Oral fish oil positively influences nutritional-inflammatory risk in patients with hematological malignancies during chemotherapy with impact on long-term survival: a randomized clinical trial

Short-title (Running Head): Fish oil and hematological malignancies

Abstract

Background: Studies suggest ingestion fish oil (FO), a source of the omega-3 polyunsaturated fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), can reduce deleterious side-effects of chemotherapy. The aim of this randomized clinical trial was to evaluate the effect of supplementation with oral FO for nine weeks on nutritional parameters and inflammatory nutritional risk in patients with hematological malignancies during the beginning of the chemotherapy. **Methods:** Twenty-two patients with leukemia or lymphoma were randomized to the unsupplemented group (UG) (n=13) or supplemented group (SG) (n=9). SG received 2 g/day of fish oil for nine weeks. Nutritional status, serum acute-phase proteins, and plasma fatty acids were evaluated before (T0) and after (T1) the intervention period. Data were analyzed by two models; model 1 - data from all patients included in the study, and model 2 - data from UG patients with no increase in the proportions of EPA and DHA in plasma and data from SG patients showing, at least, a 100% increase in plasma EPA and DHA. **Results:** SG showed increased plasma proportion of EPA and DHA in both models. In model 2, C-reactive protein (CRP) and CRP/albumin ratio showed larger reductions in the SG. Overall long-term survival in both models (465 days after the start of the chemotherapy) was higher in the group ingesting fish oil ($P<0.05$). **Conclusions:** These findings indicate an improved nutritional-inflammatory risk and potential effects on long-term survival in patients with hematological malignancies supplemented with FO during the beginning of chemotherapy.

Keywords: fish oil, n-3 PUFA, hematologic malignancies, nutritional status, leukemia and lymphoma.

Introduction

Hematologic malignancies belong to a heterogeneous group of hematologic diseases [13]. Inflammation is now recognized to be an important component in cancer prognosis, with several inflammatory markers being in use to monitor disease progression and prognosis [49]. One of the main strategies used to treat hematological malignancies is chemotherapy. The treatment aims to destroy neoplastic cells, but also affects healthy cells, like cells of the gastrointestinal tract [2]. Adverse effects caused by antineoplastic therapy include giddiness, nausea, vomiting, mucositis, dysphagia, diarrhea, and changes in taste and smell perception [7]. Such adverse effects are linked to metabolic and nutritional losses and subsequent deterioration in the quality of life [39, 15].

Dietary fatty acids (FAs) have profound physiological implications [28]. Studies suggest that ingestion of n-3 polyunsaturated fatty acids (PUFAs), especially docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) found in fish oil, can be beneficial to cancer treatment by preserving nutritional status [17], decreasing inflammatory markers (e.g. positive acute-phase proteins) [33], and increasing survival [31]. However, studies using fish oil supplementation in hematological malignancies are scarce, and there is no clear information regarding indication to use or not such nutritional strategy in these patients.

The aim of this study was to evaluate the effect of oral supplementation with 2 g/day of fish oil (a source of EPA and DHA) for nine weeks on nutritional parameters and inflammatory status in newly diagnosed patients with hematological malignancies starting chemotherapy. Overall survival to 465 days from the start of fish oil supplementation was a secondary outcome. We hypothesized that patients diagnosed with hematological malignancies would show a reduction in overall nutritional inflammatory risk and improved survival when ingesting fish oil.

Experimental Methods

Subjects

Patients with hematological malignancies assisted at the Ambulatory and Hematology Clinical Center of the University Hospital of Santa Catarina - Florianópolis, Santa Catarina, Brazil, from November 2012 to December 2013 participated in this randomized clinical trial (RCT). The eligibility criteria were: age ≥

18 years, histopathological diagnosis of leukemia or lymphoma and chemotherapy treatment indication. Exclusion criteria were: being in palliative care, taking statins or anti-inflammatory drugs, being allergic to fish and fish derivatives, being unable to perform oral ingestion of food, having received chemotherapy previously, being pregnant, ingesting fish oil or other n-3 PUFA supplements in the six months before study initiation. A non-probabilistic convenience sample was defined by time saturation.

The diagnosis and disease staging were established according to routine biopsy, immunophenotyping, cytogenetics and immunohistochemistry, conducted by the University Hospital Pathology Division.

Study design

Eligible patients were randomly allocated into one of two groups: supplemented group (SG) and unsupplemented group (UG). Randomization was performed with the online tool: Research Randomizer (http://www.randomizer.org/). Odd numbers represented patients allocated to SG and even numbers allocated to UG. The study was conducted following guidelines in the Declaration of Helsinki [(40)]. All procedures involving human patients were approved by the Research Ethics Committee of the local Institution. All participants signed a consent form. The trial is registered at www.ensaiosclinicos.gov.br, under the register RBR-7q6cqg.

