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A Systematic Review of N-3 and N-6 Polyunsaturated Fatty Acid Concentration in Childhood Cancer Patients and Associated Clinical Outcomes

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Abstract

Background: This systematic review evaluated primary research to establish blood omega-3 polyunsaturated fatty acids (n-3 PUFA); eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and omega-6 polyunsaturated fatty acids (n-6 PUFA); arachidonic acid (AA) concentration. The effectiveness of their supplementation on clinical and nutritional outcomes and associations between their concentration and clinical and nutritional outcomes were also evaluated.

Methods: Electronic databases were searched (no restriction-Dec 2018) with no language restrictions. We included studies of cancer patients aged < 18 years and reporting supplementation and/or concentration of EPA, DHA and AA. Evidence was critically appraised employing the CASP tool.

Findings: Three studies (n = 84) met the inclusion criteria, mainly of weak quality and heterogeneous in both study designs and outcomes measured. The overall median(range) n-3 and n-6 PUFA concentration were; EPA: 0.4 (0.24 - 0.4%), DHA: 1.66 (1.3 - 1.68%), AA: 7.01 (6.5 - 7.3%) and AA/EPA: 18.7 (171 - 29.2%). EPA%, DHA% and AA% were all lower than the references 0.45 - 0.77%, 2.22 - 3.76% and 7.91 - 10.46% respectively, whilst AA/EPA% was higher than the reference (< 14.59%). Both higher intake and blood concentration of EPA and DHA may reduce weight loss during initial treatment, whilst a high ratio of AA/EPA may be associated with lower BMI centiles.

Interpretation: EPA and DHA may be beneficial in children with cancer. High-quality population-based longitudinal cohort studies and clinical trials are urgently warranted.

Keywords: *Childhood Cancer; N-3 PUFA; N-6 PUFA*

Abbreviations

AA: Arachidonic Acid; AA/EPA: Ratio of Arachidonic Acid and Eicosapentaenoic Acid; BMI: Body Mass Index; C: Control; CRP: C-Reactive Protein; CS: Cross-Sectional Study; CT: Chemotherapy; CVD: Cardiovascular Disease; DHA: Docosahexaenoic Acid; EPA: Eicosapentaenoic Acid; FFQ: Food Frequency Questionnaire; HDL: High Density Lipoprotein; IQR: Interquartile Range; LC: Long Chain; LCH: Langerhans Cell Histiocytosis; LDL: Low Density Lipoprotein; MUAC: Mid-Upper Arm Circumference; NA: Non-Applicable; N-3 PUFA: Omega-3 Polyunsaturated Fatty Acids; N-6 PUFA: Omega-6 Polyunsaturated Fatty Acids; NR: Non-Reported; NS: Non-Statistically Significant; ONS: Oral

Nutritional Support; PCS: Prospective Cohort Study; RCT: Randomised Controlled Trials; RDI: Recommended Daily Intakes; SD: Standard Deviation; T: Time Of Measurement; Tch: Total Cholesterol; TEI: Total Energy Intake; TSF: Triceps Skinfold Thickness

Background

The survival rates of paediatric cancer have improved considerably in the last 40 years as a result of the implementation of more intensive treatments and the success of medical clinical trials [1]. However, malnutrition, defined as undernutrition, protein energy malnutrition, overnutrition and poor growth [2], is common during [3] and after treatment [4]. Additionally, survivors of childhood cancer are at higher risk of developing long term sequelae, including the metabolic syndrome, cardiac complications and second cancers as well as some anti-immune conditions, which can be all exacerbated by malnutrition. Consequently, attention is shifting to the reduction of treatment related side-effects during and after the completion of therapy [2,5]. Long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFFA), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), may have beneficial effects on this population during treatment as they modulate the metabolic inflammatory response that is associated with both weight loss and muscle wasting in cancer patients [6-8].

These LC n-3 polyunsaturated fatty acids (PUFA), particularly DHA and EPA, and long chain omega-6 polyunsaturated fatty acids (LC n-6 PUFA) have to be supplied from the diet as the body is unable to synthesise them de novo [9].Linoleic acid, the most abundant n-6 PUFA in the western diet, is mainly present in vegetable oils and animal fats and is a precursor of arachidonic acid (AA). The main LC n-3 PUFAs, DHA and EPA, are found in oily fish, seeds and some vegetable oils [10]. Both are essential for health, but have different effects on the body, being either pro-inflammatory (n-6 PUFA) or anti-inflammatory (n-3 PUFA). A balance between n-3 PUFA and n-6 PUFA is necessary for homeostasis of the immune system as well as optimal body and brain development, particularly among younger children [11]. However, excessive intake of n-6 PUFA has been associated with long term inflammation. This is attributed to the synthesis of AA, which is a potent precursor of inflammatory markers. Consequently, excessive intake of n-6 PUFA increases the risk of many chronic diseases, including cardiovascular disease (CVD), cancer and rheumatoid arthritis [9,10], whereas n-3 PUFA (EPA and DHA) may be protective against these conditions. DHA may also prevent cancer development by acting as an inhibitor of malignant cell growth [12,13], whilst excessive AA may promote tumour promotion and progression [12,13]. Therefore, supplementation of DHA and EPA, may be beneficial in patients with cancer.

