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Meta-analyses

A systematic review and meta-analysis of the n-3 polyunsaturated fatty acids effects on inflammatory markers in colorectal cancer

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SUMMARY

Background: Cancer and inflammation are closely related and an exacerbated inflammatory process can lead to tumor progression and a worse prognosis for the patient with cancer. Scientific literature has shown evidence that n-3 polyunsaturated fatty acids (PUFA) have anti-inflammatory action, and for this reason could be useful as an adjuvant in the treatment of some cancers.
Objective: A systematic review and meta-analysis of the literature was conducted until September, 2014, to evaluate the effects of n-3 PUFA on inflammatory mediators in colorectal cancer (CRC) patients.
Patients and methods: Clinical trials were systematically searched in three electronic databases and screening reference lists. Random meta-analysis model was used to calculate the overall and stratified effect sizes.
Results: Nine trials, representing 475 patients with CRC, evaluated effects of n-3 PUFA on cytokines (n = 6) and/or acute phase proteins (n = 5) levels. n-3 PUFA reduce the levels of IL-6 (SMD -2.34; 95% CI -4.37, -0.31; $p = 0.024$) and increase albumin (SMD 0.31; 95% CI 0.06, 0.56; $p = 0.014$) in overall analyses. In stratified analyses, reduction in IL-6 levels occurs in surgical patients that received 0.2 g/kg of fish oil parenterally at postoperative period (SMD -0.65; 95% CI -1.06, -0.24; $p = 0.002$), while, increase in albumin concentration occurs in surgical patients that received $\geq$ 2.5 g/d of EPA + DHA orally at preoperative period (SMD 0.34; 95% CI 0.02, 0.66; $p = 0.038$). In patients undergoing chemotherapy, the supplementation of 0.6 g/d of EPA + DHA during 9 week reduces CRP levels (SMD -0.95; 95% CI -1.73, -0.17; $p = 0.017$), and CRP/albumin ratio (SMD -0.95; 95% CI -1.73, -0.18; $p = 0.016$).
Conclusions: The results suggest benefits on some inflammatory mediators with the use of n-3 PUFA on CRC patients, but these benefits are specific to certain supplementation protocols involving duration, dose and route of administration, and also, the concomitant anti-cancer treatment adopted.

1. Introduction

Independently of the origin of the tumorigenesis process, many cancers share a common mechanism characterized by a generalized immunosuppression and the activation of pro-inflammatory cell signaling, which promotes progression, survival, proliferation, invasion, angiogenesis and systemic spread of the tumor tissue [1]. The participation of inflammatory mediators, as cytokines and chemokines, have a key role in this course by orchestrating the anti-cancer defense line (IL-12, Interferon − IFN) or by acting such as protumorigenic agents (IL-1, IL-6, IL-17, TNF) [2]. These inflammatory molecules are produced by immune, cancer and stroma cells through the activation of various transcription factors, such as Nuclear Factor Kappa B (NF-kB), Activator Protein-1 (AP-1), Signal Transducers and Activators of Transcription 3 (STAT3), and Smad. For details about the interaction among these cells and the role of specific cytokines and chemokines on anti or protumorigenic mechanisms in tumor microenvironment, see important reviews about the theme [2–6].

In the established tumors, the balance among inflammatory factors is profoundly tilted toward pro-tumor inflammation. Without therapeutic intervention (anti-cancer treatment),

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advanced tumors rarely regress [3,6]. Additionally, chemotherapy and radiation also activate inflammatory pathways, which subsequently can contribute to the chronic inflammation related to cancer [2]. Other repercussions caused by these procedures include chemo and radiation resistance, poor metabolism of cytotoxic anti-cancer drugs with the increase of toxic symptoms and progressive weight loss, which leads to a worse prognosis represented by the increased malignancy and low survival, and poorer quality of life [7].

Due to its impact on the course of the disease, the treatment of cancer should involve therapies targeting at inhibiting and/or resolving the chronic inflammation. Within this perspective, many studies have reported several benefits with the use of the n-3 polyunsaturated fatty acids – PUFA (eicosapentaenoic and/or docosahexaenoic acid, EPA or DHA) to cancer patients, assigning a possible action of these fatty acids for the inhibition and resolving of the inflammation, and based on that, the use of these fatty acids to support cancer treatment [8–12] (For understanding the action of n-3 PUFA on inflammation see [13–15]).

In the last years, some systematic reviews of the literature were performed in order to gather evidence and verify the efficacy of n-3 PUFA in altering the concentration of inflammatory mediators/markers in different clinical situations. There is some inconclusive evidence, that n-3 PUFA decreases the concentrations of inflammatory mediators/markers in: chronic heart failure; pancreatitis; chronic renal disease; critical illness (sepsis); and, Alzheimer's disease [16,17]. In cardiovascular disease, the results of studies are controversial and inconclusive, while in healthy subjects, the evidence suggests that there is not reduction in the inflammation with the use of n-3 PUFA [17,18].

Thus, this meta-analysis was conducted in order to verify the action of the n-3 PUFA (Intervention) on different inflammatory mediators (cytokines and acute phase proteins) (Outcomes) in colorectal cancer patients (Population), which is one of the five main types of cancer responsible for the death of people worldwide, besides being a clinical model of tumor that present a close association with inflammation [19]. Secondary objectives include verify if some clinical variables of the study patients as well as, if some supplementation protocol characteristics may influence the anti-inflammatory action of n-3 PUFA.

2. Methods

The method of this systematic review and meta-analysis was prepared in accordance with the PRISMA statement (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) [20].

2.1. Search strategy

Systematic research of trials published until September, 2014 was conducted on MEDLINE (via PubMed; National Library of Medicine, Bethesda, Maryland), Science Direct (via Scopus, Elsevier, Philadelphia, USA) and Web of Knowledge (via Web of Science, Thomson Reuters, New York, USA) using the combination of the categories of search terms shown in Table 1.

The search was elaborated according to PICO strategy considering: Patients/population: colorectal cancer patients; Intervention: n-3 PUFA; Comparisons: parallel group did not receive n-n-3 PUFA as intervention; and, Outcomes: cytokines and acute phase proteins. The search in databases was done using Boolean operators (OR and AND), parentheses, quotation marks and asterisks. Quotation marks were used to search for exact terms or expressions; parentheses were used to indicate a group of search terms or combine two or more groups of search terms enabling all possible combinations of sentences; asterisks (*) or cipher symbol ($) were used to search all words derived of the precedent inflected part. Any filters to refine search were not added. Additionally, reference lists of all identified studies and important reviews about the theme were hand-searched for relevant trials.