The patients in the SG were instructed to ingest two capsules/day of fish oil (2 g/day in total) for nine weeks. This dose has been tested effectively in previous studies [(28, 40, 41)]. Subjects were instructed to ingest capsules in a dose-fractionated form, about 20 to 30 minutes before lunch or dinner and accompanied by liquid. During the supplementation period, supplement intake was controlled by weekly phone calls (for non-hospitalized subjects) or daily contact in the hospital. Also, subjects were instructed to record capsules intake in a provided form. The supplementation started on the first day of the chemotherapy (T0). The UG did not receive any supplement.

Personal data, lifestyle information and weight loss in the previous six months were obtained by interview. Clinical data (hematologic malignant diagnosis, stage, other diseases and medication use) were obtained from patient records.

Dietary supplement

The fish oil supplement was in the form of gelatin capsules with 1,000 mg oil/capsule (Omega 3, Phytomare®, Governador Celso Ramos, SC, Brazil). The oil was extracted from salmon, mackerel, and sardines. Two fish oil capsules provided a total of 17 kilocalories, and 0.7 g saturated fatty acids, 0.5 g monounsaturated fatty acids, 0.8 g polyunsaturated fatty acids, and 4 mg of cholesterol. Two capsules provided a total of 610 mg of n-3 PUFAs (367 mg of EPA and 243 mg of DHA). Analysis of the fatty acid profile of the fish oil capsule, by high-performance liquid chromatography, was conducted and returned the following proportions (%) of the investigated fatty acids: EPA 25.06; DHA 14.58; palmitic 18.95; myristic 9.71; arachidonic < 1; palmitoleic 12.94; oleic 9.73; linoleic 2.99; stearic 5.76; α-linolenic < 1.

Anthropometric data

Weight and height were measured with an electronic platform scale with a coupled vertical stadiometer (Toledo®, Toledo Company of Brazil, São Bernardo do Campo, SP, Brazil). Usual weight was self-reported by the patient. Triceps skinfold (TS) was measured with a Compass Lange Skinfold Caliper® (Beta Technology Incorporated, Santa Cruz, California, USA) and mid-upper arm circumference (MUAC) was measured with an inelastic tape (TBW®, São Paulo, SP, Brazil). TS and AC were obtained from the arithmetic mean of three measures. All anthropometric measurements were made following standard techniques [44].

Blood collection

Blood samples (30 mL) were collected after approximately eight hours of fasting at two-time points: on the day prior to the first chemotherapy (baseline) and nine weeks later (week 9). Blood was collected into vacuum tubes (Vacutainer® System, BD Biosciences, Abingdon, UK) containing lithium-heparin. Plasma was prepared and stored at -80ºC until thawing for determination of fatty acid profile. Blood was collected into separating gel tubes and serum isolated for determination of CRP and albumin concentrations.

Determination of hemogram, albumin and CRP concentration

Hemogram parameters were obtained from whole blood using an automated method (Sysmex Xe-2100, Roche®, Kobe, Japan); the values are expressed as cells/mm^3. Albumin was quantitatively determined by an automated colorimetric

method (Siemens Healthcare Diagnostics Inc., Newark, DE, USA) employing bromocresol purple as a color reagent [29], and is expressed as g/dL. Serum CRP was quantified by a nephelometric method (Siemens Dade Behring Inc., Newark, DE, EUA) [30] and is expressed as mg/L.

Nutritional status

The variation of weight related to the previous study period was assessed by calculating the weight loss percentage, resulting from the division between ΔW (the difference between usual weight and current weight) and the usual weight multiplied by 100 [20].

Several variables were assessed to evaluate nutritional status. Arm circumference (AC), mid-upper arm circumference (MUAC), and triceps skinfold (TS) (expressed in cm and mm, respectively). Mid-upper arm muscle circumference (MUAMC) was calculated according to the expression: *MUAC - (π x TS)/10*. Body mass index (BMI) was calculated by the ratio of weight (kg) to the square of the height (m). The cutoff points for classification were proposed by the World Health Organization [44-46]. Nutritional Risk Index (NRI) was calculated based on the equation: *1.519 x serum albumin (g/dL) + 41.7 x current weight (kg)/usual weight (kg)*. The classification adopted was: No nutrition risk: >100; Borderline nutrition risk: 99.9-97.5; Mild nutrition risk: 83.5-97.5; Severe nutritional risk: <83.5 [8, 26].

