Randomized control trials

Parenteral fish oil as a pharmacological agent to modulate postoperative immune response: A randomized, double-blind, and controlled clinical trial in patients with gastrointestinal cancer

Raquel Susana Matos de Miranda Torrinhas\textsuperscript{a,b,}\textcopyright, Raquel Santana\textsuperscript{a}, Thaís Garcia\textsuperscript{a}, Maria Fernanda Cury-Boaventura\textsuperscript{c}, Maria Mirtes Sales\textsuperscript{d}, Rui Curi\textsuperscript{e}, Dan Linetzky Waitzberg\textsuperscript{a,b}

\textsuperscript{a}University of São Paulo, Department of Gastroenterology, Medical School (LIM 35), Brazil
\textsuperscript{b}University of São Paulo, Food and Nutrition Research Center (NAPAN), Brazil
\textsuperscript{c}Cruzeiro do Sul University, Institute of Physical Activity and Sports Science, Brazil
\textsuperscript{d}University of São Paulo, Section of Flow Cytometry from the Department of Hematology, Clinics Hospital, Brazil
\textsuperscript{e}University of São Paulo, Department of Biophysics and Physiology, Institute of Biomedical Sciences, São Paulo, Brazil

\textbf{A R T I C L E I N F O}

Article history:
Received 20 September 2012
Accepted 17 December 2012

Keywords:
Omega-3 fatty acids
Parenteral nutrition
Surgery
Immunology
Clinical outcome

\textbf{S U M M A R Y}

\textit{Background:} Fish oil-based lipid emulsions (FOLEs) have shown post-operative immunological and clinical benefits in parenteral nutrition.

\textit{Aim:} To assess post-operative immune response after short-term pre-operative parenteral infusion of isolated FOLE in gastrointestinal cancer patients.

\textit{Methods:} The patients (n = 63) received pre-operative peripheral infusion (0.2 g fat/kg body weight/d) of FOLE (Omegaven\textsuperscript{\textcopyright}) or control lipid emulsion (MCT/LCT; Lipovenos MCT\textsuperscript{\textcopyright}) for 3 days. Post-operative concentrations of inflammatory mediators, leukocyte functions, surface molecules, infections, and length of intensive care unit (ICU) and hospital stay were measured.

\textit{Results:} FOLE patients had a significant increase of IL-10 levels on day 3, decrease of IL-6 and IL-10 levels on day 6, lower decrease in leukocyte oxidative burst, maintenance of monocyte percentage expressing HLA-DR and CD32, and increase of CD32 neutrophil expression compared to MCT/LCT patients. No changes were observed in the frequency of post-operative infections or length of ICU and hospital stay.

\textit{Conclusions:} Short-term pre-operative infusion of FO alone improves the post-operative immune response of gastrointestinal cancer patients without significantly changing post-operative infections or length of ICU and hospital stay. ID:NCT01218841.

\textcopyright 2012 Elsevier Ltd and European Society for Clinical Nutrition and Metabolism. All rights reserved.

1. Introduction

The prevention of persistent immune dysfunction after major surgery remains a central challenge for surgeons to avoid post-operative complications from infection and multiple organ failure.\textsuperscript{1} Surgical patients with cancer have an additional risk of complications due to infections because of depressed cellular and humoral immune functions as a result of the underlying disease.\textsuperscript{2}

Immune response can be directly or indirectly influenced by long-chain omega-3 polyunsaturated fatty acids (n-3 PUFAs), mainly eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, which are present in fish oil.\textsuperscript{3} These fatty acids can prevent and mitigate inflammation by positively affecting the production of eicosanoids, cytokines, and resolvins as well as the structure of lipid raft microdomains after their incorporation into the leukocyte cell membrane.\textsuperscript{3}

Parenteral infusion of fish oil lipid emulsion (LE) avoids digestive and absorptive losses of n-3 PUFAs that can occur after oral or enteral consumption. In addition, the incorporation of n-3 PUFAs into plasma and blood cells occurs at a faster rate following parenteral supplementation (1–3 d) compared to enteral supplementation (4–7 d).\textsuperscript{4} A reduction in the release of pro-inflammatory cytokines from monocytes and adhesive interaction with the endothelium was observed 48 h after the infusion of fish oil LE in

\textcopyright Conferences: Part of the results of this study was orally presented at the ESPEN 2010 Congress, the annual meeting of The European Society for Clinical Nutrition and Metabolism, in Nice, France, 6 September.

\textcopyright Corresponding author: Faculdade de Medicina da Universidade de São Paulo, Avenida Dr. Arnaldo, 455, 2\textsuperscript{nd} andar, sala 2108, CEP 01245-903, São Paulo, SP, Brazil.
Tel.: +55 11 3061 7459.
E-mail address: torrinhas@uol.com.br (R.S.M. de Miranda Torrinhas).