The aetiology and pathophysiology of malnutrition in paediatric cancer is multifactorial and directly related to the metabolic response of the cancer itself and the intensity of the treatment [2,3]. These factors have dichotomous effects on the immune system; anti-inflammatory and pro-inflammatory, which in turn may lead to either an overall loss of body weight and muscle mass or an increase in fat mass and muscle wasting (Figure 1). For instance, dexamethasone inhibits the immune system by inhibiting the transcription of genes that code for the upregulation of inflammation [14] but it may also interfere with the metabolism of n-3 PUFA by inhibiting the synthesis of EPA and DHA [14], which in turn increases the ratio of AA to DHA (and AA to EPA) [15]. This therefore increases inflammation [15] and consequently causes protein energy malnutrition with or without obesity [16].

Given that most children and adolescents treated for cancer now survive into adulthood [17], the emerging evidence of the importance of n-3 PUFA, particularly DHA and EPA, on health [10,18] and the absence of a published systematic review examining this subject, we sought to investigate (i) blood DHA, EPA and AA concentration of paediatric cancer patients at diagnosis, during treatment and at the end of therapy; (ii) the effectiveness of n-3 PUFA supplementation on clinical and nutritional outcomes and (iii) to establish whether there are any associations between n-3 PUFA and n-6 PUFA concentration and clinical and nutritional outcomes.

Methods

Wedesigned a protocol a priori (http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42016035210; registration number: CRD42016035210). The process and reporting of this systematic review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [19].

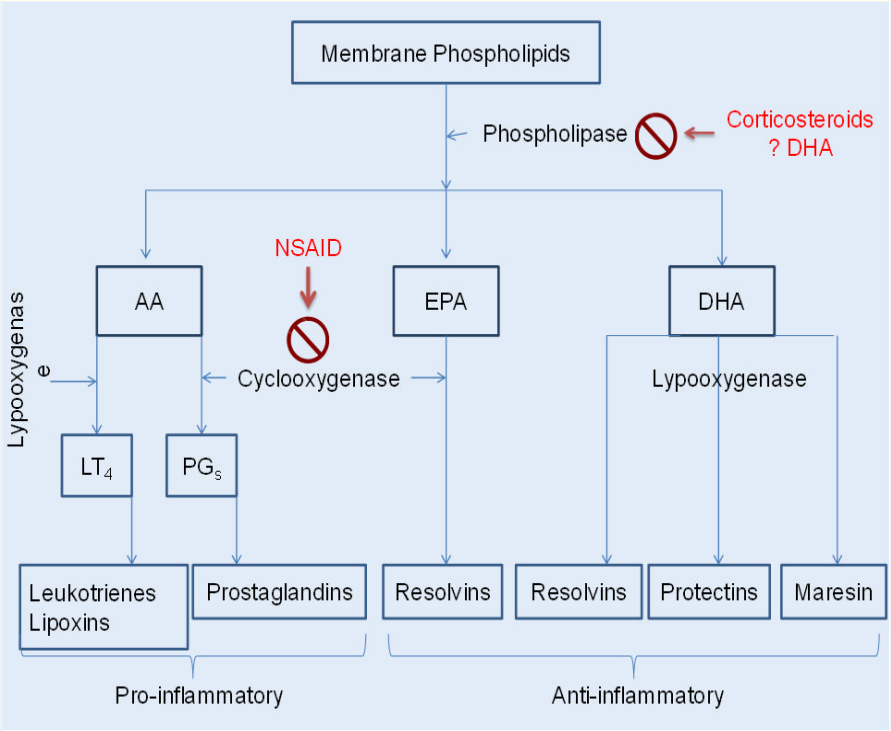

Figure 1: *Effects of Corticosteroids, Non-Steroidal Anti-inflammatory Drugs (NSAID) and DHA on inflammation.*

Outcome data

Our primary outcome was:

- Blood n-3 PUFA (DHA and EPA) and n-6 PUFA concentration in paediatric cancer at diagnosis, during treatment and at the end of therapy.

Our secondary outcomes were:

- Associations between blood n-3 PUFA (DHA and EPA) and n-6 PUFA concentration and nutritional status expressed as Body Mass Index (BMI), weight centile, height centile, mid upper arm circumference (MUAC), triceps skinfold thickness (TSF) or body composition (muscle and fact mass) expressed as a percentage
- Associations between blood n-3 PUFA (DHA and EPA) and n-6 PUFA concentration and any reported clinical and nutritional outcomes
- Effects of n-3 PUFA (DHA and EPA) rich oral nutritional supplements (ONS) on relevant clinical and nutritional outcomes