The CRP/albumin index was applied to categorize the inflammatory-nutritional prognosis of the patient. Classification adopted was: without risk: <0.4, low risk: 0.4 to 1.2; moderate risk: 1.2 to 2.0; high risk: >2.0 [16].

Plasma fatty acids profile

The plasma fatty acid profile was determined by High-Performance Liquid Chromatography (HPLC), according to Nishiyama-Naruke et al. [36]. Plasma fatty acid constituents of phospholipids, triacylglycerols, cholesterol esters, and free fatty acids were extracted using chloroform:methanol (2:1, vol:vol), adapting the method described by Folch et al. [24]. Lipid extracts were suspended in methanol, and the pH was adjusted to ≥12 with 5 mol/l NaOH. The aqueous solution was acidified with hydrochloric acid (pH ≤3) and subjected to a new lipid extraction using hexane, followed by evaporation in gas N_2 at 37 ºC. Fatty acids were derivatized with 4-bromomethyl-7-coumarin and acetonitrile according to Abushufa, Reed and Weinkove [1], and subsequently separated

on a reversed phase analytical column (Discovery BIO Wide Pore, C8, 5 microns particles, 250 x 4.6 mm (Supelco- Sigma-Aldrich®). The chromatographic analysis was performed with a Waters AllianceBIO Separation Module e2796 (Waters, Milford, MA, USA). Sixteen microliters of derivatized fatty acids were injected and then eluted by the binary gradient of acetonitrile: water from 70:30 to 90:10 at 0.5 mL/min in 80 minutes run at a temperature between 18 to 21°C. The compounds were detected by fluorescence detection (Waters 2475 Multi λ Fluorescence Detector), with excitation at 325 nm and emission at 398 nm. Chromatographic data were recorded and integrated into Empower Pro Version 2.0 software. The following fatty acids were investigated: DHA; EPA; arachidonic; stearic; oleic; linoleic; α-linolenic; palmitic; myristic; lauric. Data are expressed as a percentage of total fatty acids.

Overall survival, hospital readmissions, and number chemotherapy sessions

Overall survival (OS) was defined as the time elapsed between baseline (the day of the first chemotherapy) and death from any cause or censored if alive at follow-up date (the follow-up was standardized to 465 days - 15.5 months - after study entry). The date of death, hospital readmissions and the number of chemotherapy sessions were recorded from patient medical records. OS curves were computed using the Kaplan–Meier method and compared using log-rank tests.

Statistical analysis

For statistical analyses, the intake of fish oil was considered the exposure variable. Data normality was tested by applying the Shapiro-Wilk test. Student's unpaired *t*-test or Mann-Whitney test were used to test for differences between groups at each time point. Student's paired *t*-test or Wilcoxon test for paired data were used to test the differences between time points within a study group.

All analyses were performed in STATA® 11.0 version for Windows (StataCorp, Texas, USA) and figures were drawn using GraphPad Prism v.5.01 (Graphpad Inc.; La Jolla, USA). $P < 0.05$ was considered to indicate statistical significance.

Two models for statistical analyses were applied: Model 1 included all eligible patients that accepted to participate and finished the nine weeks follow-up; Model 2 included patients from SG that presented ≥ 100% increment in proportions of plasma EPA and DHA at 9 weeks compared to baseline and UG patients who did not show an increment (< 50% increase) in plasma EPA and DHA at 9 weeks when compared to

baseline. Such strategy was applied to reduce any potential heterogeneity related to the absorption and incorporation of fatty acids caused by the chemotherapy.

Results

Eighty-one new cases of hematologic diseases were identified at University Hospital Professor Polydoro Ernani de São Thiago/Federal University of Santa Catarina between November 2012 to December 2013. Fifty patients were not eligible for participation in the study according to the inclusion/exclusion criteria. Therefore, 31 patients were invited to take part in the trial. Three patients chose not to participate. Finally, 28 patients (90.3% of the eligible patients) were randomized to two study groups (model-1). Afterward, according to the analysis of the plasma fatty acid profile, 14 patients were randomized to the same two study groups (model 2), as shown in Figure 1. Six participants were withdrawn from the study, three per group. The reasons for withdrawal are given in figure 1.

In both analysis models, plasma EPA and DHA concentrations did not change in the UG ($P>0.05$) (Supplementary table 1). In the SG, EPA and DHA increased in the SG in both analysis models. However, the increase in plasma DHA for model 1 reached a $P=0.07$, which can be interpreted as a statistical tendency (Supplementary table 1).

