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Docosahexaenoic acid (DHA) supplementation attenuates changes in the concentration, phenotype, and response of immune peripheral blood cells in breast cancer patients undergoing neoadjuvant therapy. Secondary findings from the DHA-WIN trial

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Abstract

Background Breast cancer neoadjuvant therapy may negatively impact the immune system. As a secondary outcome of the docosahexaenoic acid (DHA) for women with breast cancer in the neoadjuvant setting (DHA-WIN trial), we sought to assess the effects of an intervention with DHA on parameters of immune function of women undergoing neoadjuvant therapy.

Methods Women with early-stage breast cancer in the neoadjuvant setting were recruited for the DHA-WIN trial and randomly assigned to receive either 4.4 g/day of DHA or a placebo for 18 weeks in conjunction with their neoadjuvant chemotherapy for breast cancer. Venous blood was collected to isolate peripheral blood mononuclear cells. Immune parameters were assessed by measuring white blood cell concentration, flow cytometry, and cytokines concentration after mitogen-stimulated immune response.

Results In the placebo group the proportion of T cells (CD3 +), and functionally active monocytes (CD14 + HLA-DR +) was reduced at the last cycle of chemotherapy (15 weeks) but remained constant in the DHA group (*P* interaction < 0.05). The neutrophil-to-lymphocyte ratio (NLR) was maintained in the DHA group but increased in the placebo at the end of chemotherapy (*P*-interaction = 0.02). An increase in this ratio was associated with lower chance of achieving pathological complete response (OR = 0.32, 95% CI [0.14,0.16], *P* = 0.01). After 15 weeks of therapy, the DHA-supplemented group had higher concentrations of stimulated cytokines IL-4, IL-10, and the T helper type 1 cytokine IFN-γ after phytohemagglutinin (PHA) challenge, and higher concentrations of TNF-α and IFN-γ cytokines after lipopolysaccharide exposure (*P* < 0.05).

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Conclusion Supplementing DHA during breast cancer neoadjuvant chemotherapy improved systemic immune function by attenuating changes in blood cell concentrations, preventing depletion of immune cells, and enhancing ex vivo cytokine secretion after stimulation.

Keywords Immune response, Pathological complete response, Long-chain polyunsaturated fatty acid, Cytokines, Immune phenotype

Introduction

Globally, breast cancer is the most diagnosed cancer in women [1]. Despite advances in diagnosis and therapy, breast cancer is estimated to account for 1 in 6 cancer deaths worldwide, representing the leading cause of cancer death in women [2]. Neoadjuvant therapy is the systemic treatment given before surgery to reduce tumour size, increase operability, decrease postoperative morbidity, and triage postoperative adjuvant therapy to improve patient outcomes [3]. Although initially recommended for inoperable and locally advanced breast cancer, neoadjuvant therapy is currently used for patients with operable tumours to increase the chances of breast conserving surgery [3, 4]. Additionally, neoadjuvant therapy allows the assessment of important endpoints that predict long-term survival and informs the next steps of treatment. Pathological complete response (pCR) is among these important outcomes, defined as the absence of invasive disease in the breast tissue and axillary lymph nodes. pCR is considered a surrogate marker of overall survival and disease-free survival in selected breast cancer subtypes (triple negative breast cancer (TNBC) and human epidermal growth factor receptor 2 (HER2) positive) [5]. This has resulted in an increased interest in identifying predictors of pCR to identify patients that would benefit the most from neoadjuvant therapy.

A growing body of evidence supports the essential role of the host's immune system in the outcome of tumour progression or therapeutic response in breast cancer patients [6–8]. Immune cells are recruited to the tumour site through the complex crosstalk between the tumour microenvironment and the periphery [9]. The balance between effector and inhibitory phenotypes of immune cells predicts the elimination or progression of tumour cells [10]. Conventional treatment relies on chemotherapy success, but the dose is often limited by patient tolerance due to the intensity of side effects, which result from toxicity to non-tumour tissues [11]. Chemotherapy-induced tissue damage is associated with tumour-promoting inflammation, which can lead to increased survival and proliferation of tumour cells in sites distant to the breast [12]. Consequently, there is a complex balance between the beneficial and adverse effects of neoadjuvant chemotherapy.