Eligibility criteria and search strategy

The eligibility criteria included studies investigating children and adolescents, aged <18 years diagnosed with cancer according to the International Classification of Childhood Cancer, third edition (ICCC-3) [20] and with Langerhams Cell Histiocytosis (LCH). To minimise the risk of bias there were no restrictions on dates of publications, follow up period or language. A summary of the PICOS criteria is presented in table 1. Electronic searches were performed (no restriction-Nov 2018) using the Cochrane Library, MEDLINE (via EBSCOhost), CINAHL (via EBSCOhost), PUBMED and Google search to identify systematic reviews, Randomised Controlled Trials (RCT), observational studies, case control studies and letters to the editor. We also examined the reference list of all relevant articles, narrative reviews and

private collections. The search strategy included the following keywords and medical subject heading searches (MeSH): "paediatric"; "cancer*"; "childhood cancer*", childhood tumour*; childhood neoplasm*", neoplasm*, "polyunsaturated fatty acids"; "eicosapentaenoic acid"; "EPA"; "docosahexaenoic acid"; "DHA"; "arachidonic acid"; "fatty acids"; "Omega-3"; "Omega-6"; "n-3 PUFA; "n-6 PUFA". Adaptations for abbreviations, acronyms and British and American English were made for all searches.

Parameter	Description
Population	Children and young people aged less than 18 years. Diagnosed with a cancer included in the International classification of Childhood cancer, third edition (ICCC-3) or with Langerhans cell histiocytosis Treated for cancer
Intervention/Exposure	Cancer treatment including: chemotherapy, radiotherapy, proton therapy, haematopoietic stem cell transplantation and surgery plus chemotherapy and/or radiotherapy. Supplemented with n-3 PUFA (EPA and/or DHA)
Comparison	Supplementation of n-3 PUFA (EPA and/or DHA) v standard nutritional treatment
Outcomes	
Primary	N-3 PUFA (DHA and EPA) and N-6 PUFA whole blood percentage in paediatric cancer at diagnosis, during treatment and at the end of therapy.
Secondary	Studies looking at associations between n-3 PUFA (DHA and EPA) and n-6 PUFA blood percentage and nutritional status. Studies investigating associations between n-3 PUFA (DHA and EPA) and n-6 PUFA blood percentage and any reported clinical outcomes.
Study design	Randomised controlled trials and observational studies (cohort studies, cross-sectional studies and case-control studies).

Table 1: *Summary of PICOS criteria for the inclusion of studies.*

Study selection, quality assessment and data extraction

Titles and abstracts from the combined searches were reviewed by two researchers independently (RRI, LW). In those cases of disagreement, an independent advisor (DW) made the final decision. Evidence was critically appraised independently by two researchers (RRI, LW) employing a standard methodological tool; the Critical Appraisal Skills Programme (CASP). In case of disagreement a third independent reviewer (DW) made the final assessment and a decision was made. The CASP comprises an assessment of: (i) Contextual information including the study objectives, study design and the patient's characteristics; (ii) Potential selection bias including inclusion and exclusion criteria, clear patient selection and an assessment of validity, reliability and accuracy of techniques used; (iii) Outcome measures including reference values; (iv) statistical analyses employed and (v) reporting of results and control for confounding factors. Quality ratings were applied to each study where a quality rating of "strong" meant that the given study met all areas of the CASP criteria, "moderate" when there was one weak area and "weak" when ≥ two weak areas were identified.

For those studies that did not report blood EPA, DHA and AA concentration, we contacted the corresponding author first on two occasions, giving two weeks in between emails to allow for some time to reply. If that failed, we then repeated the process by contacting the last or most senior author and then the second author. If no response was received after these three attempts, the article was included; however, the data did not count towards the final results as this would have been unavailable.

A meta-analysis of selected studies investigating the associations between blood n-3 PUFA (DHA and EPA) and n-6 PUFA concentration with nutritional status and relevant clinical outcomes was planned. For comprehensibility of the results, the median and range blood n-3 PUFA (DHA and EPA) and n-6 PUFA concentration was calculated from all studies. We used the reference ranges recommended by Damsgaard., et al. (2014) [21] to establish EPA, DHA and AA status (deficiency or excessive concentration). Healthy ranges for children are avai-

lable for both boys and girls between the ages of 8-11 years only. This is expressed as a percentage of whole blood total fatty acids. For the purpose of this systematic review reference ranges for both boys and girls have been combined; EPA (0.45 - 0.77%), DHA (2.22 - 3.76%), AA (7.91 - 10.46%) and AA/EPA (< 14.59) [21]. Thus those children who had values below these ranges were classified as "deficient" and those who had values above these ranges were classified as "above healthy range".

Results

Study selection and study characteristics

Three studies met our eligibility criteria [6,22,23] (Figure 2). Of these, one was a RCT [6], one was a prospective cohort study [22] and one was a cross-sectional study [23]. A total of 84 children were included in all studies with individual sample sizes ranging from 12 to 52. Two studies included all paediatric cancer diagnosis [6,22]. and one included patients diagnosed with solid tumours [23]. Two studies were written in English [6,22] and one in Spanish [23] and these were performed in Spain [23], Turkey [6,22], and the United Kingdom [22]. Whole blood n-3 PUFA (EPA and DHA) and n-6 PUFA (AA) were investigated in two studies [22,23] as a primary outcome and one study compared the effects of standard ONS against EPA rich ONS on clinical and nutritional outcomes (weight, height/length, BMI, weight centile, disease status and febrile neutropaenia [6]. One study included children diagnosed with solid tumours only [23], one included children diagnosed with cancer according to ICCC-3 and Langerhans cell histiocytosis [22] and one included children diagnosed with malignancy and treated with chemotherapy [6]. The methods used to analyse n-3 and n-6 PUFA were reported in both studies [22,23].