Characteristics of the study participants

Baseline characteristics of the 22 randomized patients for model 1 and the 14 patients in model 2 did not differ between UG and SG (table 1). Acute leukemia (AL) and Non-Hodgkin lymphoma (NHL) were the main diagnoses in patients included in both analysis models. The distribution of patients between groups according to sex was not different. The concomitant diseases presented by the patients were: osteoporosis, type 2 diabetes mellitus, hypertension, rheumatoid arthritis, depression, gastritis, gastric ulcer, hiatal hernia, esophagitis and hypothyroidism, and hyperthyroidism. Although the percentage of weight loss in the last six months before chemotherapy was numerically higher in the UG compared to the SG, this is statistically significant.

Anthropometric parameters and nutritional status

No significant changes were observed in either analysis model for weight, mid-upper arm circumference (MUAC), triceps skinfold (TS) and mid-upper arm muscle

circumference (MUAMC) (table 2). For model 2, BMI was lower in SG than in UG at the end of the nine weeks ($P<0.05$). However, NRI was higher in SG than in UG using model 2 ($P<0.05$) (Table 2).

The CRP/albumin ratio for both models of analysis is shown in figure 2. Both groups had a significant decrease in the inflammatory-nutritional risk from baseline to the ninth week (T1) for analysis model 1. UG patients changed their classification from high to the medium and low risk of complications categories. In the group ingesting fish oil, most patients were categorized as low risk or no risk, after supplementation (UG = 5.1 [2.0; 31.6] to 1.4 [1.0; 9.0]; SG = 12.6 [2.8; 18.1] to 1.1 [0.9; 6.8]; $p<0.05$). There were no significant differences between groups when analysis model 2 was applied, besides the same changes to a lower risk category (Figure 2).

Blood, serum and plasma parameters

UG showed a significant increase in red blood cell count (RBC) with both analysis models (table 3). Hematocrit and leukocytes increased significantly after nine weeks for model 1 ($P<0.05$). These changes did not occur in the SG. Additionally, using model 1, the SG had a significant reduction in serum levels of CRP ($P<0.05$), which did not occur in patients in the UG (table 3).

Overall survival of the patients that completed the study

During the nine weeks of the trial, three patients in the UG died (Figure 1). Patients that completed the nine weeks of the trial were followed for additional 14 months; outcomes were survival (Figure 3), hospital readmissions and the number of chemotherapy cycles. No significant differences were observed in the number of hospital readmissions. However, the number of chemotherapy cycles was significantly higher in the SG independent of the model of analysis (Supplementary Table 2). During the follow-up, four patients of the UG started palliative chemotherapy treatment. In the SG, two patients relapsed after one year of remission and were submitted to further chemotherapy sessions (data not shown).

There were no reported deaths during the 465 days of follow-up for the patients in the SG independent of the analysis model. In contrast, the total number of reported deaths in the UG was eight when analysis model 1 was used (log rank $P=0.005$ when compared to the SG group) (Figure 3A). Appling model 2 analysis, there were six

reported deaths in the UG (log rank P=0.008 when compared to the SG group) (Figure 3B).

Discussion

This randomized clinical trial showed that ingesting 2 g/day of supplemental fish oil improved long-term survival in patients with hematological malignancies receiving chemotherapy. Furthermore, patients receiving fish oil were also able to undertake a greater number of cycles of chemotherapy. Fish oil containing 367 mg of EPA and 243 mg of DHA for nine weeks was sufficient to alter the fatty acid composition of plasma lipid constituents, leading to a ~2-fold increment in the proportion of EPA and 1.8-fold for DHA. The same has been shown in previous studies [22, 32, 40].

Studies testing EPA and DHA effects in patients diagnosed with hematologic malignancies are scarce. One previous study was performed in early-stage Chronic Lymphocytic Leukemia [21] and another one in acute myeloid leukemia [7]. Both used fish oil as a nutritional strategy in parallel with chemotherapy. However, neither of these previous studies assessed the same outcomes assessed in the current study. Hence, it is difficult to compare our findings with the ones of these two previous studies.

In vitro and review studies have concluded that EPA and DHA can induce apoptosis in leukemic cell lineages [14, 25, 48]. Furthermore, *in vitro* studies performed with leukemic cell lines have shown an increment in the antineoplastic action of drugs used in chemotherapy when EPA and DHA are present in the culture medium [22, 47, 18]. Additionally, in dogs with Lymphoblastic lymphoma, EPA and DHA ingestion increased survival and decreased plasma IL-6 concentration [37].