There is rising interest in assessing peripheral blood immune markers that are correlated with clinical outcomes due to their advantages of being easily and sometimes routinely measured during clinical care compared to invasive procedures. Studies have associated levels of systemic immune biomarkers with the achievement of pCR [13, 14]. The neutrophil-to-lymphocyte ratio (NLR) is an emerging biomarker assessed in the peripheral blood. NLR exhibits an inverse relationship with the achievement of pCR and overall survival, whereas higher NLR are associated with poor survival outcomes [15]. However, the literature still presents inconsistencies regarding these findings, challenging the identification and utility of quantifying these systemic markers.

Docosahexaenoic acid (DHA) is an essential omega-3 long-chain polyunsaturated fatty acid that has been investigated during the treatment of various types of cancer due to its pleiotropic effects [16]. Several preclinical trials have demonstrated the efficacy of a diet high in DHA combined with chemotherapy drugs in breast cancer rodent models to mitigate side effects and improve outcomes [17–19]. Newell et al. [19] previously demonstrated an improvement in chemotherapy success, measured as reduced tumor weight and Ki67 activity (proliferation) in animals fed DHA (3.9% w/w of total fat) in combination with chemotherapy (docetaxel) in a patient-derived xenografts (PDXs) model of TNBC. This treatment regime was compared to chemotherapy alone in animals fed a control diet.

Despite the limited number of interventional studies, clinical evidence suggests that DHA supplementation effectively increases DHA incorporation in breast adipose tissue [20] and, when "highly incorporated" into plasma phospholipids, is associated with improved overall survival in metastatic breast cancer [21]. To date, no clinical trials have evaluated the impact of DHA supplementation on immune parameters during early breast cancer therapy. The purpose of this research was to evaluate the effects of chemotherapy alone and in combination with DHA supplementation on circulating peripheral blood immune cells during neoadjuvant chemotherapy. Moreover, we investigated these effects on changes in the concentration of blood immune cells, immune cell types, and the immune response. Additionally, we investigated if there was a relationship between

clinic-pathological factors and changes in immune function. We hypothesized that supplementing DHA during neoadjuvant therapy during early treatment in patients with breast cancer will benefit the immune system by maintaining the dynamics of peripheral blood immune cells and mitigating other negative impacts of chemotherapy drugs on immune cell function.

Methods

Study design

The docosahexaenoic acid (DHA) for women with breast cancer in the neoadjuvant setting (DHA-WIN trial) was a two-arm parallel double-blinded randomized clinical trial conducted on women undergoing neoadjuvant therapy for breast cancer at the Cross Cancer Institute (University of Alberta, Edmonton, AB) between 2019 to 2022. The trial compared the supplementation of 4.4 g/day of DHA-enriched algae triacylglycerol form (Life's DHA S40-O400, DSM Nutritional Products, Columbia, MD, USA) (DHA, $n=23$) to a placebo (a mixture of corn/soy oil, capsules from DSM Nutritional Products) (placebo, $n=26$). The protocol of the trial was previously described (Newell et. al. 2019), and the baseline characteristics of the participants included in this study are described in Table 1. The trial was registered at ClinicalTrials.gov Identifier: NCT03831178.

Blood collection, complete blood cell analysis and peripheral blood mononuclear cell isolation

Venous blood samples were obtained at the beginning of each of the 6 cycles of chemotherapy (every 3 weeks for 18 weeks). Immune analyses were performed, as described below, at baseline (pre-treatment), cycle 4 (9 weeks), and either cycle 6 or at the end of treatment (15 or 18 weeks, as indicated in each section). Peripheral blood samples were collected from participants (6 mL total) in EDTA tubes through an arm puncture. Whole blood was assessed for Complete Blood Count (CBC) and Differential at the Cross Cancer Institute using the XN-1000 Automated Hematology Analyzer (Sysmex Corporation, Kobe, Japan). Blood samples (3 mL) were diluted to 6 mL with 10 g/L bovine serum albumin (BSA; fraction V; Sigma Aldrich, St. Louis, MO, USA) in phosphate-buffered saline (PBS). Cells were separated by a density gradient of Histopaque 1077 (Sigma), as previously described [22]. Briefly, the diluted blood solution was added to 5 mL of Histopaque 1077 and centrifuged at $700\times g$ at 21 °C for 30 min. After centrifugation, the lymphocyte band was recovered, and cells were resuspended in complete cell culture media. Cells were counted on a hemocytometer, and trypan dye (Sigma) exclusion was used to determine viability. Fresh cells were diluted to 1×10^6 cells/mL and aliquoted for stimulation with mitogens (i.e. cells were not frozen prior to stimulation).