0261-5614/$ – see front matter © 2012 Elsevier Ltd and European Society for Clinical Nutrition and Metabolism. All rights reserved.
http://dx.doi.org/10.1016/j.clnu.2012.12.008
healthy volunteers, suggesting that parenteral delivery of n-3 PUFA may induce fast immune-modulatory effects.\(^5\)

As an integral part of parenteral nutrition (PN), LEs containing fish oil have been shown to provide benefits to patients undergoing major surgery, mainly by inducing a better post-operative balance of inflammatory mediators with a concomitant decrease in post-operative infection rates as well as length of stay in hospitals and intensive care units (ICUs).\(^6\) However, the parenteral route is currently only indicated for patients who cannot meet their nutritional needs through oral or enteral pathways.\(^7\) Therefore, the majority of surgical patients would currently not benefit from the potential advantages of parenteral infusion of n-3 PUFA.

In order to provide the immune and anti-inflammatory properties of n-3 PUFAs to surgical patients, we propose the use of parenteral fish oil LE independent of PN therapy. In this prospective, randomized, double-blind, parallel, and controlled clinical trial, we assessed the effect of peripheral infusion of parenteral fish oil LE as a pre-operative pharmacological agent on the post-operative immune response and its impact on clinical outcomes of patients with gastrointestinal cancer.

2. Methods

2.1. Ethical considerations

The current study was conducted following the ethical recommendations of the Declaration of Helsinki and the Ethical Committee of the University of São Paulo “CAPPesq – Comissão de Ética para Análise de Projetos de Pesquisa” do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP), which approved its protocol and informed consent forms. All enrolled patients had previously provided written informed consent.

2.2. Patients

Adult patients who were 18–75-years-old admitted to the gastrointestinal surgery division of the HCFMUSP from April 2006 to October 2008 for elective surgery for resection of gastric or colon cancer were screened for eligibility in our study. Additional inclusion criteria included a Karnofsky performance status score \(\geq 60\) and peripheral venous access suitable for continuous access during parenteral therapy and blood collection. Exclusion criteria included intolerance or allergy to any ingredient of LE, infection (i.e., acquired immune deficiency syndrome), inflammatory disease (i.e., arthritis), immunologic disease (i.e., lupus), metabolic disease (i.e., insulin-dependent diabetes), dementia or other cognitive and behavioral problems, ingestion of drugs that significantly modulate intermediary metabolism, implanted electromagnetic instruments, and refusal to sign the informed consent. A randomization sequence with two blocks of 50 patients each (1:1 allocation) was computer-generated (GraphPad statistical software, Graphpad, USA) prior to initiation of the study by an independent investigator (D.A., from Farmoterápica, SP, Brazil) to assign the participants to either experimental and control groups. With the exception of this independent pharmacist, investigators and staff were kept blind to each participant’s assignment. The patients were enrolled and assigned into the two groups by two trained investigators (R.S. and T.G.) following this randomization sequence, which was concealed until the end of the statistical analysis.

2.3. LE treatment

An independent pharmacist (D.A., from Farmoterápica, SP, Brazil) prepared LE bags that were identical in appearance. The infusion of fish oil LE [experimental group (FO – Omegaven\(^\text{®}\) 10%, Fresenius-Kabi, Germany)] or LE rich in medium-chain triglycerides [control group (MCT/LCT – Lipovenos MCT\(^\text{®}\) 10%, Fresenius-Kabi, Germany)] was performed in a double-blind manner. Three days before surgery, 0.2 g fat/kg of total body weight of LEs was continuously infused for 6 h per day. The location of the exclusive peripheral venous access was changed daily.

2.4. Analysis of immune response

Immune response markers were analyzed in at least 7 patients from each group. For this purpose, peripheral blood samples were collected in Vacutainer\(^\text{®}\) tubes containing heparin (BD-Becton & Dickinson Co Franklin Lakes, NJ USA) immediately before the start of the LE infusion (baseline, T0), at the end of the LE infusion (pre-operative, T1), and on the third post-operative day (post-operative, T2). Due to the particular kinetics, exclusive blood samples for interleukin (IL) analyses were also collected on the 6th post-operative day (post-operative, T3).

2.4.1. Inflammatory mediators

The plasma concentration of IL-6 and IL-10 was evaluated by using the human IL-6 ELISA set and human IL-10 ELISA set kits (BD Biosciences, Sparks, MD, USA), respectively, following a standard ELISA “sandwich” technique for cytokine detection with minor modifications.\(^8\) The plasma concentration of C Reactive Protein (CRP) and prostaglandin E\(_2\) (PGE\(_2\)) was assessed using the immunoturbidimetric method with a commercial kit (Biolink\(^\text{®}\), São Paulo, SP Brazil) and the Biotrak\(^\text{®}\) enzyme immune assay system (GE Healthcare, Piscataway, NJ USA), respectively, in a microplate spectrophotometer reader (SpectraMax plus, Sunnyvale, CA USA) following the manufacturers’ recommendations.