RCTs conducted in different types of cancer patients receiving chemotherapy and supplemented with n-3 PUFAs have shown positive effects of this strategy on inflammatory and nutritional outcomes. For example, a double-blind RCT with patients with lung cancer observed increased body weight and a reduction in inflammatory indexes in the group that received fish oil containing 510 mg EPA and 310 mg DHA [23]. A RCT testing colorectal cancer patients found that fish oil containing 367 mg EPA and 243 mg DHA, given daily for nine weeks, decreased serum levels of CRP [34], reduced inflammatory and nutritional risk [34, 42], and maintained or increased BMI and body weight during chemotherapy [42].

A systematic review regarding the effects of n-3 PUFA in cancer patients receiving chemotherapy concluded that the main beneficial effect of this supplementation is the preservation of body weight and body composition [17]. However, in our study, there were no significant changes in body weight or BMI with fish oil supplementation. Nevertheless, we observed a trend increment in MUAMC in the subjects supplemented with fish oil, suggesting preservation or gain of lean mass. In addition, NRI was higher in patients receiving fish oil.

Studies with different cancer patients demonstrated a decrease in inflammation after supplementation with n-3 PUFAs [6, 50, 12, 19]. These effects may be partly due to inflammatory and immune response modulation by n-3 PUFAs as a result of an altered pattern of production of lipid mediators including eicosanoids, such as prostaglandins, leukotrienes, thromboxanes, resolvins (E and D), and D1 protectins [10, 27, 41]. Additionally, some n-3 PUFAs anti-inflammatory effects seem to be exerted through the decreased activation of the pro-inflammatory transcription factor NF-κB and perhaps increased activation of the anti-inflammatory transcription factor peroxisome proliferator activated receptor-gamma (PPARγ) [9, 43, 11].

In this study, chemotherapy affected the CRP/albumin ratio. In the UG the risk classification changed from high to moderate. However, in patients who received fish oil, the risk classification changed from high to low. This risk classification decline can be an indicative of a positive effect of supplementation with fish oil. The same effect had been shown previously in patients with colorectal cancer [34, 42]. Thus, the CRP/albumin ratio may be a sensitive marker of the ability of fish oil supplementation to improve the inflammatory-nutritional status in these patients.

Survival up to 465 days of follow-up was greater when patients ingested fish oil independent of the model of analysis. Similarly, some previous studies showed prolonged survival after n-3 PUFA supplementation in patients with pancreatic cancer [31], advanced non-small cell lung cancer [35], and metastatic breast cancer [5]. The longer survival might be attributed to the effects fish oil in the nutritional-inflammatory risk. Additionally, the increment of the antineoplastic action and reduction of the toxicity of the chemotherapy[38], could explain such observations.

The data presented here suggest that it will be important for future trials to perform medium or long-term follow-up of some variables relevant to the clinical environment (e.g. survival, hospital readmissions, etc.). Furthermore, it may be possible for researchers involved in previous trials to retrospectively check clinical

variables from the enrolled patients looking for a medium or long-term changes that were not part of the original follow-up in some trials.

Studies with oral fish oil supplementation have a specific limitation: regular fish oil's odor and aftertaste affect the performance of double-blind placebo-controlled trials. Although some studies try to minimize this limitation using deodorized fish oil capsules, in medium to low-income countries, regular fish oil is easily assessable when compared to other forms available in the market. Therefore, no placebo was offered to the control group. Other limitations include the study sample, which was composed by patients with different onco-hematological diagnosis, with different disease staging, chemotherapy regimes, and co-morbidities. However, the cohort was a result of a careful screening according to our inclusion and exclusion criteria. We believe our criteria were important to reduce additional confounding factors. Nevertheless, despite these limitations, the data from the present study provide relevant information to guide future research in these health conditions.

In conclusion, the ingestion of fish oil concomitant with chemotherapy increases long-term survival (465 days after the beginning of the chemotherapy) potentially by reducing the inflammatory-nutritional risk, in patients with hematological malignancies. Bigger clinical trials, focusing these patients, need to be conducted to test such findings globally.

Acknowledgments

We are grateful to the Graduate Program in Nutrition – Federal University of Santa Catarina, Brazil; Fellowship Program Social Demand / Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES); and Phytomare for the donation of dietary supplements. We thank Professor André Bafica for making the HPLC equipment facility (funded by Nanobiotec CAPES) available to us.

Financial Support

This research was supported by a grant from the National Counsel of Technological and Scientific Development (CNPq) – Universal call - CNPq Nº 14/2011 – project 473321/2011-4. CNPq had no role in the design, analysis or writing of this article.