The searches were conducted in the online database and the results exported to the reference manager software EndNote® version X7 (Thomson Reuters, New York, USA).

2.2. Selection criteria

Titles and abstracts of the articles, and when clear information was not presented, the full text, were reviewed in order to choose those which were eligible. The eligibility criteria were: controlled or randomized clinical trials performed in humans; use of n-3 polyunsaturated fatty acids as intervention, isolated or added in dietary formulas or as lipid emulsion; sample composed of subjects with over 18 years of age and, only affected by malignant colorectal neoplasm; and those trials that had assessed the cytokines or acute phase proteins levels *in vivo*.

Trials that did not meet the inclusion criteria, duplicated or triplicated publications from the same trial, as well as, trials that were originally published in languages other than English, Spanish or Portuguese, were excluded.

2.3. Data extraction

Data were extracted from eligible articles independently by two reviewers and cross-checked. Articles were consulted again in case of divergence of opinions. For qualitative synthesis, the following data were extracted: locality, methodological characteristics (study design, blinding, randomization technique), patient characteristics (mean age, Body Mass Index -BMI, cancer stage), sample size enrolled, anti-cancer treatment adopted, intervention characteristics (formulation, dose, duration of supplementation and route of administration), and, proportion of loss to follow-up. Outcomes data extracted were mean and standard deviation of the inflammatory mediators/markers (cytokines and acute phase proteins). For the presentation of results, we consider the biomarker concentration at the final moment of supplementation or at any other moment during the supplementation period, which could be significantly lower or higher than control.

Quantitative analysis of the selected trials was performed for interleukins 6 and 1β (pg/mL), tumor necrosis factor – TNF (pg/mL), C-reactive protein – CRP (mg/L), albumin (g/dL) and CRP/Albumin ratio (inflammatory and nutritional risk of complications [21]). For

Table 1
Groups of search terms (PICO strategy) used for search strategy.

PICO's criteria	Descriptions and *search terms* used for each criteria
Patient/population	Patients with colorectal cancer *((cancer OR neoplasm OR tumor) AND (colorectal OR colon OR rectal))*
Intervention	n-3 polyunsaturated fatty acids (EPA and DHA) *("fish oil" OR "n-3 polyunsaturated fatty acids" OR "Omega-3" OR "eicosapentaenoic acid" OR EPA OR "docosahexaenoic acid" OR DHA OR "linolenic acid" OR "polyunsaturated fatty acids" OR Immunonutrition)*
Comparisons	Parallel group did not receive n-3 polyunsaturated fatty acids *("controlled clinical trial" OR "randomized clinical trial")*
Outcomes	Inflammatory markers (cytokines and acute phase proteins) *(cytokine OR interleukin OR "C-reactive protein" OR CRP OR "tumor necrosis factor" OR TNF OR albumin OR "inflammatory markers" OR inflammation OR "acute phase protein")*

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trials that did not present the mean and standard deviation values for any outcome of interest, the corresponding authors were contacted to request these values, and only articles from authors who provided these data were included. From trials that presented the mean and their respective standard deviation in graphic format, the values were estimated by inspection of their spatial distribution in the graphic area.

The data were organized in a Microsoft Office Excel® 2013 document (Microsoft Corporation, Washington, USA).

2.4. Validity assessment

An evaluation of quality was conducted by two independent reviewers, according to Cochrane Collaboration's tool for assessing quality and risk of bias [22] and CONSORT-based checklist [23]. The first tool analyzes the risk of occurrence of the six domains of bias: selection bias, performance bias, detection bias, attrition bias, reporting bias and other bias. Within each domain, an independent judgment by the two reviewers of high, low or unclear risk of bias was made. If insufficient details were reported of what was performed in the study, a judgment of unclear risk was made. If what was done was clearly stated, a judgment of high or low risk was made.

The CONSORT-based checklist consists of 37 items that could be characterized as "yes" if it was clearly and adequately reported or "no" if it was partially reported, unclear, or not reported at all. Each "yes" answer received a score of "1" and each "no" answer was scored as "0". The overall quality scoring of the trial was calculated as a proportion of the "yes" rated applicable items (possible range 0–37 points).

Disagreements between authors in assigning methodological quality scores were resolved by discussion after consulting the conflicting information in the article, until consensus was reached.

2.5. Statistical analysis

All statistical analyses were performed with the use of Data Analysis and Statistical Software – STATA, version 11.0 (StataCorp, Texas, USA).

Individual analysis was performed for each inflammatory mediator/marker. Pooled and stratified analyses were made. The trials were primary stratified in two categories: surgical or chemotherapy trials. The only trial [24] that n-3 PUFA supplementation occurred in follow-up was excluded from the stratified analyses. Secondary stratification included the route of supplementation and it was performed only in surgical trials.

Analyses were performed considering only the data presented in the trials, without any imputation of additional data. We assessed the heterogeneity among the trials included in each analysis by: 1) visually inspecting the forest plots to detect those trials with very disparate SMD and confidence interval of others; 2) performing a Chi^2 test ($p<0.1$ was considered statistically significant); and, 3) performing an I-square test (I^2) (value higher than 50% indicated a substantial heterogeneity). Publication bias were not assessed because the meta-analysis included few trials.

We used the random effects meta-analysis package from STATA with the inverse-variance method to test the significance of the analysis because the trials included were clinically heterogeneous (different countries, doses of n-3 PUFA, duration of supplementation, concurrent treatment anti-cancer and other characteristics). The standardized mean difference – SMD (effect size) and corresponding 95% confidence intervals (CI) were calculated. We adopted the p value < 0.05 for statistical significance of the test and SMD values of 0.2, 0.5 and 0.8 to represent a small, moderate or large effect sizes, respectively [25]. Despite being a group of similar outcomes, we choose to consider the results of the individual analysis for each marker, and therefore, we do not orient the adoption of the lower p value than 0.05.

