L, Braga M, Vignali A, et al. Effect of route of delivery and formulation of postoperative nutritional support in patients undergoing major operations for

malignant neoplasms. *Arch Surg.* 1997;132(11):1222–1229. discussion 1229–30.
[9] Weimann A, Braga M, Harsanyi L, et al. ESPEN Guidelines on Enteral Nutrition: Surgery including organ transplantation. *Clin Nutr. (Edinburgh, Scotland).* 2006;Apr25(2):224–244.
[10] Klek S, Sierzega M, Szybinski P, et al. The immunomodulating enteral nutrition in malnourished surgical patients - a prospective, randomized, double-blind clinical trial. *Clin Nutr.* 2011;30(3):282–288.
[11] Wong CS, Aly EH. The effects of enteral immunonutrition in upper gastrointestinal surgery: A systematic review and meta-analysis. *Int J Surg (London, England).* 2016;29:137–150.
[12] Yan X, Zhou FX, Lan T, et al. Optimal postoperative nutrition support for patients with gastrointestinal malignancy: A systematic review and meta-analysis. *Clin Nutrit.* 2017;36(3):710–721.
[13] Chen DW, Wei Fei Z, Zhang YC, et al. Role of enteral immunonutrition in patients with gastric carcinoma undergoing major surgery. *Asian J Surg.* 2005;28(2): 121–124.
[14] Lind DS. Arginine and cancer. *J Nutr.* 2004;134(10): 2837S–2841S. discussion 2853S.
[15] Aiko S, Yoshizumi Y, Ishizuka T, et al. Enteral immunoenhanced diets with arginine are safe and beneficial for patients early after esophageal cancer surgery. *Dis Esophagus: Official J Int Soc Dis Esophagus.* 2008;21(7): 619–627.
[16] Braga M, Gianotti L, Vignali A, et al. Preoperative oral arginine and n-3 fatty acid supplementation improves the immunometabolic host response and outcome after colorectal resection for cancer. *Surgery* 2002;132(5): 805–814.
[17] Giger U, Buchler M, Farhadi J, et al. Preoperative immunonutrition suppresses perioperative inflammatory response in patients with major abdominal surgery-a randomized controlled pilot study. *Ann Surg Oncol.* 2007;14(10):2798–2806.
[18] Cheng Y, Zhang J, Zhang L, et al. Enteral immunonutrition versus enteral nutrition for gastric cancer patients undergoing a total gastrectomy: a systematic review and meta-analysis. *BMC Gastroenterol.* 2018;18(1):11.
[19] Mortensen K, Nilsson M, Slim K, et al. Consensus guidelines for enhanced recovery after gastrectomy: Enhanced Recovery After Surgery (ERAS(R)) Society recommendations. *Br J Surg.* 2014;101(10):1209–1229.
[20] Zhao Y, Wang C. Effect of omega-3 polyunsaturated fatty acid-supplemented parenteral nutrition on inflammatory and immune function in postoperative patients with gastrointestinal malignancy: a meta-analysis of randomized control trials in China. *Medicine* 2018; 97(16): e0472.
[21] Bozzetti F, Gianotti L, Braga M, et al. Postoperative complications in gastrointestinal cancer patients: the joint role of the nutritional status and the nutritional support. *Clin Nutr (Edinburgh, Scotland).* 2007;26(6):698–709.
[22] 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 randomized controlled clinical trials. . *Ann. Surg.* 1999; 229(4):467–477.
[23] Zheng Y, Li F, Qi B, et al. Application of perioperative immunonutrition for gastrointestinal surgery: a meta-analysis of randomized controlled trials. *Asia Pacific J Clin Nutr.* 2007;16 (Suppl 1):253–257.
[24] Klek S, Scislo L, Walewska E, et al. Enriched enteral nutrition may improve short-term survival in stage IV gastric cancer patients: A randomized, controlled trial. *Nutrition (Burbank, Los Angeles County, Calif).* 2017;36: 46–53.
[25] Hamza N, Darwish A, O'Reilly DA, et al. Perioperative enteral immunonutrition modulates systemic and mucosal immunity and the inflammatory response in patients with periampullary cancer scheduled for pancreaticoduodenectomy: a randomized clinical trial. *Pancreas* 2015;44(1):41–52.
[26] Braga M, Gianotti L, Nespoli L, et al. Nutritional approach in malnourished surgical patients: a prospective randomized study. *Arch Surg. (Chicago, Ill: 1960)* 2002;137(2):174–180.
[27] Gianotti L, Braga M, Nespoli L, et al. A randomized controlled trial of preoperative oral supplementation with a specialized diet in patients with gastrointestinal cancer. *Gastroenterology* 2002;122(7):1763–1770.
[28] Klek S, Kulig J, Sierzega M, et al. The impact of immunostimulating nutrition on infectious complications after upper gastrointestinal surgery: a prospective, randomized, clinical trial. *Ann Surg.* 2008;248(2):212–220.