367

References

1. Chen GQ, Wang LS, Wu YL, et al. Leukemia, an effective model for chemical biology and target therapy. Acta Pharmacol Sin. 2007; 28: 1316-24.
2. Zhu J, Paul WE. CD4 T cells: fates, functions, and faults. Blood. 2008; 112: 1557-69.
3. Ingenbleek Y, Carpentier YA. A prognostic inflammatory and nutritional index scoring critically ill patients. Int J Vitam Nutr Res. 1985; 55: 91-101.
4. Jain S, Gautam V, Naseem S. Acute-phase proteins: As diagnostic tool. J Pharm Bioallied Sci. 2011; 3: 118-27.
5. Soeters PB, Schols AM. Advances in understanding and assessing malnutrition. Curr Opin Clin Nutr Metab Care. 2009; 12: 487-94.
6. Acs (2014) American Cancer Society. A Guide to Chemotherapy. http://www.cancer.org/treatment/treatmentsandsideeffects/treatmenttypes/chemotherapy/understandingchemotherapyaguideforpatientsandfamilies/understanding-chemotherapy-intro (accessed 22 de janeiro de 2015).
7. Ravasco P, Monteiro-Grillo I, Vidal PM, et al. Nutritional deterioration in cancer: the role of disease and diet. Clin Oncol (R Coll Radiol). 2003; 15: 443-50.
8. Coronha AL, Camilo ME, Ravasco P. [The relevance of body composition in cancer patients: what is the evidence?]. Acta Med Port. 2011; 24 Suppl 4: 769-78.
9. Kalish BT, Fallon EM, Puder M. A tutorial on fatty acid biology. JPEN J Parenter Enteral Nutr. 2012; 36: 380-8.
10. Silva JaP, Fabre MES, Waitzberg DL. Omega-3 supplements for patients in chemotherapy and/or radiotherapy: A systematic review. Clin Nutr. 2015; 34: 359-366.
11. Mocellin MC, Camargo CQ, Nunes EA, et al. A systematic review and meta-analysis of the n-3 polyunsaturated fatty acids effects on inflammatory markers in colorectal cancer. Clinical Nutrition.
12. Ma YJ, Yu J, Xiao J, et al. The consumption of omega-3 polyunsaturated fatty acids improves clinical outcomes and prognosis in pancreatic cancer patients: a systematic evaluation. Nutr Cancer. 2015; 67: 112-8.
13. Rickham PP. Human Experimentation. Code of Ethics of the World Medical Association. Declaration of Helsinki. Br Med J. 1964; 2: 177.

14. Who (1995) Physical status: the use and interpretation of anthropometry. IN COMMITTEE, R. O. A. W. E. (Ed. *WHO Techinical Report series 854.* Geneva, WHO.
15. Lasky FD, Li ZM, Shaver DD, et al. Evaluation of a bromocresol purple method for the determination of albumin adapted to the DuPont aca discrete clinical analyzer. Clin Biochem. 1985; 18: 290-6.
16. Ledue TB, Weiner DL, Sipe JD, et al. Analytical evaluation of particle-enhanced immunonephelometric assays for C-reactive protein, serum amyloid A and mannose-binding protein in human serum. Ann Clin Biochem. 1998; 35 (Pt 6): 745-53.
17. Detsky AS, Mclaughlin JR, Baker JP, et al. What is subjective global assessment of nutritional status? JPEN J Parenter Enteral Nutr. 1987; 11: 8-13.
18. Who (2000) Obesity: preventing and managing the global epidemic. IN CONSULTATION, R. O. A. W. (Ed. *WHO Technical Report Series 894.* Geneva, WHO.
19. Who. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet. 2004; 363: 157-63.
20. Buzby GP, Knox LS, Crosby LO, et al. Study protocol: a randomized clinical trial of total parenteral nutrition in malnourished surgical patients. Am J Clin Nutr. 1988; 47: 366-81.
21. The Veterans Affairs Total Parenteral Nutrition Cooperative Study Group. Perioperative total parenteral nutrition in surgical patients. The Veterans Affairs Total Parenteral Nutrition Cooperative Study Group. N Engl J Med. 1991; 325: 525-32.
22. Corrêa C, Angeleli A, Camargo N, et al. Comparison of PCR/albumin ratio with prognostic inflammatory nutritional index (PINI). Jornal Brasileiro de Patologia e Medicina Laboratorial. 2002; 38: 183 - 190.
23. Nishiyama-Naruke A, Souza JA, Carnelós M, et al. HPLC Determination of Underivatized Fatty Acids Saponified at 37°C Analysis of Fatty Acids in Oils and Tissues. Analytical Letters. 1998; 31: 2565-2576.
24. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem. 1957; 226: 497-509.
25. Abushufa R, Reed P, Weinkove C. Fatty acids in erythrocytes measured by isocratic HPLC. Clin Chem. 1994; 40: 1707-12.