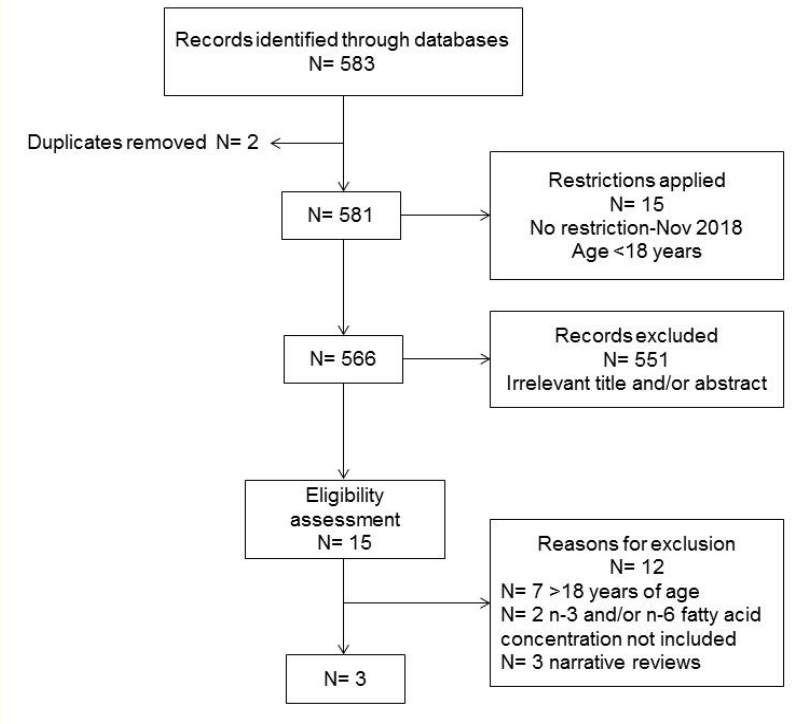

Figure 2: *Flow diagram of studies identified, screened and selected.*

Whole blood n-3 PUFA (EPA and DHA) and n-6 PUFA (AA) status

Two out of three studies [22,23] investigated whole blood n-3 PUFA (EPA and DHA), n-6 PUFA (AA) and AA/EPA concentration and 1/3 reported also the patients' fatty acid status (Table 2) [23]. For comprehensibility of the results, the overall median (range) values (at

diagnosis and during treatment) were calculated from these two studies [22,23] The overall median (range) whole blood n-3 PUFA and n-6 PUFA percentages were; EPA: 0.4 (0.24 - 0.4%), DHA: 1.66 (1.3 - 1.68%), AA: 7.01 (6.5 - 7.3%) and AA/EPA: 18.7 (17.1 - 29.2%). EPA%, DHA% and AA% were all less than the reference ranges 0.45 - 0.77%, 2.22 - 3.76% and 7.91 - 10.46% respectively, whilst whole blood AA/EPA% was higher than the reference range (< 14.59%) [21].

Studies	Quality	Patients diagnosis	N, age at diagnosis (years)	Method/ time of measureents	Method		Results	
					Design	Paramters Outcome Intervention	At diagnosis	During Treatment
de la Torre Aguilar., *et al.* 2012 Spain [23]	Weak	Solid tumours	n = 12 between 0-16 years; mean (± SD) 8.10 (± 5.42) (n = 8 females; n = 4 males) Controls n = 20 age, gender NR	T: At least following one CT cycle before the study M: Assay chromatography gas liquid (Lepage and Roy 1986) Assay coefficient of variation NR	CS	weight, height, 24h dietary recall and FFQ biochemical parameters: total protein, albumin, prealbumin, CRP, TCh, LDL, HDL, apolipoprotein (Apo A-1, Apo B), urea, iron, ferritin, transferritin, insulin Fatty acid profile including AA, EPA, DHA and AA/EPA	NA	No child (solid tumour group) was undernourished (under 3rd percentile); n = 1 was obese (>97th percentile) Protein intake was below the RDI (11.25 ± 4.61) v 15-30g/day (p<0.01). Lipids, carbohydrates and TEI were within range Only CRP (8.94 ± 7.62mg/L) and ferritin (1254 ± 1196 mg/ml) were significantly higher than the reference Prevalence of deficiency NR EPA was significantly lower in the solid tumour group compared with the control group (median ± SD (0.24 ± 0.17% v 0.38 ± 0.20%; p = 0.01)) DHA did not statistically differ between the solid tumour group and the control group (median ± SD (1.66 ± 0.65% v 1.63 ± 0.50%; p>0.05) AA did not statistically differ between the solid tumour group and the control groups (7.01 ± 2.12% v 7.85 ± 1.5; p>0.05) AA/EPA ratio solid tumour v control group (29.2 ± 12.4% v 20.65 ± 7.5%) *