2.4.2. Leukocyte functions and surface molecule expression

The analysis of leukocyte functions as well as HLA-DR and CD32 expression was performed using a flow cytometer (FACSCALIBUR\(^\text{™}\)) that was calibrated daily with 1 \(\mu\)m fluorescent latex beads (Calibrate\(^\text{™}\) 3) and the CELLQuestPro\(^\text{®}\) software (all from BD-Becton & Dickinson Co, Franklin Lakes, NJ, USA). Chemotaxis, phagocytosis, and oxidative burst were evaluated by using the kits Migratest\(^\text{®}\), Phagotest\(^\text{®}\), and Phagoburst\(^\text{®}\) (Orpegen Pharma, Heidelberg, Germany), respectively, following the manufacturer instructions for preparing, acquiring, and analyzing samples by flow cytometry, as described elsewhere.\(^9\) For HLA-DR and CD32 expression analysis, whole blood was incubated in a dark room at 4°C for 30 min with 10 \(\mu\)L of human AB+ serum and 10 \(\mu\)L of the samples together with the phycoerythrin-labeled anti-HLA-DR, fluorescein isothiocyanate-labeled CD32, R-phycocerythrin plus cyanine-labeled anti-CD16, and allophycocyanin-labeled anti-CD14 monoclonal antibodies together with their respective isotype controls (all from BD Pharmigen, San Diego, CA, USA). After the incubation, blood samples were washed three times with 2 mL of PBS (pH 7.4), lysed for 10 min with 1.8 mL of 1% lysis solution (BD FACSTM Lysing Solution\(^\text{®}\), BD Biosciences, San Jose, CA USA), and then washed three more times with 2 mL of PBS. The samples were then fixed with 250 \(\mu\)L of 1% paraformaldehyde solution (Sigma–Aldrich Co, St. Louis, MO USA). Leukocytes were gated based on side scatter vs. the CD14 dot plot for monocytes and CD16 dot plot for neutrophils. The percentage and intensity of HLA-DR\(^\text{+}\) and CD32\(^\text{+}\) cells were assessed by recording the mean of 10,000 events per sample.

2.5. Analysis of clinical outcome

We recorded post-operative infectious complications according to the criteria described by Buzby et al. as well as the length of ICU
and hospital stay. The primary clinical endpoint was to assess post-operative clinical outcome in all patients, but we also later separately assessed patients with an additional risk of having complications, namely the elderly (age ≥ 60-years-old) and/or malnourished patients [scored patient-generated-subjective global assessment (PG-SGA)].

2.6. Sample size and statistical analysis

The sample size (minimum of 28 patients per treatment) was calculated by considering post-operative complications as the main clinical endpoint, a significance level of α = 0.05, test power of 80%, standard deviation of 30% of the arithmetic mean for samples, and a difference of group means of 20% as the highest average value. Statistical discussion was guided by a previous statistical plan supervised by a senior statistician (J.P.). For all statistical analyses, the significance level was set at 5%. Demographic/descriptive data were compared between treatments by the Wilcoxon test for continuous variables and the chi-squared test for categorical variables. Values and changes in immunological data between the time of assessment and type of treatment were evaluated by the Wilcoxon test. For leukocyte function, we grouped the different variables provided by each flow cytomtry kit to describe an individual function (i.e., phagocytosis = percentage of phagocytizing monocytes + intensity of phagocytosing monocytes + percentage of phagocytosing neutrophils + intensity of phagocytosing neutrophils) and tested their compliance by using Cronbach’s alpha test. When leukocyte functions had compliances ≥50%, they were treated as their respective Cronbach’s alpha scores, which were calculated from the arithmetic average of their standardized component variables, obtained by subtracting the mean divided by the standard deviation. Clinical data were compared between treatments by the chi-squared and binomial tests. For elderly and/or malnourished subgroup of patients we analysed changes in immunological data between the time of assessment as a result of anastomotic dehiscence; and b) infection and septic shock, caused by self-inflicted lesion at the surgical wound. These patients were not assessed for clinical variables, except patient b, whose duration of ICU stay was assessed because he experienced the complication during his stay in the ward. A CONSORT diagram shows the flow of participant’s assignment and analysis (Fig. 1). The study groups were adequately matched for descriptive characteristics of demography, tumor site, and type of surgery (Table 1). For the patients where immunological markers were assessed, there was no significant difference in baseline values, except for IL-6, which was higher in FO patients than in MCT/LCT patients, and IL-10, which was higher in MCT/LCT patients than in FO patients (Table 2).

3.2. Immune response markers

Changes in immunological measurements that occurred within the same group of patients in relation to their baseline values are shown in Fig. 2. In addition, Table 3 shows changes and p values that occurred in immunological measurements during the study period between FO and MCT/LCT patients. After infusion and before the surgery (T1), FO patients exhibited a decrease in IL-6 levels (p = 0.018) and MCT/LCT patients demonstrated an increase in IL-6 (p = 0.003) and IL-10 (p = 0.053) levels compared to baseline.