26. Fahrmann JF, Hardman WE. Omega 3 fatty acids increase the chemo-sensitivity of B-CLL-derived cell lines EHEB and MEC-2 and of B-PLL-derived cell line JVM-2 to anti-cancer drugs doxorubicin, vincristine and fludarabine. Lipids Health Dis. 2013; 12: 36.
27. Malihi Z, Kandiah M, Chan YM, et al. Nutritional status and quality of life in patients with acute leukaemia prior to and after induction chemotherapy in three hospitals in Tehran, Iran: a prospective study. J Hum Nutr Diet. 2013; 26 Suppl 1: 123-31.
28. Bonatto SJ, Oliveira HH, Nunes EA, et al. Fish oil supplementation improves neutrophil function during cancer chemotherapy. Lipids. 2012; 47: 383-9.
29. Fahrmann JF, Ballester OF, Ballester G, et al. Inhibition of nuclear factor kappa B activation in early-stage chronic lymphocytic leukemia by omega-3 fatty acids. Cancer Invest. 2013; 31: 24-38.
30. Bukki J, Stanga Z, Tellez FB, et al. Omega-3 poly-unsaturated fatty acids for the prevention of severe neutropenic enterocolitis in patients with acute myeloid leukemia. Nutr Cancer. 2013; 65: 834-42.
31. Chiu LC, Wong EY, Ooi VE. Docosahexaenoic acid modulates different genes in cell cycle and apoptosis to control growth of human leukemia HL-60 cells. Int J Oncol. 2004; 25: 737-44.
32. Gillis RC, Daley BJ, Enderson BL, et al. Eicosapentaenoic acid and gamma-linolenic acid induce apoptosis in HL-60 cells. J Surg Res. 2002; 107: 145-53.
33. Yamagami T, Porada CD, Pardini RS, et al. Docosahexaenoic acid induces dose dependent cell death in an early undifferentiated subtype of acute myeloid leukemia cell line. Cancer Biol Ther. 2009; 8: 331-7.
34. Altenburg JD, Harvey KA, Mccray S, et al. A novel 2,6-diisopropylphenyl-docosahexaenoamide conjugate induces apoptosis in T cell acute lymphoblastic leukemia cell lines. Biochem Biophys Res Commun. 2011; 411: 427-32.
35. Betiati DDSB, De Oliveira PF, Camargo CDQ, et al. Effects of omega-3 fatty acids on regulatory T cells in hematologic neoplasms. Revista Brasileira de Hematologia e Hemoterapia. 2013; 35: 119-125.
36. Wirtitsch M, Roth E, Bachleitner-Hofmann T, et al. Omega-3 and omega-6 polyunsaturated fatty acids enhance arsenic trioxide efficacy in arsenic trioxide-resistant leukemic and solid tumor cells. Oncol Res. 2009; 18: 83-94.

37. De Lima TM, Amarante-Mendes GP, Curi R. Docosahexaenoic acid enhances the toxic effect of imatinib on Bcr-Abl expressing HL-60 cells. Toxicol In Vitro. 2007; 21: 1678-85.
38. Ogilvie GK, Fettman MJ, Mallinckrodt CH, et al. Effect of fish oil, arginine, and doxorubicin chemotherapy on remission and survival time for dogs with lymphoma: a double-blind, randomized placebo-controlled study. Cancer. 2000; 88: 1916-28.
39. Finocchiaro C, Segre O, Fadda M, et al. Effect of n-3 fatty acids on patients with advanced lung cancer: a double-blind, placebo-controlled study. Br J Nutr. 2012; 108: 327-33.
40. Mocellin MC, Silva JaP, Camargo CQ, et al. Fish oil decreases C-reactive protein/albumin ratio improving nutritional prognosis and plasma fatty acid profile in colorectal cancer patients. Lipids. 2013; 48: 879-88.
41. Silva JaP, Trindade EB, Fabre ME, et al. Fish oil supplement alters markers of inflammatory and nutritional status in colorectal cancer patients. Nutr Cancer. 2012; 64: 267-73.
42. 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: 805-14.
43. Zhu MW, Tang DN, Hou J, et al. Impact of fish oil enriched total parenteral nutrition on elderly patients after colorectal cancer surgery. Chin Med J (Engl). 2012; 125: 178-81.
44. 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: 121-4.
45. De Miranda Torrinhas RS, Santana R, Garcia T, et al. Parenteral fish oil as a pharmacological agent to modulate post-operative immune response: a randomized, double-blind, and controlled clinical trial in patients with gastrointestinal cancer. Clin Nutr. 2013; 32: 503-10.
46. Calder PC. Polyunsaturated fatty acids and inflammatory processes: New twists in an old tale. Biochimie. 2009; 91: 791-5.
47. Hardman WE. (n-3) fatty acids and cancer therapy. J Nutr. 2004; 134: 3427S-3430S.
48. Serhan CN. Novel chemical mediators in the resolution of inflammation: resolvins and protectins. Anesthesiol Clin. 2006; 24: 341-64.