PBMCs phenotype analyses by flow cytometry

The phenotype of immune cells from whole blood collected from participants was assessed by direct immunofluorescence assay as previously described [22, 23]. Briefly, immune cells from aliquots (100 uL) from whole blood were incubated for 30 min with the following fluorophore-conjugated monoclonal antibodies (mAbs; all from Biolegend): CD1c, CD3, CD4, CD8, CD11b, CD11c, CD14, CD16, CD20, CD25, CD28, CD45RA, CD45RO, CD56, CD86, CD95, CD103, CD107, CD141, CD152, CD183, CD196, CD279, HLA-DR. The description of the specific mAbs added to each well and the combination of antibodies can be found in the Supplementary Table 1 and Table 2. Cells were fixed with paraformaldehyde (1% wt/wt in PBS) and then measured in the flow cytometer (BD LSRFortessa, Becton Dickinson, Franklin Lakes, NJ, USA) at the University of Alberta, Faculty of Medicine and Dentistry Flow Cytometry Facility. Compensation controls were included on each day of acquisition. The proportion of positive cells were determined using FlowJo software v10.10 (BD Biosciences) using the gating strategies illustrated in the Supplementary Fig. 1.

In vitro stimulation of PBMCs and measurements of cytokine production

To assess whether changes in blood cell concentration and immune cell phenotypes also reflect changes in the immune response, we isolated PBMCs from the whole blood of the participants and stimulated the cells either with phytohemagglutinin (PHA) to evaluate the response of T cells, or lipopolysaccharide (LPS) to investigate the antigen-presenting cells and B cells. Isolated PBMCs (3×10^6 cells) were added to 3 mL of complete culture media and cells were stimulated in vitro with PHA (25 µg/mL, Sigma) or LPS (10 µg/mL, O111:B4, Sigma). Cells were incubated at 37 °C in a controlled atmosphere (5% CO2, 95% relative humidity) for 48 h. After incubation, tubes were centrifuged (200 g at 4 °C for 5 min), and the supernatant was collected and stored at -80 °C for cytokine analysis.

Cytokine concentration in the supernatant was quantified using sandwich enzyme-linked immunosorbent assay (ELISA) kits (Biolegend and R&D Systems, Minneapolis, MN, USA) following the manufacturer's instructions. The ranges of detections were as follows: tumour necrosis factor (TNF)-α (7.8–500 pg/mL, 430204), interleukin (IL)-4 (3.9–250 pg/mL, 430304), IL-10 (2.9–250 pg/mL, 430604), interferon (IFN)-γ (7.8–500 pg/mL, 430104), IL-6 (7.8–500 pg/mL, 430104), IL-8 (15.6–1,000 pg/mL, 431504), IL-2 (7.8–500 pg/mL, 431804),

Table 1 Sociodemographic and clinicopathological characteristics of participants in the placebo and DHA groups