3. Results

3.1. Patients

Of the patients who met the inclusion criteria, 84 agreed to participate in the study. From these patients, 21 were excluded from the protocol for various reasons [voluntary withdraw not associated with any discomfort or side effect of LE infusion (FO: n = 3; MCT/LCT: n = 4), voluntary withdrawal after reference of abdominal pain and tachycardia (MCT/LCT: n = 2), change of surgery date (FO: n = 5; MCT/LCT: n = 2), incomplete infusion of LE (FO: n = 1; MCT/LCT: n = 4), and 63 patients completed the infusion protocol [FO (n = 31) and MCT/LCT (n = 32)]. However, 2 patients who completed the MCT/LCT infusion protocol died post-operatively after a) septic shock during the immediate post-operative period as a result of anastomotic dehiscence; and b) infection and septic shock, caused by self-inflicted lesion at the surgical wound. These patients were not assessed for clinical variables, except patient b, whose duration of ICU stay was assessed because he experienced the complication during his stay in the ward. A CONSORT diagram shows the flow of participant’s assignment and analysis (Fig. 1). The study groups were adequately matched for descriptive characteristics of demography, tumor site, and type of surgery (Table 1). For the patients where immunological markers were assessed, there was no significant difference in baseline values, except for IL-6, which was higher in FO patients than in MCT/LCT patients, and IL-10, which was higher in MCT/LCT patients than in FO patients (Table 2).

3.2. Immune response markers

Changes in immunological measurements that occurred within the same group of patients in relation to their baseline values are shown in Fig. 2. In addition, Table 3 shows changes and p values that occurred in immunological measurements during the study period between FO and MCT/LCT patients. After infusion and before the surgery (T1), FO patients exhibited a decrease in IL-6 levels (p = 0.018) and MCT/LCT patients demonstrated an increase in IL-6 (p = 0.003) and IL-10 (p = 0.053) levels compared to baseline.

![Fig. 1. CONSORT diagram of gastrointestinal cancer patient’s randomization to receive 3 days of pre-operative peripheral infusion of a fish oil parenteral lipid emulsion (FO) that was rich in omega-3 fatty acids, or a control parenteral lipid emulsion (MCT/LCT) that was rich in medium-chain triglycerides.](image-url)
MCT/LCT patients presented a post-operative decrease of PGE2 levels (3.2.1. Effect of LCT patients before or after surgical trauma (data not shown). expression on neutrophils surface were observed in FO and MCT/LCT patients compared to FO patients (p < 0.05). MCT/LCT patients also presented with a post-operative baseline values, as well as FO patients on the 3rd day for IL-6 levels (p < 0.0001) on the 3rd day and of the variation in the length of ICU stay than those receiving FO (15.76 vs. 2.42 for FO patients, p = 0.016), Fig. 3.

4. Discussion

This is the first study exploring pre-operative parenteral infusion of isolated fish oil LE as an adjuvant pharmacological agent for the treatment of patients with gastrointestinal cancer, regardless of the PN indication. We found that this treatment favorably modulated post-operative immune mediators, which was accompanied by the preservation or improvement in leukocyte phenotype.

The isolated use of parenteral fish oil LE as a pharmacological agent has been discussed as an approach for quickly modulating immune functions and improving clinical outcomes in different clinical settings. As recently demonstrated in patients with rheumatoid arthritis, infusion of fish oil LE at a dose of 0.2 g of fat per body weight was safe, well tolerated, and efficient for improving clinical symptoms.13 In addition, treatment of critically ill patients with severe sepsis with fish oil-based lipid emulsion was considered safe and helpful for the rapid reduction of the clinical severity of the disease.14 In this study, our infusion protocol was safe and well tolerated, with mild adverse effects (nausea, vomiting, and local phlebitis) occurring at a low frequency (9.7% for fish oil LE, n = 3; and 6.2% for control LE, n = 2), which disappeared without LE treatment interruption.

The optimal timing of immunonutrition intervention in surgery is a critical issue that remains to be fully determined. Experimentally, anti-inflammatory effects on lung vasculature and changes in membrane fatty acids pattern were observed after only 3 h of fish oil LE infusion.15 We chose to induce fish oil LE pre-operatively based on emerging evidence that suggests that this window is more effective than the post-operative period for supplying nutrients that modulate immune functions, and provide clinical benefits.16 In addition, a retrospective study showed greater benefit in surgical patients receiving PN with fish oil LE infused perioperatively than when given only post-operatively, including a lower mortality rate.17 It is possible that immunonutrients need to reach suitable tissue and plasma levels for maximal effect, and pre-operative administration may be the best approach for achieving this goal.