499 49. Calder PC. Polyunsaturated fatty acids, inflammatory processes and inflammatory
500 bowel diseases. Mol Nutr Food Res. 2008; 52: 885-97.
501 50. Vanden Berghe W, Vermeulen L, Delerive P, et al. A paradigm for gene regulation:
502 inflammation, NF-kappaB and PPAR. Adv Exp Med Biol. 2003; 544: 181-96.
503 51. Calder PC. Omega-3 polyunsaturated fatty acids and inflammatory processes:
504 nutrition or pharmacology? Br J Clin Pharmacol. 2013; 75: 645-62.
505 52. Murphy RA, Mourtzakis M, Chu QS, et al. Supplementation with fish oil increases
506 first-line chemotherapy efficacy in patients with advanced nonsmall cell lung cancer.
507 Cancer. 2011; 117: 3774-80.
508 53. Bougnoux P, Hajjaji N, Ferrasson MN, et al. Improving outcome of chemotherapy
509 of metastatic breast cancer by docosahexaenoic acid: a phase II trial. Br J Cancer.
510 2009; 101: 1978-85.
511 54. Pappalardo G, Almeida A, Ravasco P. Eicosapentaenoic acid in cancer improves
512 body composition and modulates metabolism. Nutrition. 2015; 31: 549-55.

513

Legends

Figure 1 - Flowchart of patients in the study. Model 1 included all eligible patients that accepted to participate and finished the nine weeks of follow-up. Model 2 included patients from SG that presented ≥ 100% increment in the proportion of plasma EPA and DHA at 9 weeks compared to baseline and UG patients who did not show an increment (< 50%) in plasma EPA and DHA at 9 weeks when compared to baseline.

Figure 2 - CRP/albumin ratio of patients with hematological neoplasms supplemented with fish oil during chemotherapy on different moments: T0 - Data before the first session of chemotherapy; T1 - Data after 9 weeks of chemotherapy. A) Model 1 included all eligible patients that accepted to participate and finished the nine weeks of follow-up; Model 1: UG: Unsupplemented group n=13; SG: Supplemented Group n=9 and, B) Model 2: included patients from SG that presented ≥ 100% increment in the proportion of plasma EPA and DHA at 9 weeks compared to baseline, and UG patients who did not show an increment (< 50%) in plasma EPA and DHA at 9 weeks when compared to baseline. Model 2: UG group n=8; SG n=6. C and D (model 1), E and F (model 2) line representation to visualize individual changes in nine weeks. CRP/albumin ratio: values represent an inflammatory - nutritional risk index. Without risk: <0.4, low risk: 0.4–1.2; moderate risk: 1.2–2.0, high risk: > 2.0. # P value for Wilcoxon test for paired data.

Figure 3 - Overall survival (OS) was defined as the time elapsed between baseline (the day of the first chemotherapy) and death from any cause until 465 days of follow-up. OS curves according to fish oil supplementation were computed using the Kaplan–Meier method and compared using log-rank tests. A) Model 1: US: n=13; SG: n=9 included all eligible patients that accepted to participate and finished the nine weeks of follow-up. B) Model 2: UG: n=8; SG, n=6 included patients from SG that presented ≥ 100% increment in the proportion of plasma EPA and DHA at 9 weeks compared to baseline and UG patients who did not show an increment (< 50%) in plasma EPA and DHA at 9 weeks when compared to baseline. Abbreviations: UG: Unsupplemented group, SG: Supplemented Group.

547
548

549 **Table 1 -** Characteristics of the patients at baseline.

550 **Table 2 -** Nutritional status parameters of patients with hematological malignancies
551 supplemented or not with fish oil during chemotherapy.

552 **Table 3 -** Blood parameters of study's patients supplemented or not with fish oil during
553 chemotherapy.

554

555 **Supplementary Table 1 -** Percentage (%) of plasma fatty acids in patients with
556 hematological malignancies supplemented or not with fish oil during chemotherapy.

557 **Supplementary Table 2 -** Number of hospital readmissions and chemotherapy cycles
558 during 465 days of follow-up.
559

560