	Total ($n=49$)	Placebo ($n=26$)	DHA ($n=23$)	*P* value[3]
Age (years)[2]	50.8 ± 10.7	51.2 ± 12.0	50.4 ± 9.3	0.80
BMI [kg/m^2][1]	28.8 ± 6.7	27.5 ± 6.0	30.3 ± 7.3	0.60
Underweight (< 18.5)	1 (2.0)	1 (3.8)	0 (0.0)	
Healthy weight (18.5–24.9)	13 (26.5)	8 (30.8)	5 (21.7)	
Overweight (25–29.9)	18 (36.7)	10 (38.5)	8 (34.8)	
Obese (≥ 30)	17 (34.7)	7 (26.9)	10 (43.5)	
Ethnicity [count (%)][1]				0.99
Caucasian	32 (65.3)	17 (65.4)	15 (65.2)	
Not Caucasian	17 (34.7)	9 (34.6)	8 (34.8)	
Menopausal status[1]				0.31
No	25 (51.0)	13 (50.0)	12 (52.2)	
Yes	22 (44.9)	13 (50.0)	9 (39.1)	
Missing	2 (4.1)	0 (0.0)	2 (8.7)	
Age at menarche (years)[2]	12.8 ± 1.5	12.7 ± 1.7	12.8 ± 1.3	0.82
Missing	4 (8.2)	2 (7.7)	2 (8.7)	
Histology[1]				0.87
HER2 +	24 (51.0)	13 (50.0)	11 (47.8)	
TNBC	12 (24.5)	6 (23.1)	7 (30.4)	
Luminal A	10 (20.4)	6 (23.1)	4 (17.4)	
Luminal B	2 (4.1)	1 (3.8)	1 (4.3)	
Estrogen receptor status[1]				0.21
Positive	26 (53.1)	16 (61.5)	10 (43.5)	
Negative	23 (46.9)	10 (38.5)	13 (56.5)	
Progesterone receptor status[1]				0.24
Positive	17 (34.7)	11 (42.3)	6 (26.1)	
Negative	31 (63.3)	14 (53.8)	17 (73.9)	
Missing	1 (2.0)	1 (3.8)	0 (0.0)	
HER2 Status[1]				0.99
Positive	24 (49.0)	13 (50.0)	11 (47.8)	
Negative	25 (51.0)	13 (50.0)	12 (52.2)	
Disease Stage[1]				0.63
IIA	13 (26.5)	6 (23.1)	7 (30.4)	
IIB	10 (20.4)	4 (15.4)	6 (26.1)	
IIIA	14 (28.6)	9 (34.6)	5 (21.7)	
IIIB	4 (8.2)	2 (7.7)	2 (8.7)	
IIIC	1 (2.0)	0 (0.0)	1 (4.3)	
Missing	7 (14.3)	5 (19.2)	2 (8.7)	
Tumour Size[1]				0.92
T1	1 (2.0)	1 (3.8)	0 (0.0)	
T2	27 (55.1)	15 (57.7)	12 (52.2)	
T3	11 (22.4)	5 (19.2)	6 (26.1)	
T4	5 (10.2)	3 (11.5)	2 (8.7)	
Missing	5 (10.2)	2 (7.7)	3 (13.0)	
Axillary Node Status[1]				1.00
N0	12 (24.5)	6 (23.1)	6 (26.1)	
N1	21 (42.9)	11 (42.3)	10 (43.5)	
N2	5 (10.2)	3 (11.5)	2 (8.7)	
N3	2 (4.1)	1 (3.8)	1 (4.3)	
Missing	9 (18.4)	5 (19.2)	4 (17.4)	
Pathological complete response (pCR)				0.22
Yes	17 (34.7)	7 (26.9)	10 (43.5)	
No	32 (65.3)	19 (73.1)	13 (56.5)	

DHA docosahexaenoic acid, *BMI* body mass index, *HER2* human epidermal growth factor receptor 2, *TNBC* triple negative breast cancer

[1] Count (% of total or given group)

[2] Mean ± SD

[3] Comparisons between Placebo and DHA groups. Pearson Chi-Square test was used to compare categorical variables (exact test if cell count less than 5) and independent t-test was used to compare continuous variables (mean ± SD)

TGF (transforming growth factor)-β1 (7.8–500 pg/mL, 436707), IL-21 (31.2–2,000 pg/mL, 433804), IL-17A (3.9–250 pg/mL, 433914), and IL-1β (3.91–250 pg/mL, DY201). Supernatants were measured for the concentration of IL-5 (3.9–250 pg/mL, 4304) and IL-27 (156–10,000 pg/mL, DY2526), but were below detection. Samples were diluted to fit the appropriate detection ranges, and half of the lowest detection value was used for samples with concentrations below the detection ranges. Plates were read in the spectrophotometer Sinergy H1 (Agilent BioTek, Santa Clara, CA, USA). All samples were measured in duplicate with a coefficient of variation (CV) less than 15%, and the concentrations were determined based on the standard curves.