3. Results

3.1. Search results

From 1446 identified reports, 436 were excluded for being duplicated or triplicated. Thus, 1011 reports were included on initial review (title and abstract review). 965 reports were excluded by presenting titles that seemed irrelevant, and 24 were excluded after abstract review. The full-text articles of the remaining 22 reports were retrieved and reviewed. From these 22, 13 were excluded because they did not have a parallel control group (n = 1) or n-3 PUFA as intervention (n = 1); also because of full text was published in Japanese language (n = 1); lack of inflammatory outcomes (n = 1); or because the sample was composed by subjects with other cancer types (n = 6) (Fig. 1). Three trials were published only as abstract. Despite nominally meeting inclusion criteria, they did not contain sufficient data for qualitative and quantitative analysis, and due to this cause, they were also excluded.

3.2. Overview of trial characteristics

The key characteristics of the nine included trials are summarized in Tables 2 and 3. Four trials were conducted in European countries [24,26–28], two in China [29,30], one in Japan [31], and the other two in Brazil [32,33]. Most trials (n = 7) were published in the last eight years, while one was published in the 1990s [24] (this trial was the first documentation of such cytokine changes *in vivo* in patients with cancer). All trials were parallel designs. One [26] was conducted with four groups (intervention in perioperative; the same intervention in postoperative; control with placebo; and

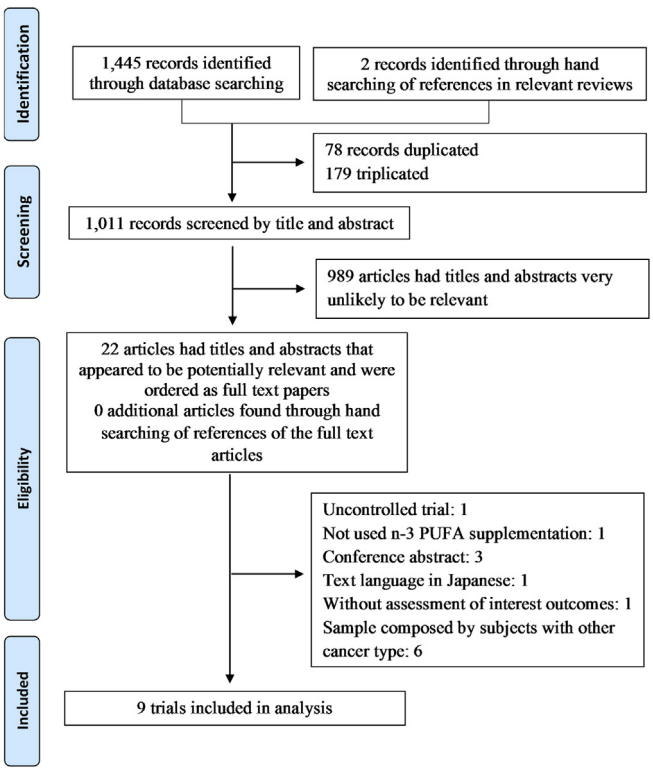

Fig. 1. Flow chart of the literature search, screening, and selection process for controlled clinical trials.

Table 2
Basic characteristics of the study design of included clinical trials.

Trial (year)	Country	Design	Blinding	Randomization	EPA + DHA, n	Control, n	Mean age, y
Braga et al. (2002)[a] [26]	Italy	Parallel	NR	Computer program	50	50	I: 60.5 C: 61.8
Horie et al. (2006) [31]	Japan	Parallel	NR	NR	33	34	I: 69[b] C: 63
Liang et al. (2008) [29]	China	Parallel	Double	Computer program	20	21	I: 55.8 C: 59.2
Mocellin et al. (2013) [32]	Brazil	Parallel	NR	Computer program	6	5	I: 55.2 C: 53.6
Pastore-Silva et al. (2012) [33]	Brazil	Parallel	NR	According to even or odd days of the week	10	8	I: 50.1 C: 54.3
Purasiri et al. (1994)[c] [24]	United Kingdom	Parallel	NR	NR	14	6	63.0
Sorensen et al. (2014) [27]	Denmark	Parallel	Double	Sealed non-transparent envelopes	74	74	I: 69 C: 71
Zhu et al. (2012) [30]	China	Parallel	Double	Computer program	29	28	I: 69.9 C: 70.8
Trabal et al. (2010) [28]	Spain	Paralel	No	Block randomization	6	7	I: 61.5 C: 68.2

Abbreviations: NR – Not reported; I – Intervention group; C – Control group; FA – Fatty acids.

[a] The trial protocol contained four groups (intervention in perioperative; intervention in postoperative; control with placebo; and control without placebo), each one with 50 individuals. For our analysis the control group that did not receive a control diet was not considered. The results for intervention groups were similar. The analysis of serum IL-6 levels, was performed with the first ten individuals enrolled into each group.

[b] significant difference ($p < 0.05$).

[c] This trial contained three groups (patients with clinically localized CRC that received intervention in preoperative period; patients with advanced CRC that received intervention in follow-up; and patients with advanced CRC that no received interventions). For this review, only groups with advanced CRC, were considered, since the group with localized CRC did not have a control.

control without placebo), and another [24] with three groups (patients with clinically localized CRC that received intervention in preoperative period; patients with advanced CRC that received intervention in follow-up; and control patients with advanced CRC that did not receive interventions in follow-up). In three trials [27,29,30], patients and investigators were blinded, and only two [24,31] did not report the technique used for allocation of the patients in the study groups.

Five trials [26,27,29–31] used a control diet or placebo, while the remaining trials did not use any type of control diet. In total, the trials treated 242 patients with n-3 PUFA (range 6–74 patients per trial), and 233 patients with placebo/control diet or without (range 5–74 patients per trial). The median age in the intervention group was 61.6 years (range 50.1–69.9 years) and, in control group, 62.8 years (range 53.6–71 years). Most trials (n = 8) were conducted with adults and elderly patients, while one [30] was conducted only with patients > 65 years old. Two trials [24,28] enrolled only patients with advanced colorectal cancer (IV stage). In four trials [27,28,32,33], the average of BMI was >25 kg/m^2 in both study groups, indicating overweight.