Reuelta Inita*., et al.*.2015 UK [22]	Moder-ate	Paediatric cancer patients (and LCH)	n = 20 median (IQR) age 4.2 (1.5 - 8.5) years; 50% males	T: Baseline, median (IQR) 15.5 (11.0-21.5) days and 6 months, 101 (72.2-160) days M: AA, DHA, EPA –assay FAME (Bell*., et al.* 2011) Assay coefficient of variation NR Reference ranges for AA, DHA, EPA and AA/EPA Damsgaard*., et al.* (2014)	PCS	CRP, AA, DHA, EPA –assay FAME (Bell*., et al.*2011) DHA and EPA% were compared between patients on nutritional support and those who were not	CRP mg/L median (IQR) 4.0(1.0-8.0) DHA (22 6n-3) % median (IQR)1.3 (0.9-1.9) 95% (19/20) were deficient EPA (20 5n-3) % 0.4 (0.3-0.5) % 70% (14/20) deficient AA (20 4n-6) 6.0(5.4-6.8) % 60% (12/20) deficient AA/EPA ratio (%) 17.1 (10.5-22.2) 100% (20/20) high EPA and DHA% did not statistically differ between nutritionally supported and non-nutritionally supported patients	CRP mg/L median (IQR) 1.0(1.0-4.0) DHA (22 6n-3) % median (IQR)1.8 (1.3-2.1) p = 0.001 87.5% (14/16) were deficient EPA (20 5n-3) % 0.4 (0.3-0.6) NS 60% (12/16) were deficient AA (20 4n-6) 7.3 (5.5-8.1) % p = 0.05 50% (8/16) deficient AA/EPA ratio (%) 18.7(13.3-25.0) NS 75% (12/16) high EPA and DHA% did not statistically differ between nutritionally supported and non-nutritionally supported patients

Table 2: *Studies reporting DHA and EPA concentration in paediatric cancer patients.*

**These values have been calculated for this systematic review i.e. they have not been presented in the original article; AA: Arachidonic acid; AA/EPA: CRP: C-Reactive Protein; CS: Cross Sectional Study; Arachidonic Acid/ Eicosapentaenoic Acid Ratio; CT: Chemotherapy; DHA: Docohexanoic Acid; EPA: Eicosapentaenoic Acid; FFQ: Food Frequency Questionnaire; HDL: High Density Lipoprotein; International Classification of Childhood Cancer, 3rd edition; IQR: Interquartile Range; LCH: Langerhans Cell Histiocytosis; LDL: Low Density Lipoprotein; M: Method; NS: Non Statistically Significant; NA: Non-Applicable; NR: Non-Reported; PCS: Prospective Cohort Study; RDI: Recommended Daily Intakes; SD: standard Deviation; T: Time of Measurements; TCh: Total Cholesterol; TEI: Total Energy Intake.*

The prevalence of n-3 PUFA (EPA and DHA) and n-6 PUFA (AA) deficiency was established in one study performed in Scotland at baseline and following 6 months of treatment [21]. At baseline, 70% of children were deficient in EPA, 95% in DHA and 60% were deficient in AA. At 6 months of treatment, 60% were still deficient in EPA, 87.5% in DHA and 50% in AA. In contrast, AA/EPA ratio was above reference ranges in 100% of children at baseline and in 75% of children at 6 months.

In order to establish whole blood n-3 PUFA and n-6 PUFA at different time points, we stratified results from the studies by time points (diagnosis, during treatment and after the completion of therapy) and then calculated the median and range (Table 3). No study reported results at three time points or after the completion of therapy. One study reported results at two time points; baseline (median length of time between diagnosis and measurement 15.5 days) and 6 months post diagnosis [22] and one study reported results at only one time point: during treatment [23] (Table 2 and 3). EPA, DHA and AA% were all lower than the reference ranges at both time points, whilst EPA/AA was above the reference range also at both time points.

	At diagnosis			During treatment			After treatment		
	N of studies	Median	Range	N of studies	Median	Range	N of studies	Median	Range
EPA%	1	0.40	0.30 - 0.50	2	0.32	0.24 - 0.40	0	-	-
DHA%	1	1.30	0.90 - 1.90	2	1.67	1.66 - 1.68	0	-	-
AA%	1	6.00	5.40 - 6.80	2	7.15	7.01 - 7.30	0	-	-
AA/EPA%	1	17.10	10.50 - 22.20	2	23.95	18.70 - 29.20	0	-	-

Table 3: *Whole blood n-3 PUFA (EPA and DHA) and n-6 PUFA (AA) percentages at different stages of paediatric cancer treatment. Reference ranges for EPA: 0.45 - 0.77%; DHA: 2.22 - 3.76%; AA: 7.91 - 10.46 and AA/EPA: < 14.59 (Damsgaard., et al. 2014).*

Additionally, one study [22,23] compared n-3 PUFA (EPA and DHA) and n-6 PUFA (AA) with a matched healthy control. EPA (median ± SD: 0.24 ± 0.17% v 0.38 ± 0.20%; $p = 0.01$) was significantly lower in the solid tumour than in the control group, whilst DHA (median ± SD: 1.66 ± 0.65% v 1.63 ± 0.50%; $p > 0.05$)) and AA (median ± SD: 7.01 ± 2.12% v 7.85 ± 1.5; $p > 0.05$) did not statistically differ between the two groups (Table 2).