Parenteral MCT/LCT lipid emulsion, which was infused as a control for this study, is identical to fish oil LE in appearance and rich in MCTs. MCTs are not susceptible to peroxidation and do not directly participate in eicosanoid synthesis, and therefore they may have a neutral impact on the reticuloendothelial system and even after the post-hoc analysis in the sub-groups of elderly and/or malnourished patients (Table 4). However, elderly and/or malnourished patients receiving MCT/LCT had a significant higher variation in the length of ICU stay than those receiving FO (15.76 vs. 2.42 for FO patients, p = 0.016), Fig. 3.

Table 1 Descriptive/demographic characteristics of patients with gastrointestinal cancer who were pre-operatively treated for 3 days with a peripheral infusion of a fish oil parenteral lipid emulsion (FO) that was rich in omega-3 fatty acids, or a control parenteral lipid emulsion (MCT/LCT) that was rich in medium-chain triglycerides.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>FO</th>
<th>MCT/LCT</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor site</td>
<td>Stomach</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Colon</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>18</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>Nutritional Status</td>
<td>Eutrophic</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Malnutrition</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>Open Surgery</td>
<td>Curative</td>
<td>25</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Palliative</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Age</td>
<td>&lt;60</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>&gt;60</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>Comorbidities</td>
<td>Yes</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>17</td>
<td>17</td>
</tr>
</tbody>
</table>

a Scored patient-generated-subjective global assessment (scored PG-SGA), performed in 55 patients.

b Hypertension, non-severe dyslipidemia, or non-insulin-dependent diabetes mellitus II, in addition to gastrointestinal cancer.

values. Importantly, these changes were significant between the two patient groups (p < 0.05).

During the post-operative period, FO patients exhibited an increase (3rd day, p = 0.004) followed by a decrease (6th day, p = 0.032) in IL-10 levels and an increase in CD32 expression on the neutrophil surface (p = 0.017) compared to baseline values, as well as MCT/LCT patients. The MCT/LCT patients had a trend of increasing IL-6 levels on the 3rd day (p = 0.072) and a significant increasing of IL-6 (p = 0.003) and CRP (p = 0.005) levels on the 6th day compared to baseline values, as well as FO patients on the 3rd day for IL-6 levels only. MCT/LCT patients also presented with a post-operative decrease of IL-10 levels (p < 0.0001) on the 3rd day and of the percentage of monocytes expressing HLA-DR (p = 0.023) and CD32 (p = 0.017) compared to baseline values, and FO patients. Both FO and MCT/LCT patients presented a post-operative decrease of PGE2 levels and leukocyte oxidative burst compared to baseline values (p < 0.05), but the decrease in the leukocyte oxidative burst was higher in MCT/LCT patients compared to FO patients (p < 0.05). In addition, no changes in leukocyte chemotaxis or phagocytosis, percentage of neutrophils expressing HLA-DR and CD32, or intensity of HLA-DR expression on neutrophils surface were observed in FO and MCT/LCT patients before or after surgical trauma (data not shown).

3.2.1. Effect of fish oil LE on post-operative clinical outcome

We did not detect significant differences in the frequency of infectious complications between FO and MCT/LCT patient groups, even after the post-hoc analysis in the sub-groups of elderly and/or malnourished patients (Table 4). However, elderly and/or malnourished patients receiving MCT/LCT had a significant higher variation in the length of ICU stay than those receiving FO (15.76 vs. 2.42 for FO patients, p = 0.016), Fig. 3.

Table 2 Baseline values (mean ± standard deviation) of immunological markers of patients with gastrointestinal cancer who were pre-operatively treated for 3 days with a peripheral infusion of a fish oil parenteral lipid emulsion (FO) that was rich in omega-3 fatty acids, or a control parenteral lipid emulsion (MCT/LCT) that was rich in medium-chain triglycerides.