Concomitant anti-cancer treatment was surgery in five trials [26,27,29–31] and chemotherapy in three trials [28,32,33]. For trials conducted in surgical patients, n-3 PUFA supplementation occurred in five preoperative days [26,31] or seven postoperative days [29,30], or 9–14 perioperative days [26,27]. In the trials with patients undergoing chemotherapy, the patients were supplemented with n-3 PUFA for 9 weeks [32,33] or 12 weeks [28]. Oral intake of the n-3 PUFA occurred in five trials [24,27,28,32,33], while, in two trials [29,30], n-3 PUFA was administered parenterally, in one [31], was enterally, and other [26], was orally at pre-operative and enterally at post-operative period.

n-3 PUFA dosage lower than 2 g/day occurred in two trials [32,33]. Median daily dose was 2.2 g (range 0.6–4.8 per day). In two trials [29,30], the dosage was dependent to the patients' weight, and in this case it was not possible to determine the ingested amount of the n-3 PUFA. In another trial [24], the dose was increased gradually: 2.4 g/d in the first 15 days to 3.6 g/d in the 16–30 days and to 4.8 g in the 31–182 days of the supplementation protocol. Supplement compliance was assessed in six trials. The methods adopted to determine the supplementation protocol compliance were: daily consumption register performed by the patient [32,33]; and assessment of supplementation formulas constituents (i.e., EPA, DHA or arginine) in plasma or serum blood [24,26,32] or in phospholipids of granulocytes [27].

Fish oil was used in eight trials as a vehicle formula of the n-3 PUFA: in two of these trials [32,33], fish oil was coated in capsules, in four fish oil was added to the liquid diet [26,27,28,31], and in other two [29,30], it was administered parenterally as lipid emulsion. Isolated n-3 PUFA were used in remaining trials coated in capsule [24]. Antioxidants, arginine and/or nucleotides were administered in the same formula that n-3 PUFA in three trials [26,28,31].

In seven trials, the information about dropouts and withdraws was presented. In one surgical trial, the rate of withdrawal was 12.8% [27], with similar proportion in both study groups. The causes for loss of patients from study were: postoperative death, logistic reasons, refusal, no operation and not taking the formula containing the n-3 PUFA. In another trial, with patients receiving chemotherapy [33], 21.8% of the participants allocated were removed from the study (non-significant difference between groups) due to decision of the patient or because they ingested less than 80% of the recommended amount of fish oil capsules. In the remaining studies, there was no loss after allocation of intervention. Only the first trial used intent-to-treat analysis [27].

Presence or absence of adverse effects was not stated in three trials [24,28,31]. In the six remaining trials, only one [26] presented surgical patients with related symptoms (e.g. abdominal cramping/bloating, diarrhea and vomiting) in all groups in the postoperative period. It was indicated that the possible cause of them was the surgical procedures.

3.3. Quality assessment results

The results of quality assessment by Cochrane Collaboration's tool are presented in Figs. 2 and 3. According to this tool, three trials were completely free of bias [27,29,30]. In all trials attrition or reporting bias were not identified. In one [28], the information was not sufficient to judge it as free of detection bias, and in another

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Table 3
Characteristics of the n-3 PUFA supplementation protocol and results of the inflammatory markers of included clinical trials.

Trial (year)	Patient	EPA + DHA, g/d	Period	Formulation	Route of administration	Dropout, %	Quality score[a]	Results
Braga et al. (2002) [26]	Surgical	3.3	9 perioperative days or 5 preoperative days	Liquid diet enriched with arginine and n-3 FA	Oral on preoperative days and Enteral on postoperative days	0	45	↓ IL-6 POD 1, 4 and 8
Horie et al. (2006) [31]	Surgical	2.5	5 preoperative days	Liquid diet enriched with arginine, n-3 FA and ribonucleic acids	Enteral	0	47	↑ Albumin POD 3
Liang et al. (2008) [29]	Surgical	0.2 g/kg[b]	7 postoperative days	Fish oil	Parenteral	0	48	↔ IL-6 ↔ TNF-α
Mocellin et al. (2013) [32]	Undergoing chemotherapy	0.6	9 weeks	Fish oil	Oral	0	83	↔ IL-1β ↔ TNF-α ↔ IL-10 ↔ IL-17A ↔ Albumin ↓ CRP ↓ CRP/albumin ratio
Pastore-Silva et al. (2012) [33]	Undergoing chemotherapy	0.6	9 weeks	Fish oil	Oral	21.8	59	↔ CRP ↔ Albumin ↔ IL-6 ↔ IL-1β ↔ TNF-α ↔ CRP/albumin ratio
Purasiri et al. (1994) [24]	Follow-up	2.4 − 4.8[d]	6 months	γ-linolenic acid, EPA and DHA isolated	Oral	NR	28	↓ IL-1β ↓ IL-2[c] ↓ IL-4 ↓ Il-6 ↓ TNF-α ↓ IFN-γ
Sorensen et al. (2014) [27]	Surgical	3.0	14 perioperative days	Liquid diet enriched with fish oil	Oral	12.8	88	↔ Albumin POD 4 ↔ CRP POD 4
Zhu et al. (2012) [30]	Surgical	0.2 g/kg[b]	7 postoperative days	Fish oil	Parenteral	0	50	↓ IL-6 ↓ TNF-α
Trabal et al. (2010) [28]	Undergoing chemotherapy	2	12 weeks	Liquid diet enriched with EPA and antioxidants	Oral	NR	52	↔ Albumin

Abbreviations: NR − Not reported; FA − Fatty acids; POD − Postoperative day; ↔ no significant difference between the intervention and control groups after intervention; ↓ − significantly lower than control group after intervention or in the moment specified; ↑ − significantly higher than control group after intervention or in the moment specified.

[a] CONSORT checklist for quality assessment of trials. The presented value correspond to the proportion (%) of "yes" attributed for the applicable requirements.

[b] Dose refer to fish oil and not EPA + DHA.

[c] Concentration of this cytokine was significantly ($p < 0,05$) lower on intervention group than control group at baseline.

[d] These dose corresponds to essentials fatty acids, including γ-linolenic acid, EPA and DHA.