N-3 PUFA (EPA and DHA) and n-6 PUFA (AA) and clinical outcomes

Two of the three studies [6,22]. investigated n-3 PUFA and n-6 PUFA and clinical outcomes (Table 4). The following clinical outcomes were measured: weight loss, weight percentile, height, BMI, BMI percentile, disease status, febrile neutropaenia, lipid peroxidation (inflammatory marker) and remission rates. It was not possible to perform a meta-analysis due to the reduced number of studies and the heterogeneity of the outcomes measured. Therefore, evidence was described narratively. Two studies [6,22] reported data on BMI; of these one study [6] showed that those children supplemented with EPA rich ONS had a significantly lower reduction in BMI than those supplemented with standard nutritional care at 3 months. The other study [22] reported no correlation between whole blood EPA% and BMI; however, the same study reported a positive strong correlation between whole blood DHA% and BMI at baseline. Neither of the studies [6,22] found these effects following 6 months of treatment.

One study showed that patients supplemented with EPA rich ONS had fewer rates of both weight loss and remission rates when these were compared with children treated with standard ONS at 3 and 6 months post diagnosis [6]. Higher whole blood AA% was associated with higher lipid peroxidation, a marker of inflammation, in one study [22]. There was no difference reported in febrile neutropaenia rate between those children who received rich EPA ONS and those who received standard care [6].

One study investigated whether there was any difference in whole blood n-3 PUFA (EPA and DHA) and n-6 PUFA (AA) concentration between children treated with nutritional support and those who did not received any treatment [22]. No statistical significant differences were found in whole blood EPA and DHA% between the two groups at neither baseline or 6 months.

Studies	Quality	Patients diagnosis	N, age at diagnosis (years)	Time of measurements Method Intervention Control	Methods			Results
					Design	Variables	Outcome	Associations between n-3 PUFA (EPA and DHA) and n-6 PUFA (AA) and clinical outcomes
Bayram., *et al.* 2009 Turkey [6]	Weak	Paediatric malig-nancy and receiving CT	n = 52 mean (±SD): 7.5 (±3.0)	T: Diagnosis, once per month for 3 and 6 months I:2 x ONS pro-tein and energy dense EPA (Pro-SureTM, Abbot) 1.09g EPA per day C: usual dietary care, ONS 240 ml (300kcal, 16g protein):	RCT	Paediatric cancer Intensive chemo-therapy Age Gender	Primary outcome: weight loss Second-ary out-comes: height BMI weight per entile disease status and febrile neutro-paenia Remis-sion ates	At 3 months (n = 52) Fewer patients showed weight loss (6.1% vs 47.6%; p = 0.001), BMI (12.1% vs 52.6%; p = 0.002) and negative deviation in weight centile (6.1% vs 31.6%; p = 0.02) in the intervention group vs control group At 6 months (n = 23) Weight loss was significantly lower (6.7% vs 50%; p = 0.03) in the intervention group vs control group. There was not significant group differences in loss of BMI or negative deviation from weight percentiles (p > 0.05) and in cases of febrile neutropaenia Remission rates were greater in the treatment vs control group (87.9 vs 63.2%)
Revuelta Iniesta., *et al.* 2015 UK [22]	Moder-ate	Paediatric cancer patients, ICCC-3 (and LCH)	n = 20 median (IQR) age 4.2 (1.5-.5) years; 50% males	T: Baseline, median (IQR) 15.5 (11.0-21.5) days and 6 months, 101 (72.2-160) days M: AA, DHA, EPA –assay FAME (Bell., *et al.* 2011) Lipid peroxida-tion: TBARS assay kit (Cayman) Reference rang-es for AA, DHA, EPA and AA/EPA Damsgaard ., *et al.*. (2014)	PCS	Whole blood percent-age EPA DHA, AA, AA/EPA	BMI centile at baseline and at 6 months Lipid peroxida-tion	Whole blood DHA positively correlated with BMI centile at baseline (r = 0.9; very strong; p < 0.001) at baseline only AA negatively correlated with BMI centile (r = -0.5; moderate; p = 0.04) and positively correlated with lipid peroxidation (r = 0.8; very strong; p<0.001) at baseline onlyThere was not correlation between EPA and BMI centile at neither at baseline or 6 months

Table 4: *Associations between n-3 PUFA (EPA and DHA) and n-6 PUFA (AA) and clinical outcomes.*

AA: Arachidonic Acid; AA/EPA: Arachidonic Acid/ Eicosapentaenoic Acid Ratio; BMI: Body Mass Index; C: Control; CT: Chemotherapy; DHA: Docohexanoic Acid; EPA: Eicosapentaenoic Acid; FFQ: Food Frequency Questionnaire; HDL: High Density Lipoprotein; I: Intervention; ICCC-3: International Classification of Childhood Cancer, 3rd edition; IQR: Interquartile Range; LCH: Langerhams Cell Histiocytosis; LDL: Low Density Lipoprotein; M: Method; n-3 PUFA: Omega-3 Polyunsaturated Fatty Acids; n-6 PUFA: Omega-6 Polyunsaturated Fatty Acids; NS: Non Statistically Significant; NA: Non-Applicable; NR: Non-Reported; PCS: Prospective Cohort Study; RCT: Randomised Control Trial; SD: Standard Deviation; T: Time of Measurements; TCh: Total Cholesterol.