[29] Lobo DN, Williams RN, Welch NT, et al. Early postoperative jejunostomy feeding with an immune modulating diet in patients undergoing resectional surgery for upper gastrointestinal cancer: a prospective, randomized, controlled, double-blind study. *Clin Nutr. (Edinburgh, Scotland).* 2006;25(5):716–726.
[30] Sunpaweravong S, Puttawibul P, Ruangsin S, et al. Randomized study of antiinflammatory and immunemodulatory effects of enteral immunonutrition during concurrent chemoradiotherapy for esophageal cancer. *Nutr Cancer.* 2014;66(1):1–5.
[31] Miyata H, Yano M, Yasuda T, et al. Randomized study of the clinical effects of omega-3 fatty acid-containing enteral nutrition support during neoadjuvant chemotherapy on chemotherapy-related toxicity in patients with esophageal cancer. *Nutrition (Burbank, Los Angeles County, Calif).* 2017;33:204–210.
[32] Helminen H, Raitanen M, Kellosalo J. Immunonutrition in elective gastrointestinal surgery patients. *Scand J Surg.* 2007;96(1):46–50.
[33] Alivizatos V, Athanasopoulos P, Makris N, et al. Early postoperative glutamine-supplemented parenteral nutrition versus enteral immunonutrition in cancer patients undergoing major gastrointestinal surgery. *J BUON* 2005; 10(1):119–122.
[34] Calder PC. Immunonutrition in surgical and critically ill patients. *Br J Nutr.* 2007;98(Suppl 1):S133–S139.
[35] Guidelines for the use of parenteral and enteral nutrition in adult and pediatric patients. *J Parenteral Enteral Nutr.* 2002;26(1 Suppl):1sa–138sa.
[36] Song GM, Liu XL, Bian W, et al. Systematic review with network meta-analysis: comparative efficacy of different enteral immunonutrition formulas in patients underwent gastrectomy. *Oncotarget* 2017;48(14):23376–23388. doi: 10.18632/oncotarget.15580.
[37] De Luis DA, Izaola O, Terroba MC, et al. Effect of three different doses of arginine enhanced enteral nutrition on nutritional status and outcomes in well nourished postsurgical cancer patients: a randomized single blinded prospective trial. *Eur Rev Med Pharmacol Sci.* 2015;19(6): 950–955.
[38] De Luis DA, Izaola O, Cuellar L, et al. A randomized double-blind clinical trial with two different doses of arginine enhanced enteral nutrition in postsurgical cancer patients. *Eur Rev Med Pharmacol Sci.* 2010;14(11): 941–945.

[39] Klek S, Szybinski P, Szczepanek K. Perioperative immunonutrition in surgical cancer patients: a summary of a decade of research. *World J Surg.* 2014;38(4):803–812.
[40] Song GM, Tian X, Zhang L, et al. Immunonutrition support for patients undergoing surgery for gastrointestinal malignancy: preoperative, postoperative, or perioperative? a bayesian network meta-analysis of randomized controlled trials. *Medicine* 2015;94(29):e1225.
[41] Koller M, Senkal M, Kemen M, et al. Impact of omega-3 fatty acid enriched TPN on leukotriene synthesis by leukocytes after major surgery. *Clin Nutr.* 2003;22(1):59–64.
[42] Kojima M, Wakai K, Tokudome S, et al. Serum levels of polyunsaturated fatty acids and risk of colorectal cancer: a prospective study. *Amer J Epidemiol.* 2005;161(5):462.
[43] Perez CE, Reyes MA, Asbun Bojalil J, et al. Effectiveness of immunonutrition on inflammatory markers in patients with cancer; randomized clinical trial. *Nutr Hosp.* 2015; 132(4):1676–1682.
[44] Leung L, Cahill CM. TNF-alpha and neuropathic pain–a review. *J Neuroinflamm.* 2010;7(1):27.
[45] Han T, Li XL, Cai DL, et al. Effects of glutamine-supplemented enteral or parenteral nutrition on apoptosis of intestinal mucosal cells in rats with severe acute pancreatitis. *Eur Rev Med Pharmacol Sci.* 2013;17(11): 1529–1535.
[46] Bell SG. Immunomodulation. Part IV: Glutamine. *Neonatal Netw.* 2006;25(6):439–443.
[47] Bonhomme S, Belabed L, Blanc MC, et al. Arginine-supplemented enteral nutrition in critically ill diabetic and obese rats: a dose-ranging study evaluating nutritional status and macrophage function. *Nutrition (Burbank, Los Angeles County, Calif).* 2013;29(1):305–312.
[48] Shirakawa H, Kinoshita T, Gotohda N, et al. Compliance with and effects of preoperative immunonutrition in patients undergoing pancreaticoduodenectomy. *J Hepato-Biliary-Pancreatic Sci.* 2012;19(3):249–258.