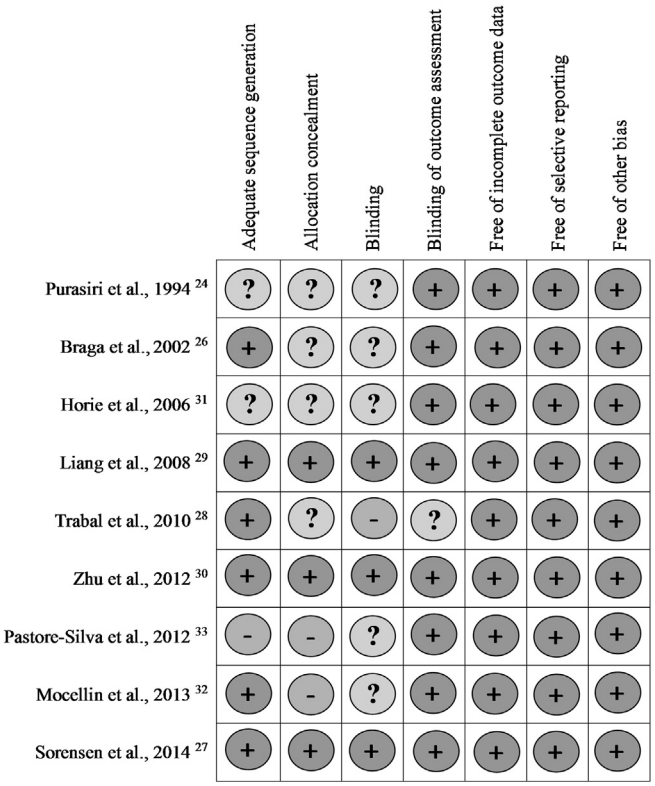

Fig. 2. Summary of risk of bias by Cochrane tools. Legend: (+) Low risk; (−) High risk; (?) Unclear risk.

[33], potential selection bias was detected. Two [32,33] did not have allocation concealment and six [26–30,32] used adequate techniques for sequence generation.

According to the CONSORT checklist, five trials fulfilled 50% or more of the applicable requirements [27,28,30,32,33] (Table 2). One trial identified the trial design in the title. This same trial was the only that presented how the sample size was determined [27]. No trials fulfilled all requirements in their abstract. On the other hand, all presented scientific background and explanation of rationale and specific objectives or hypotheses in the introduction, and the eligibility criteria for participants. Three trials had information about settings and locality where the data were collected [27,32,33]. In the trial of Purasiri et al. [24], analyses between groups were not made to test differences in the cytokine levels during the study protocol. Due to this situation, we performed a new analysis using the initial and final averages with their respective standard deviation using the STATA software to identify differences between time points to the outcomes of interest. The same trial did not present the process of enrollment and allocation for study groups. None of the nine trials mentioned the cause of the study end. All trials accordingly reported the results for primary and secondary endpoints. In only three trials, the limitations or potential bias were discussed [27,28,32]. Generalizability of the findings was demonstrated in Mocellin's trials [32]. Sources of funding were reported in five trials [24,26,27,32,33] and only two reported the trial registry number [27,32].

3.4. Effect of n-3 PUFA on inflammatory markers

All trials that investigated the effects of n-3 PUFA on IL-6, TNFα, IL-1β, CRP, albumin levels and CRP/albumin ratio were included in the meta-analyses.

The pooled analyses showed a significant reduction in levels of IL-6 (SMD -2.34; 95% CI -4.37, -0.31; $p = 0.024$), while TNF-α, IL-1β and CRP did not change with n-3 PUFA supplementation in CRC (SMD -0.77; 95% CI -2.10, 0.57; $p = 0.260$; SMD -0.52; 95% CI -1.79, 0.74; $p = 0.420$; and SMD -0.48; 95% CI -1.18, 0.21; $p = 0.171$, respectively). On the other hand, albumin levels increased significantly with n-3 PUFA supplementation (SMD 0.31; 95% CI 0.06, 0.56; $p = 0.014$).

Stratified analyses showed no reduction on levels of IL-6 (SMD -1.81; 95% CI -3.95, 0.33; $p = 0.098$) and TNF (SMD -0.46; 95% CI -0.97, 0.06; $p = 0.202$) in surgical trials. The same was observed for TNF (SMD 0.29; 95% CI -0.71, 0.28; $p = 0.546$) and IL-1β (SMD 0.13; 95% CI -0.60, 0.87; $p = 0.732$) in trials in which subjects received 0.6 g/day of n-3 PUFA for 9 weeks on concomitant chemotherapy treatment. However, sub analyses performed with surgical trials that offered 0.2 g/kg of fish oil parenterally at least 7 days on postoperative period showed a significant reduction on IL-6 levels (SMD -0.65; 95% CI -1.06, -0.24; $p = 0.002$), but not TNF-α (SMD -0.46; 95% CI -0.97, 0.06; $p = 0.202$). In trials composed by subjects undergoing chemotherapy, the n-3 PUFA supplementation (0.6 g/day for 9 week) decreased CRP levels (SMD -0.95; 95% CI -1.73, -0.17; $p = 0.017$), and CRP/albumin ratio (SMD -0.95; 95% CI -1.73, -0.18; $p = 0.016$), but did not increase the albumin levels (SMD 0.23; 95% CI -0.39, 0.85; $p = 0.460$). Increase in albumin levels was observed in stratified analysis for surgical trials that offered ≥2.5 g/d of EPA + DHA via oral administration at least five days in the preoperative period (SMD 0.34; 95% CI 0.02, 0.66; $p = 0.038$). All forest plots of the analyses are presented in Fig. 4.

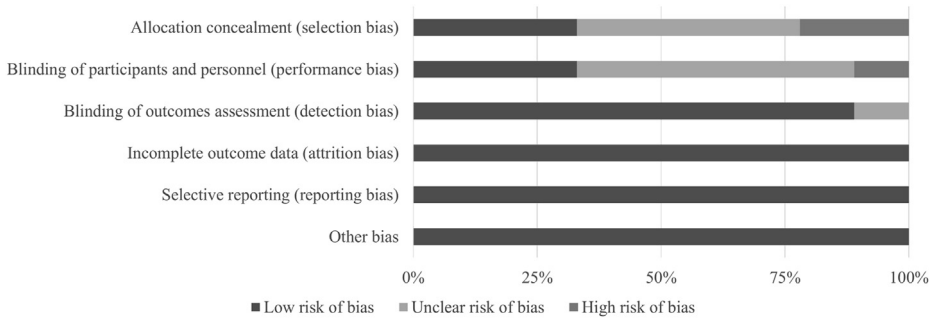

Fig. 3. Proportion of risk of bias detected after quality assessment according to Cochrane tool. Legend: Across trials, information is either from trials at a low risk of bias (dark gray), or from trials at unclear risk of bias (light gray), or from trials at high risk of bias (gray). For each study, every bias domain was checked, the given summary represents an assessment of bias risk across studies. For each bias domain, low risk of bias means that information is from studies at low risk of bias, high risk of bias indicates the proportion of information from studies at high risk of bias which might be sufficient to affect the interpretation of the results, and unclear risk of bias refers to information from studies at low or unclear risk of bias.