Risk of bias

According to the assessment of quality of evidence using CASP methods, the evidence for the whole blood n-3 PUFA (EPA and DHA) and n-6 PUFA (AA) concentration, status and associations with clinical outcomes in paediatric cancer patients was mainly of poor quality. The main issues identified with studies scored as "weak" (n = 2) and/or "moderate" (n=1) were as follows:

- The sample size was very small in all studies.
- The precision of the results was difficult to establish due to the absence of confidence intervals and relative risk [6,23].
- Confounding factors were not taken into consideration in the design and in the analysis of the data [6,22,23].
- Exposure bias, such as dietary intake and nutritional treatment, was not taken into consideration [6].
- Not all outcomes described in the methods were reported as results [6,23] making it impossible to report some clinical outcomes highlighted in the studies.

Despite this, all selected studies were included in order to help draw some general conclusions.

Discussion

This is the first systematic review appraising international evidence of the prevalence of blood n-3 PUFA (EPA and DHA) and n-6 PUFA (AA) status in paediatric cancer patients. We also investigated associations between fatty acid profile (EPA, DHA and AA) and clinical and nutritional outcomes as well as the effectiveness of n-3 PUFA supplementation. As we only found 3 studies of small sample sizes and mainly of poor quality, at present there is insufficient evidence to accurately determine the prevalence of n-3 PUFA and n-6 PUFA in this population. Moreover, it was impossible to perform a meta-analysis because of the heterogeneity of the study designs and the outcomes measured in the two eligible studies. Despite these limitations, our systematic review raises the possibility of a high prevalence of blood EPA and DHA deficiency and of an excessive AA to EPA ratio, which may appear to be associated with lower BMI centiles and lower remission rates.

Whole blood EPA, DHA and AA concentration and status

Despite having only found two studies of small sample size and ranging from poor [23] to moderate quality [22], both reported very similar blood n-3 PUFA and n-6 PUFA concentration, which are below healthy reference ranges [21-23] at the time of treatment. These were translated into prevalence of deficiency in only one study [22], which ranged between 50% for AA to 87.5% for DHA. Furthermore, the fact that there was only one study reporting blood fatty acid profile at the time of diagnosis made it very difficult to establish longitudinal patterns of change. However, from this single study [22], it appears that these are even lower at diagnosis, which is corroborated by the higher prevalence of deficiency reported at this stage (EPA: 70%; DHA: 95% and AA: 60%). In contrast, the ratio between AA and EPA was higher than the reference range [22,23] at both time points with reported prevalence of 100% at diagnosis and 75% following 6 months of treatment [22].

To our knowledge, no other study, apart from the one included in this review, has reported deficiency rates for n-3 and n-6 PUFA in neither healthy or populations diagnosed with chronic conditions, which may be explained by the lack of published healthy reference ranges [24,25]. and the reported difficulties in their development [24]. Of note and contrary to the findings from this systematic review, a RCT performed in 200 adults diagnosed with pancreatic cancer reported a high concentration of blood EPA in 14% of patients at baseline [26]. however, reference ranges were obtained from a separate population of pancreatic cancer patients, which may have also been deficient, and not from the healthy population. In agreement with our systematic review, evidence from adults diagnosed with advanced malignancies [8,27,28]. has also reported low blood EPA, DHA and AA and a high ratio between AA and EPA. In children, these fatty acid profile anomalies maybe be explained by a deficit in total energy intake, particularly during the initial phases of treatment [29-32] and by a higher consumption of n-6 PUFA, often obtained from processed foods, as opposed to n-3 PUFA [23]. As linoleic acid and α-linolenic acid both compete for the same enzymes to synthesise AA and EPA and DHA, this means that the higher intakes of n-6 PUFA require higher intakes of n-3 PUFA to achieve a beneficial AA and EPA ratio [24].