<table>
<thead>
<tr>
<th>Immunological marker</th>
<th>FO</th>
<th>MCT/LCT</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin-6</td>
<td>0.28 ± 0.14</td>
<td>0.08 ± 0.20</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Interleukin-10</td>
<td>0.11 ± 0.08</td>
<td>0.23 ± 0.02</td>
<td>0.016</td>
</tr>
<tr>
<td>C reactive protein</td>
<td>0.08 ± 0.06</td>
<td>0.07 ± 0.04</td>
<td>0.930</td>
</tr>
<tr>
<td>Prostaglandin E2</td>
<td>122.98 ± 80.38</td>
<td>132.58 ± 89.66</td>
<td>1.000</td>
</tr>
<tr>
<td>Phagocytosis</td>
<td>-0.05 ± 0.81</td>
<td>0.08 ± 0.50</td>
<td>0.963</td>
</tr>
<tr>
<td>Oxidative burst</td>
<td>-0.17 ± 0.82</td>
<td>0.26 ± 0.74</td>
<td>0.205</td>
</tr>
<tr>
<td>Monocytes Intensity of HLA-DR</td>
<td>80.62 ± 29.52</td>
<td>130.61 ± 96.09</td>
<td>0.124</td>
</tr>
<tr>
<td>Monocytes Intensity of CD32</td>
<td>2283.15 ± 1578.38</td>
<td>1141.87 ± 1126.44</td>
<td>0.668</td>
</tr>
<tr>
<td>Neutrophils Intensity of HLA-DR</td>
<td>66.88 ± 47.03</td>
<td>59.98 ± 28.28</td>
<td>0.787</td>
</tr>
<tr>
<td>Neutrophils Intensity of CD32</td>
<td>277.56 ± 124.75</td>
<td>355.6 ± 208.61</td>
<td>0.412</td>
</tr>
<tr>
<td>Monocytes percentage of HLA-DR</td>
<td>87.03 ± 21.26</td>
<td>91.91 ± 14.70</td>
<td>0.872</td>
</tr>
<tr>
<td>Monocytes percentage of CD32</td>
<td>92.21 ± 22.14</td>
<td>94.41 ± 8.37</td>
<td>0.477</td>
</tr>
<tr>
<td>Neutrophils percentage of HLA-DR</td>
<td>86.28 ± 29.17</td>
<td>78.42 ± 37.23</td>
<td>0.882</td>
</tr>
<tr>
<td>Neutrophils percentage of CD32</td>
<td>93.03 ± 11.73</td>
<td>97.50 ± 1.92</td>
<td>0.679</td>
</tr>
</tbody>
</table>
immune response. The effect of fish oil LE supplementation in surgical patients under PN is often compared to LEs based on soybean oil as a control. However, soybean oil LEs are rich in n-6 PUFA, and therefore they are potentially inflammatory and immunosuppressive. In surgical patients, MCT/LCT has been shown to prevent abdominal abscesses and improve or maintain immune response markers compared to soybean oil LE.

Changes in immune response to the surgical trauma are marked by increased production of both pro-inflammatory and anti-inflammatory mediators. Therefore, we evaluated key mediators of these effects as well as leukocyte functions and surface molecules that may be influenced by their imbalance in order to identify targets of post-operative immune response that could be modulated by fish oil LE.

We found that the post-operative plasma concentration of IL-6 was decreased in patients treated with fish oil LE compared to patients treated with control LE. In agreement with this finding, previous studies have shown that surgical patients under standard PN also presented decreased post-operative levels of serum IL-6 after the supplementation with LE containing fish oil compared to pure soybean oil LE. However, it was hypothesized that the post-operative decrease of IL-6 levels observed in these studies may be a consequence of the n-6 PUFA infusion, which is present in large amounts in the LE soybean oil used as a control. Omega-6 PUFAs are precursors of the pro-inflammatory leukotriene (LT) B4, which has been experimentally associated with a dose-dependent accumulation of IL-6 mRNA and cytokine release, while omega-3 PUFAs compete with the same enzymatic pathways for the less inflammatory LTB5 synthesis.

The control LE used in our study contains 50% less omega-6 PUFA than soybean oil LE, and therefore we suggest that the decreased post-operative levels of IL-6 found may be due to the high content of n-3 PUFA provided by fish oil LE rather than the decrease of omega-6 PUFAs. In addition, our findings support recent studies that have also shown decreased IL-6 levels in surgical

Table 3

<table>
<thead>
<tr>
<th>Immunological marker</th>
<th>Group</th>
<th>T1–T0</th>
<th>P value</th>
<th>T2–T0</th>
<th>P value</th>
<th>T3–T0</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin-6</td>
<td>FO</td>
<td>-0.05 ± 0.30</td>
<td><strong>0.0001</strong></td>
<td>0.09 ± 0.52</td>
<td><strong>0.029</strong></td>
<td>0.22 ± 0.60</td>
<td>0.202</td>
</tr>
<tr>
<td></td>
<td>MCT/LCT</td>
<td>0.15 ± 0.26</td>
<td></td>
<td>1.29 ± 1.18</td>
<td></td>
<td>0.60 ± 0.99</td>
<td></td>
</tr>
<tr>
<td>Interleukin-10</td>
<td>FO</td>
<td>-0.005 ± 0.05</td>
<td>0.019*</td>
<td>0.76 ± 0.32</td>
<td>&lt;0.0001*</td>
<td>-0.07 ± 0.13</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>MCT/LCT</td>
<td>0.01 ± 0.03</td>
<td></td>
<td>-0.10 ± 0.03</td>
<td></td>
<td>0.20 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Oxidative burst</td>
<td>FO</td>
<td>-0.06 ± 0.65</td>
<td>0.632</td>
<td>-0.96 ± 0.70</td>
<td>-1.16 ± 0.73</td>
<td></td>
<td>-1.21 ± 0.78</td>
</tr>
<tr>
<td></td>
<td>MCT/LCT</td>
<td>0.11 ± 0.78</td>
<td></td>
<td>-2.31 ± 1.83</td>
<td></td>
<td>-0.13 ± 0.48</td>
<td>-0.028*</td>
</tr>
<tr>
<td>Percentage HLA-DR (Mφ)</td>
<td>FO</td>
<td>2.67 ± 15.94</td>
<td>0.464</td>
<td>-15.86 ± 18.92</td>
<td>0.052*</td>
<td>-15.11 ± 16.72</td>
<td><strong>0.010</strong></td>
</tr>
<tr>
<td></td>
<td>MCT/LCT</td>
<td>-8.11 ± 31.20</td>
<td></td>
<td>-15.11 ± 16.72</td>
<td></td>
<td>-15.11 ± 16.72</td>
<td><strong>0.010</strong></td>
</tr>
<tr>
<td>Percentage CD32 (Mφ)</td>
<td>FO</td>
<td>1.17 ± 12.98</td>
<td>0.253</td>
<td>0.19 ± 12.48</td>
<td>-0.025*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MCT/LCT</td>
<td>-4.56 ± 7.84</td>
<td></td>
<td>-15.11 ± 16.72</td>
<td></td>
<td>-15.11 ± 16.72</td>
<td><strong>0.010</strong></td>
</tr>
<tr>
<td>Intensity CD32 (Nφ)</td>
<td>FO</td>
<td>-0.14 ± 99.87</td>
<td>0.200</td>
<td>65.00 ± 138.84</td>
<td></td>
<td>65.00 ± 138.84</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MCT/LCT</td>
<td>-1.23 ± 35.68</td>
<td></td>
<td>20.26 ± 91.61</td>
<td></td>
<td>20.26 ± 91.61</td>
<td></td>
</tr>
</tbody>
</table>