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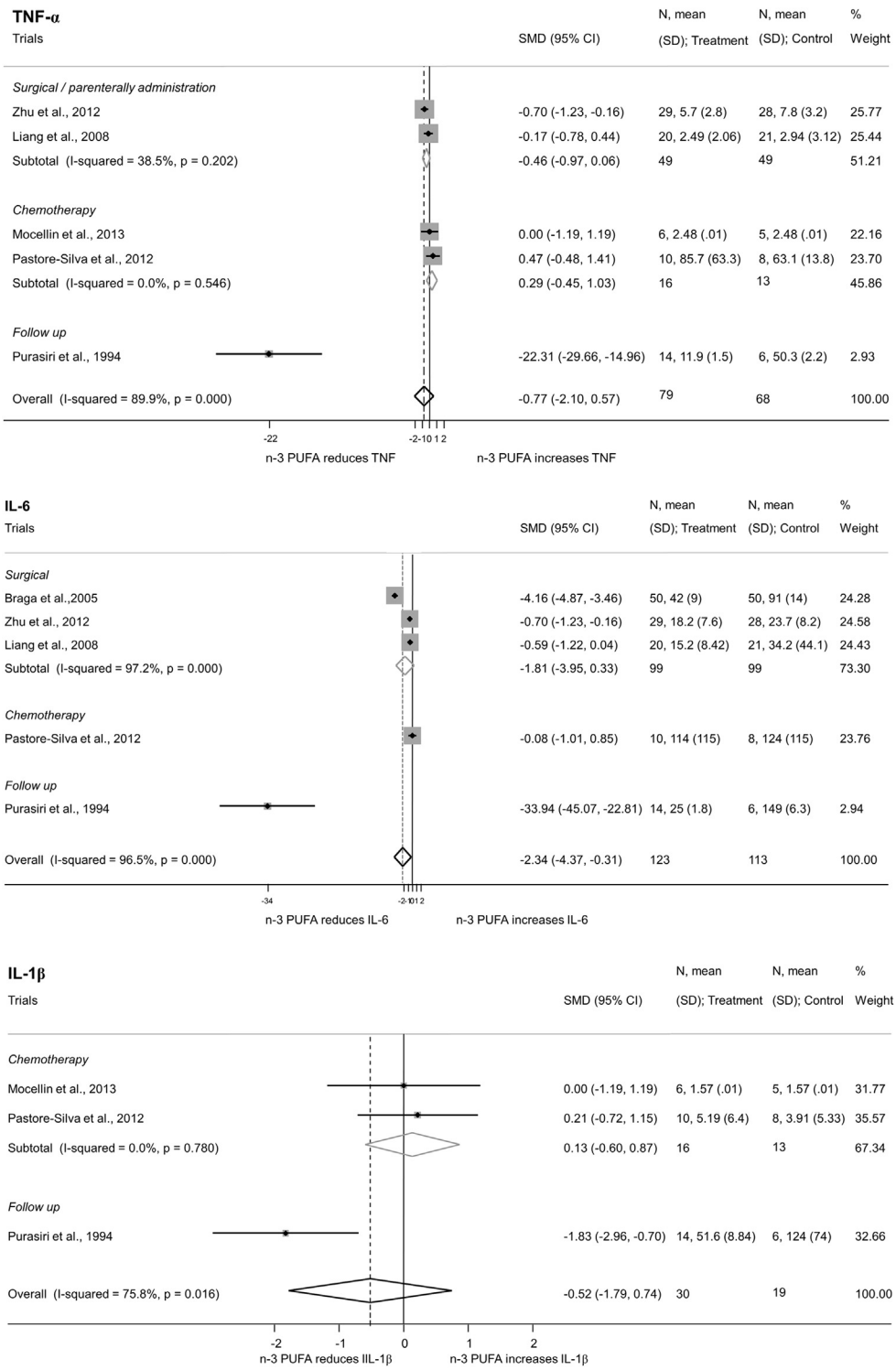

Fig. 4. Forest plot for TNF-α, IL-6, IL-1β, albumin, CRP and CRP/albumin ratio. Legend: Standard mean differences (SMD) between intervention and control groups. Pooled effect size (ES) is indicated by black diamonds; effect size of the stratified analysis is indicated by grey diamonds; percentage weighting of each study towards the overall effect is indicated by the size of grey squares; 95% confidence interval is indicated by horizontal lines; the overall treatment effect lies at the center of the diamond with left and right endpoints indicating the 95% confidence interval (CI). The left side of vertical line of the absence effect favors intervention, and the right side, the control, with exception for albumin.

Heterogeneity ($I^2 > 50\%$) was observed in the pooled analyses for IL-6, TNF-α, IL-1β and CRP, and in the stratified analysis for surgical trials considering IL-6. Despite of possible heterogeneity among them, the exclusion of the trials that were heterogeneity sources did not modify the significance of the analyses.

4. Discussion

In this systematic review of the limited trials currently available, regarding the use of n-3 PUFA in CRC patients, we found nine trials that investigated the effects of this supplementation on circulating