N-3 PUFA (EPA and DHA) and n-6 PUFA (AA) and clinical outcomes

There is a paucity of evidence examining n-3 PUFA and n-6 PUFA and associated clinical outcomes, as we only found two studies of small sample population and of low [6] and moderate quality [22] making it impossible to draw firm conclusions. Nonetheless, both studies indicated that a higher intake of EPA [6] was associated with both lower weight loss and lower remission rates and that a higher blood

DHA concentration [22] was associated with higher BMI centiles. In contrast blood AA concentration was negatively associated with BMI centile and positively associated with lipid peroxidation at early stages of treatment. These findings are supported by a recent narrative review [33] and a systematic review [9] of adult cancer patients and by studies performed in terminal cachectic cancer patients [8,26]. In contrast a RCT of 200 patients with advanced cancer cachexia found that n-3 PUFA enriched supplementation provided no advantage over standard supplementation with regards to survival duration and weight loss [26]. However, the authors reported compliance issues and undisclosed n-3 PUFA supplementation in both groups as demonstrated by the high EPA concentration at baseline, which may have hampered their results. Reduced blood n-3 PUFA and high AA concentration have both been associated with inflammation in otherwise healthy individuals [10]. however, evidence in cancer patients are equivocal and those inflammatory markers investigated differ in each study. For instance, one study included in this review investigated lipid peroxidation and CRP [22], whilst evidence from adults with advanced cancer have examined cytokines as a measured of inflammatory markers [8]. It is therefore advised that future studies incorporate both inflammatory markers as well as markers of cell damage (lipid peroxidation) to establish whether n-3 PUFA reduces inflammation in this population.

No study has investigated the effects of n-3 PUFA and n-6 PUFA supplementation on either blood fatty acid profile or body composition in paediatric cancer patients. Patients receiving treatment and survivors of childhood cancer are at higher risk of being undernourished and protein energy malnourished with or without obesity. This in turn increases the risk of developing early sarcopenic obesity [32,34]. As n-3 PUFA supplementation may contribute to skeletal muscle preservation possibly by reducing inflammation [33], future research should investigate the effects of n-3 PUFA on body composition in both patients receiving treatment and in survivors of childhood cancer who have also received this nutritional treatment (n-3 PUFA).

Quality of evidence, strengths and limitations of this systematic review

Following the risk of bias assessment using the CASP tool, the main limitations identified in this review relate to the small number of studies published to date, all of which were of weak and moderate quality, the heterogeneity of both the populations studied and the study designs and the different outcomes measured. Consequently, we were unable to perform a meta-analysis. Despite these limitations, the strength of this systematic review lies in its methodology. We conducted a comprehensive search of five electronic databases without language and date restrictions, contacted experts in the field and searched the reference list of the studies we identified. However, it is possible that potential eligible studies may not have been identified. Finally, at present reference ranges for EPA, DHA and AA are only available for children within the ages of 8 to 11 years old [21], which may have under or overestimated the prevalence of deficiency.

Taking into consideration the results from this systematic review, the limited number of studies published to date and the limitations of the studies included, we suggest key recommendations to be considered in future research (See table 5).

- Data from different forms of paediatric cancer should be presented separately as these are different diseases requiring different forms of treatment
- Interventional studies that investigate the effects of rich n-3 PUFA, EPA and DHA on clinical (remission, relapse, death, survival, length of treatment, number of hospital admissions, infections, cognitive function and quality of life) and nutritional outcomes (blood fatty acid profile, BMI and height centiles, weight loss, and body composition) in patients during and after the completion of treatment
- Develop reference ranges for AA, DHA and EPA for children of all ages
- Blood n-3 PUFA and n-6 PUFA should also be assessed at different stages of treatment (before, during and after the completion of treatment) to establish optimal blood concentration in this population
- Blood n-3 PUFA and n-6 PUFA samples should be collected in fasted patients
- In epidemiological studies, healthy reference ranges or a matched healthy control should be used. Those studies that use healthy reference ranges should report blood fatty acid status (deficiency and healthy range), particularly for EPA, DHA and AA
- The following details need to be clearly specified: (i) the timings of when the measurements are taken; (ii) clinical and demographic data; (iii) dietary intake and specific nutritional treatment, including vitamin and mineral supplementation; (iv) treatment induced side-effects (v) and assay's coefficient of variation.

Table 5: Recommendations to consider for the future.

Conclusion

This systematic review represents the first attempt, to date, to review epidemiological and interventional evidence of blood n-3 PUFA and n-6 PUFA concentration and their blood status and the effectiveness of n-3 PUFA supplementation on clinical and nutritional outcomes. Unfortunately, we were unable to make recommendations on the ideal blood n-3 PUFA and n-6 PUFA profile and on optimal n-3 PUFA intakes in paediatric cancer patients. Nonetheless, this systematic review raises the possibility of a high prevalence of EPA and DHA deficiency and that higher blood EPA and/or DHA concentration may prevent weight loss. The most important conclusion is the urgent need for high-quality population based longitudinal cohort studies and for high-quality clinical trials investigating the effectiveness of n-3 PUFA supplementation.

Declarations

Ethics Approval and Consent to Participate

Non-applicable.

Consent for Publication

Non-applicable.

Availability of Data and Material

Non-applicable.

Competing Interest

The authors declare that they have no competing interest.

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Author's Contribution

Raquel Revuelta Iniesta: design of systematic review, first selection of literature, critical appraisal, interpretation of literature, drafting of the article, figures and tables and final approval; Laura.

Wyness

Literature search, first selection of literature, critical revision and final approval; David Wilson: critical appraisal, guidance with manuscript, critical revision and final approval.

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