Statistical analysis

Mean and standard deviations were reported to describe continuous variables. The objective of this study was to evaluate the effects of DHA supplementation compared to a placebo on the immune function of participants undergoing neoadjuvant chemotherapy in the DHA-WIN trial. Secondary analyses aimed to assess and compare differences in the immune cell numbers and function from peripheral blood samples of participants during treatment (time effects). The number of participants varies in each analysis due to participants who missed a visit for blood draw, problems during the collection of blood, an insufficient number of cells for immune analysis, or the exclusion of samples with problems during acquisition in the flow cytometry analysis. Please note that all analyses, with the exception of cytokines quantification, were performed in fresh blood and missing samples were completely at random. Therefore, no method of imputation was used. Data were tested for normal distribution and due to the non-normality, non-parametric tests were performed. The Generalized estimating equations (GEE) with a repeated measures model and with a gamma distribution and log link function were employed to evaluate the changes promoted by DHA supplementation. Interaction between time points (baseline, 9 weeks, and 15 or 18 weeks) and intervention (placebo or DHA), and the main effects of each variable were included in the model for each outcome analysed. Statistically significant interaction effects between time and group were followed by post hoc testing using Bonferroni adjustment. Comparisons were made either within groups or between groups at the same time point. When only significant time effects were observed, the participants were combined to evaluate the effects of chemotherapy on the immune outcomes. Due to the exploratory nature of this study, small sample size, and the absence of imbalance of baseline characteristics (Table 1), we did not perform adjustments by other covariables. Logistic regression analysis was performed for univariate and multivariate analysis to evaluate dichotomous outcomes (pCR) and continuous variables (changes in blood cells concentrations). Group comparisons stratified by clinic-pathological characteristics were performed by Man-Whitney test. Statistical significance was considered at $P < 0.05$ (two-tailed). Statistical software SPSS (IBM Corp. IBM SPSS Statistics, Version 28.0. Armonk, NY, USA) was used to perform all statistical analyses, and the software GraphPad Prism (GraphPad Software Version 10.0.3, Boston, MA, USA) was used to generate the graphics.

Results

Whole blood cells quantification

To assess whether DHA supplementation attenuates changes in blood cell concentrations that are clinically relevant during chemotherapy, we investigated blood cell concentrations in the whole blood of the participants. Regardless of the group, the concentration of total white blood cells (WBCs), red blood cells (RBCs), lymphocytes, neutrophils, and eosinophils were reduced over time (P-time < 0.05), while the concentration of monocytes and basophils did not significantly change during treatment (Table 2).

The concentration of RBCs decreased from baseline at 9 and 18 weeks of treatment in both groups to concentrations below the reference values for healthy people (Table 2). Similarly, the concentration of lymphocytes was reduced in both the placebo and DHA groups at 9 and 18 weeks of chemotherapy, but there was a trend towards a lower reduction in the DHA group compared to the placebo group (P- interaction $= 0.073$) (Table 2). Further analysis revealed a significant effect of DHA supplementation on the NLR (P-interaction $= 0.020$) (Table 2). Specifically, the placebo group exhibited a significant increase in the NLR from baseline to 9 weeks of treatment, while it did not significantly change in the DHA-supplemented group (Fig. 1A).

Utilizing univariate logistic regression analysis, we observed that changes from baseline to 18 weeks of therapy in the NLR (OR = 0.32, 95% CI [0.14, 0.76], $P = 0.01$) were correlated with the achievement of a complete pathological response (pCR, Fig. 1B). Additionally, upon stratifying participants based on their pCR status, those who achieved it exhibited a greater reduction in the NLR compared to those who did not ($P = 0.001$, Fig. 1C).