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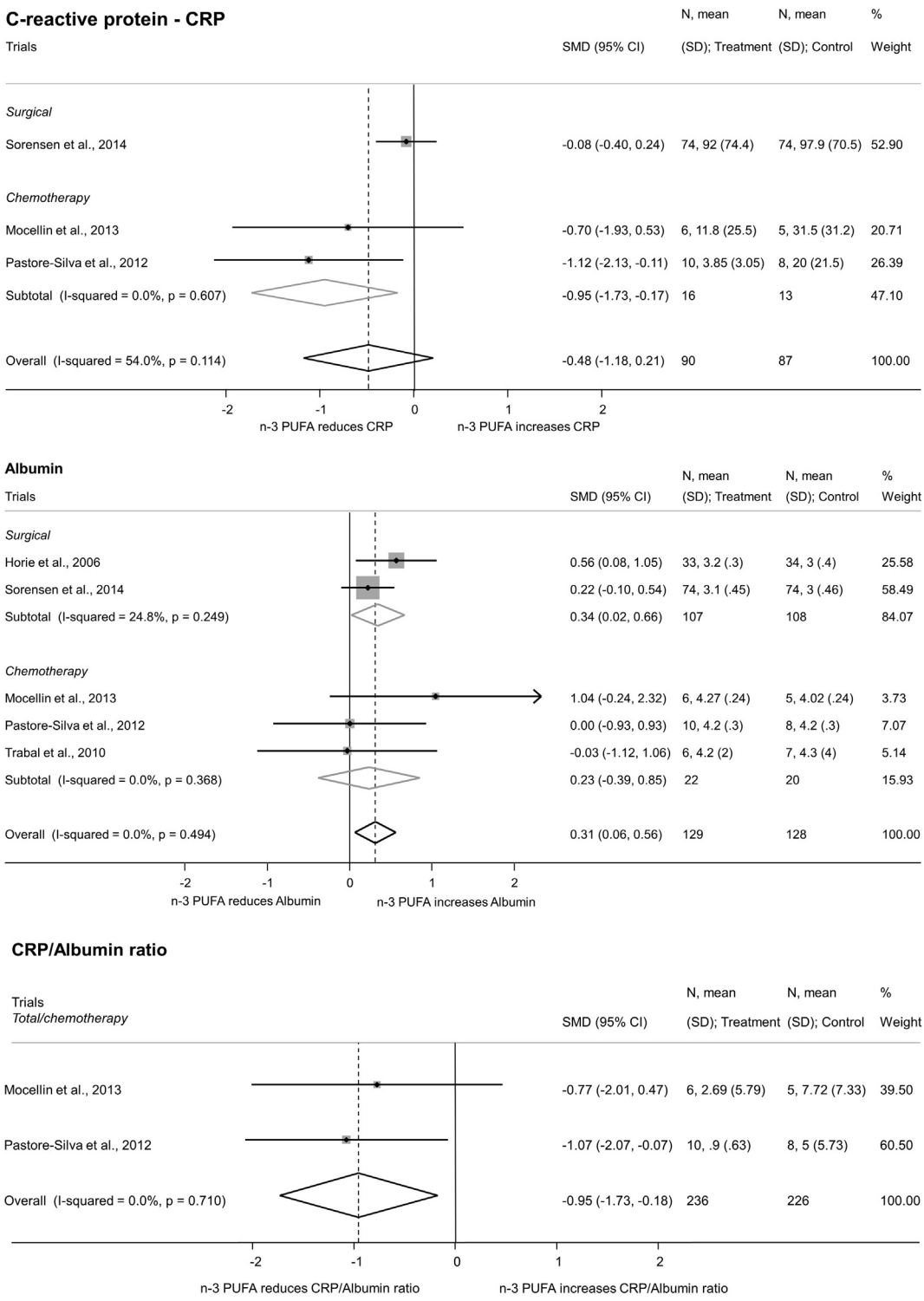

Fig. 4. (*continued*).

levels of inflammatory mediators. Five trials assessed these effects in surgical patients, two in patients undergoing chemotherapy, and one in follow-up patients with advanced cancer. Generally, in five trials, the n-3 PUFA ingestion resulted in improvement in the levels of some inflammatory mediators (cytokines or acute phase protein). Our meta-analysis, shows a reduction in IL-6 and an increase in albumin in pooled analyses with n-3 PUFA supplementation, and no significant changes in TNF-α, IL-1β and CRP concentrations. Subgroup analyses shown a reduction on IL-6 only in surgical patients that received 0.2 g/kg of fish oil parenterally at least seven days at postoperative period and an increase on albumin concentration also in surgical patients that received ≥2.5 g/d of EPA + DHA orally in the preoperative period. Furthermore, in patients undergoing chemotherapy, the supplementation of 0.6 g/d of EPA + DHA orally during 9 week reduces CRP levels and CRP/albumin ratio, but did not improve TNF-α, IL-1β and albumin.

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The sub-analysis that demonstrated increase in albumin concentration after n-3 PUFA supplementation in surgical patients requires caution regarding its interpretation. Despite the fact that the clinical situation is the same, the protocol of n-3 supplementation presented distinct characteristics. In the Horie's trial [31] the formula containing n-3 PUFA was enriched with arginine and nucleotides (immunomodulatory agents [34]), in the Sorensen's trial [27], the formula contained only n-3 PUFA. The period of supplementation was 5 days at preoperative period in the Horie's trials, while in Sorensen's it was 14 perioperative days. The effect of the intervention on albumin concentration in Horie's trial was greater than in the Sorensen's trial, despite the lower dose of EPA and DHA and shorter time of supplementation, indicating that the combination of n-3 PUFA with arginine and nucleotides is better and more effective for improving albumin concentration than n-3 PUFA alone in CRC surgical patients. However, this hypothesis requires further controlled clinical trials in order to verify the effects on inflammation of a formula containing only n-3 PUFA and other enriched with more immunomodulatory agents in addition to n-3 PUFA.

Results of prospective studies demonstrated that fish consumption or n-3 polyunsaturated fatty acids intake from fish oil was inversely associated with incidence of colorectal cancer in the general population [35–37]. n-3 PUFA may protect against colorectal cancer by the same mechanism that explains their anti-inflammatory activity, since colorectal carcinogenesis involves inflammatory pathways [12]. One of the main mechanisms by which n-3 PUFA confer anti-inflammatory properties involves enzymatic pathways (cyclooxygenase – COX, lipoxygenase – LOX and cytochrome-450), which are up-regulated and overexpressed in colorectal cancer [38,39]. COX-2 and 5-LOX use fatty acids present in membrane phospholipids as substrates to release bioactive metabolites (eicosanoids, lipoxins, maresins and protectins) that either contribute to the inflammation (metabolites derived of n-6 PUFA) or to the resolution of the inflammation (metabolites derived of n-3 PUFA). Additional mechanisms involves the modulation of intracellular signaling pathways by n-3 PUFA binding to membrane (GPR120) or intracellular (PPAR-γ) receptors that inhibit the action of pro-inflammatory transcription factors [13,15].

Benefits provided by n-3 PUFA on inflammation depend partly on their incorporation in membrane phospholipids of tumor, immune and structural cells. Findings suggest that this incorporation is time and dose-dependent [13] and appears to reach its peak after 3 weeks of daily intake [12]. Concerning the ideal dose, it has been suggested that a dose $\geq$2 g/day is necessary for providing anti-inflammatory effects [13]. Following this logic, it can be suggest that short supplementation periods require a higher dose of n-3 PUFA for changes in inflammatory status. Meanwhile, for longer periods, a lower dose could potentially provide similar results. In our systematic review, we found that the supplementation protocols that provide 2 g/day or more of n-3 PUFA, even for a short period, were more susceptive for changes at inflammatory mediator concentrations.