Mφ – Monocytes; Nφ – Neutrophils.
patients treated post-operatively with fish oil LE compared to MCT/LCT and olive oil-based LEs.\textsuperscript{25-27}

We also assessed the post-operative levels of CRP, which is a hepatic pro-inflammatory component of immune response regulated by IL-6. Accordingly, post-operative CRP significantly increased only in patients treated with the control LE, but no different post-operative levels of this mediator were observed between both studied groups. In agreement with our data, Badía-Tahull et al. did not find between treatment changes in the post-operative levels of CRP of surgical patients receiving fish oil-supplemented PN compared to a standard olive oil-based LE.\textsuperscript{28}

The post-operative plasma concentration of IL-10 was also assessed as an anti-inflammatory marker in this study. Post-operative production of IL-10 is desirable for controlling hyper-inflammation, but the persistence of high levels of this cytokine can adversely contribute to immune paralysis. Overexpression of IL-10 has been correlated with decreased post-operative expression of HLA-DR on monocytes, which is considered to be a central marker of immune paralysis after surgical trauma.\textsuperscript{29}

Our data suggest that treatment of patients with fish oil LE induces favorable post-operative physiological modulation of IL-10 concentrations, with an increase in the cytokine on the 3rd post-operative day followed by a decrease by the 6th post-operative day. Previous studies did not detect changes in post-operative levels of IL-10 after post-operative infusion of fish oil LE compared to both soybean oil LE and MCT/LCT. These observations suggest that the period of parenteral infusion of fish oil LE may be important for detecting changes in post-operative levels of IL-10. In addition, while our patients treated with control LE had decreased post-operative monocytes expressing HLA-DR, patients treated with fish oil LE maintained this immunological marker at baseline levels. Our data are in agreement with the findings of Weiss et al. who showed that PN with fish oil LE maintained the post-operative expression of leukocyte HLA-DR, which was decreased in patients that received the infusion of PN with soybean oil LE.\textsuperscript{30}

A decrease in the number of HLA-DR positive monocytes indicates the functional deactivation of these leukocytes, which is considered to be a central marker of immune paralysis after surgical trauma.\textsuperscript{29} In fact, the decrease in the number of monocytes expressing HLA-DR in our study was accompanied by a decrease in the number of monocytes expressing CD32 and decreased oxidative burst in patients treated with control LE, while fish oil LE preserved these leukocyte phenotype markers and reduced the negative impact on oxidative burst function. Decreased leukocyte oxidative burst as well as HLA-DR and CD32 expression on monocytes are frequently observed after surgical trauma and are associated with post-operative infectious and non-infectious complications. Therefore, our immunological data suggest that pre-operative infusion of fish oil LE may contribute to improve clinical outcomes.

Our original clinical endpoint was to evaluate the frequency of post-operative infections and the length of ICU and hospital stay of surgical patients treated with pure fish oil LE. There were not significant differences between treatment and control groups, in contrast to previous studies assessing parenteral regimens supplemented with fish oil LE.\textsuperscript{6} Heller et al., in surgical patients treated with parenteral regimens supplemented with fish oil LE, observed a tendency of shorter ICU stays only in those patients with sepsis risk after stratification of total patient population in “higher risk groups”.\textsuperscript{30} Based on this previous study, we further analyzed (post-hoc) clinical outcome separately for more susceptible elderly (≥60 years of age) and/or malnourished patients.\textsuperscript{31,32} A smaller variation of length of ICU stay was observed in this subgroup of patients treated with fish oil LE, in relation to control LE. These clinical data may support previous evidence by Heller et al. showing the benefit of treating debilitated patients undergoing major cancer surgery with fish oil LE in order to improve clinical outcomes.\textsuperscript{29} Therefore, additional studies are needed to explore this issue.