In our meta-analyses IL-6, albumin and CRP had their circulating levels significantly altered by n-3 PUFA supplementation in CRC patients or in specific groups of CRC patients, while TNF-α and IL-1β were not affected. The main stimulus for production and release of these cytokines is the activation of NF-κB [40]. If n-3 PUFA inhibits its activation, it would be expected that there would be some reduction on the levels of all these cytokines. However, it was not what we found. It suggests that anti-inflammatory activity of n-3 PUFA, specially its modulation on pro-inflammatory cytokines, involves other mechanisms independent of NF-κB activation. On the other hand, IL-6 is the main stimulus for production of CRP by the liver [41]. In our meta-analysis, we found a reduction on the levels of IL-6 that was not accompanied by a reduction in CRP concentration (overall analysis). Lower means of the CRP were observed in the groups that received n-3 PUFA than control, although in two of three trials included in this analysis, the difference in the CRP concentrations after n-3 PUFA supplementation between the groups was not statistically significant.

There is no difference on the concentration of IL-6 and CRP according to the I to III stages of malignancy [42], but there is a significant increase in the levels of IL-1β, IL-6, IL-8 and TNF-α in the first 12 h after the surgery for resection of colorectal cancer, remaining elevated 72 h after the surgery [43]. This increase seems to be less pronounced than in those patients undergoing chemotherapy. Sex also influences the levels of IL-6 in CRC patients: women with CRC seem to have higher IL-6 than men [42]. Due to these influences on the levels of inflammatory markers, it is necessary to consider sub-analyses by sex and anticancer treatment in meta-analysis like this. It also depends on the cytokine, which is important to be stratified by the stage of malignancy. Thus, to minimize the heterogeneity among included trials in this meta-analysis, we stratified the analysis by anti-cancer treatment, but not by sex, because the trials did not present this sub-analysis. This information is important for the new clinical trials, which must consider a sub-analysis by sex or randomization by sex, to equilibrate the proportion of male and female in the groups of study. n-3 PUFA offered orally or parenterally may have different effects on the outcomes, and must also be considered in the sub-analysis.

A major risk of bias seen in almost half of the studies included the lack of blinding. n-3 fatty acids supplements, especially dietary supplements containing fish oil, frequently possess remarkable sensory characteristics, making it difficult to blind individuals allocated to intervention groups when the route of administration is oral. Many studies use artificially flavored industrialized supplements as a source of n-3 fatty acids with the intention to mask their taste, which usually contains other nutrients besides n-3 PUFA that can potentiates or not their effects, being confounders. Likewise, the use of soybean oil or other oil containing higher proportion of n-6 PUFA as a placebo formula for fish oil can represent a potential confounder in this type of study, considering that n-6 fatty acids can also exert influences on inflammation mediators. Furukawa et al. [44] supplemented patients undergoing esophagectomy with three parenteral formulas: one fat-free (control), another formula containing soybean oil and the other with 1.8 g of EPA plus soybean oil for 21 perioperative days (seven pre and 14 postoperative days). The authors observed that the concentration of IL-6 and CRP were significantly lower on EPA plus soybean group compared to soybean alone, but it did not differ from the control group. These findings suggest that the use of n-6 PUFA as a replacement for n-3 in placebo formulas may direct the production of inflammatory mediators/markers in opposite directions and, therefore, should not be used. In this systematic review, two blinded trials [29,30] used vegetable oil containing n-6 PUFA in placebo formula as a substitute for fish oil and, in only one study, inflammatory mediators/markers concentration were significantly lower in the group that received n-3 PUFA.

Some important information about the methodology, especially regarding randomization, allocation concealment, settings and local of data collection, were superficially or not described in many studies, which directly influenced on quality assessment and risk of bias scores. Considering this, we emphasize the need for more details on methodologies, as well as a discussion of the limitations and potential bias of trials, and the generalizability of the results.

4.1. Strengths and limitations

This meta-analysis was undertaken with the use of a robust design. Effort was made to search for various sources to minimize

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publication bias, and no filters were applied. Only RCTs, with exception of Purasiri's study [24] were included, and investigators sought additional information from authors.

There was significant heterogeneity in many of the reported outcomes, especially in pooled analyses, indicating variation between the studies in the estimates of the effect of n-3 PUFA on the measured outcomes. This can be due to methodological difference of trials, including different formulas used with different dose of EPA and DHA, different duration of consumption and distinct clinical situations wherein supplementation occurs. Small sample sizes enrolled were also considered as a heterogeneity cause. For minimizing heterogeneity among the trials, sub-analyses by clinical situation (surgery or chemotherapy) and route of administration (oral or parenteral) were performed, and the heterogeneity decreased considerably or ceased to exist. Furthermore, we were careful when simulating the analyses by removing the trials that were heterogeneity sources, and the significance of the results was maintained.

In addition, the use of two tools to assess the quality allowed us to assign greater reliability for the presence or absence of bias in included studies. This procedure demonstrated the complete absence of attrition and reporting bias in all included trials.

Potential limitations include the relatively small number of studies and patients included in the overall and stratified analysis, suggesting caution in interpreting the results of the effects of n-3 PUFA supplementation on IL-6, CRP, albumin and CRP/albumin ratio. This meta-analysis included three trials [26,28,31] in which the supplementation of n-3 PUFA was combined with another nutrient with immunomodulatory activity (arginine), probably influencing part of the outcomes. Furthermore, data (mean and standard deviation) for IL-6 were estimated by inspection of the chart area in one trial [26] that presented this outcome in graphical form. We did this not to delete this study the analysis, while avoiding the need for data imputation. In addition, we must consider that the patients enrolled in the trials experienced different stages of malignancy which may have different responses after an immunomodulatory intervention.

5. Conclusion

In conclusion, our systematic review and meta-analysis showed improvement on levels of some inflammatory mediators (reduction in IL-6 and CRP/albumin ratio and increase in albumin) with the use of n-3 PUFA on CRC patients, contributing to the findings of other studies and systematic reviews that demonstrate the anti-inflammatory effects of these fatty acids. These findings have great therapeutic importance and for this reason, it can be adopted as an adjuvant to anti-cancer treatment therapy. However, the benefits of n-3 PUFA on CRC patients are specific to certain supplementation protocols involving duration, dose and route of administration, and they are also concomitant to the anti-cancer treatment adopted. Additionally, well-designed controlled trials are warranted in the future to confirm these effects.

Conflicts of interest

The authors have declared no conflicts of interest.

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