This study had several limitations. It included only adult surgical patients with gastrointestinal cancer and similar results may therefore not be achieved in other surgical groups. Patients were included without consideration of their nutritional status or alimentary habits (even antioxidants consume) because the main contribution of our study was to assess whether, regardless these nutritional factors, post-operative immune response could benefit from pre-operative infusion of fish oil LE. At baseline our

### Table 4

<table>
<thead>
<tr>
<th>Lipid emulsion</th>
<th>Complications (%)</th>
<th>Length of stay (mean of days ± standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infectous</td>
<td>Intensive care unity</td>
</tr>
<tr>
<td>T</td>
<td>R</td>
<td>T</td>
</tr>
<tr>
<td>FO</td>
<td>6.5\textsuperscript{a}</td>
<td>10\textsuperscript{a}</td>
</tr>
<tr>
<td>MCT/LCT</td>
<td>15.6\textsuperscript{b}</td>
<td>27.8\textsuperscript{b}</td>
</tr>
<tr>
<td>p value</td>
<td>0.426</td>
<td>0.222</td>
</tr>
</tbody>
</table>

T: total patient; R: patient with additional risk (elderly and/or malnourished).

\textsuperscript{a} Local surgical wound (n = 1) and pneumonia (n = 1).

\textsuperscript{b} Local surgical wound (n = 3) and sepsis (n = 2).

Fig. 3. Variation (95\% confidence interval — CI) of intensive care unit (ICU) discharge of elderly and/or malnourished surgical patients with gastrointestinal cancer who were pre-operatively treated for 3 days with a peripheral infusion of a fish oil parenteral lipid emulsion (FO) that was rich in omega-3 fatty acids, or a control parenteral lipid emulsion (MCT/LCT) that was rich in medium-chain triglycerides.
two groups of patients were, demographically, clinically and immunologically, similar. However, they had different levels of basal IL-6 and IL-10. There is not a specific explanation why this has occurred, except by chance. The difference of these cytokines levels probably had not influenced our findings because an inversion of their values, between the two groups, occurred at the 3rd post-operative day. We used an active control instead saline solution in order to preserve the double-blind design of our study, but changes observed in the control arm had been previously associated to surgical trauma. Treatment evaluation was assessed only in patients who completed the protocol of LE infusion, without performing an intention to treat analysis. In addition, it is not possible to attribute the immunological benefits observed after pre-operative infusion of fish oil LE only to their omega-3 fatty acids contend, as long as this LE emulsion also have large amounts of the antioxidant alpha-tocopherol.

In conclusion, our immunological data show that pre-operative infusion of pure fish oil LE may attenuate immune dysfunction after surgical trauma, which is a critical step for avoiding post-operative immune paralysis and multiple organ failure. Because most surgical patients do not require PN, our findings could be applicable to general surgical patient care and may lead to new approaches in pre-operative adjuvant therapy.

Funding sources

This study was funded by a grant from the FAPESP (2004/06259-7, 2007/56474-0, and 2008/50944-7) and CNPq (94/50633-3) and supported by Fresenius-Kabi Germany, which kindly provided parenteral lipid emulsions, and Farmoterápica-Brazil, which kindly prepared lipid emulsions bags.

Statement of authorship

RST participated in study design and coordination, performed leukocyte functions assays, database management, overall data analysis, and interpretation and wrote the manuscript; RS participated in the enrollment, assignment, and clinical evaluation of patients and performed cytokines assay; TG participated in the enrollment, assignment, and clinical evaluation of patients and performed the assays of leukocyte surface molecules; MFC performed the CRP and PGE2 assays and participated in the analysis and interpretation of these data; MMS created the acquisition panels and analytic histograms for each individual kit and antibody used in flow cytometric analysis and guided the collection and analysis of data obtained by this methodology; RC participated in the study design and conception, oriented the data collection, and critically revised the manuscript; DLW conceived the study, participated in its design and coordination, assisted in data interpretation, and drafted the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors do not have any additional conflict of interest to declare.

Acknowledgments

We thank Patricia A. de Oliveira for the overall technical support during the flow cytometry analysis, and Demerson Poli, João Italo Dias França, Márcio Augusto Diniz, and Prof. Julio Cesar Rodrigues Pereira for discussions on the statistical plan and analysis.

References

4. van der Meij BS, van Bolchorst-de van der Schueren MA, Langius JA, Brouwier IA, van Leenen PA. n-3 PUFAs in cancer, surgery, and critical care: a systematic review on clinical effects, incorporation, and washout of oral or enteral compared with parenteral supplementation. Am J Clin Nutr 2011 Sep 21 [Epub ahead of print].
11. Health Statistics and Health Information Systems. De-