To evaluate the influence of clinical-pathological characteristics and adjust for covariables, we investigated the association of estrogen receptor status, progesterone receptor status, human HER2 status, age, BMI, menopausal status, tumor grade, and disease stage with pCR. Only estrogen receptor status was significantly correlated

Table 2 Effects of DHA intervention on concentrations of blood cell counts from patients in the DHA-WIN trial

Cell types	Reference values	Placebo (Mean ± SD)				DHA (Mean ± SD)				Group × Time	Time	Group
		0 weeks (*n* = 26)	9 weeks (*n* = 26)	18 weeks (*n* = 22)	mean change (95% CI)[4]	0 weeks (*n* = 23)	9 weeks (*n* = 23)	18 weeks (*n* = 17)	mean change (95% CI)[4]	*P* value[1]	*P* value[2]	*P* value[3]
WBC 10^9 cells/L	4.0–11.0	6.65 ± 1.87	6.05 ± 2.61	5.14 ± 1.92	-1.29 (-2.09, -0.49)	6.87 ± 1.66	5.39 ± 1.54	5.34 ± 2.20	-1.69 (-3.09, -0.29)	0.274	**< 0.001**	0.768
RBC 10^{12} cells/L	4.3–6.0	4.57 ± 0.31	3.56 ± 0.41	3.43 ± 0.49	-1.15 (-1.36, -0.94)	4.49 ± 0.28	3.65 ± 0.37	3.42 ± 0.36	-1.09 (-1.26, -0.92)	0.308	**< 0.001**	0.966
Lymphocytes 10^9 cells/L	0.5–4.5	1.92 ± 0.76	1.15 ± 0.46	1.15 ± 0.57	-0.64 (-0.93, -0.35)	1.74 ± 0.47	1.21 ± 0.44	1.39 ± 0.46	-0.36 (-0.58, -0.14)	0.073	**< 0.001**	0.563
Neutrophils 10^9 cells/L	1.8–7.5	4.13 ± 1.42	4.24 ± 2.40	3.42 ± 1.71	-0.65 (-1.35, 0.05)	4.45 ± 1.45	3.59 ± 1.35	3.14 ± 1.92	-1.18 (-2.24, 0.12)	0.185	**0.018**	0.508
Basophils 10^9 cells/L[5]	0.0–0.3	0.02 ± 0.04	0.04 ± 0.05	0.03 ± 0.05	0.01 (0, 0.03)	0.04 ± 0.05	0.03 ± 0.05	0.04 ± 0.05	-0.01 (-0.02, 0.01)	0.054	0.137	0.360
Eosinophils 10^9 cells/L[5]	0.0–0.7	0.14 ± 0.13	0.02 ± 0.05	0.10 ± 0.12	-0.03 (-0.08, 0.03)	0.13 ± 0.08	0 ± 0.02	0.14 ± 0.16	0.00 (-0.06, 0.06)	0.117	**< 0.001**	0.824
Monocytes10^9 cells/L	0.0–1.1	0.43 ± 0.14	0.53 ± 0.29	0.44 ± 0.18	0.03 (-0.04, 0.09)	0.48 ± 0.16	0.51 ± 0.21	0.48 ± 0.21	-0.03 (-0.16, 0.09)	0.637	0.077	0.696
NLR		**2.37 ± 0.92[a]**	**4.2 ± 3.22[b]**	**4.03 ± 4.15[ab]**	1.55 (-0.15, 3.26)	2.72 ± 1.13	3.46 ± 2.13	2.52 ± 1.36	-0.05 (-0.68, 0.58)	**0.020**	**< 0.001**	0.124

Values represent the mean ± standard deviation. Data were analyzed using Generalized Estimating Equations (GEE) to assess the interaction of DHA supplementation and changes over time. [a,b]Labelled means without a common letter differ ($P < 0.05$) based on post hoc with Bonferroni adjustment comparing changes within each group

Significant differences are in bold. *DHA* docosahexaenoic acid, *SD* standard deviation, *WBC* white blood cells, *RBC* red blood cells, *CI* confidence interval, *NLR* Neutrophil-to-lymphocyte ratio

[1] *P* value for the interaction between supplementation and time in the GEE

[2] *P* value for the main effect of time (0, 9, and 18 weeks) in the GEE

[3] *P* value for the main effect of the group (DHA or placebo) in the GEE

[4] Mean value and confidence interval of the reduction from 18 weeks to baseline

[5] Due to the high frequency of zeros, data was categorized as "zero" or "above zero" and compared by GEE with a binomial probability distribution and logit link function

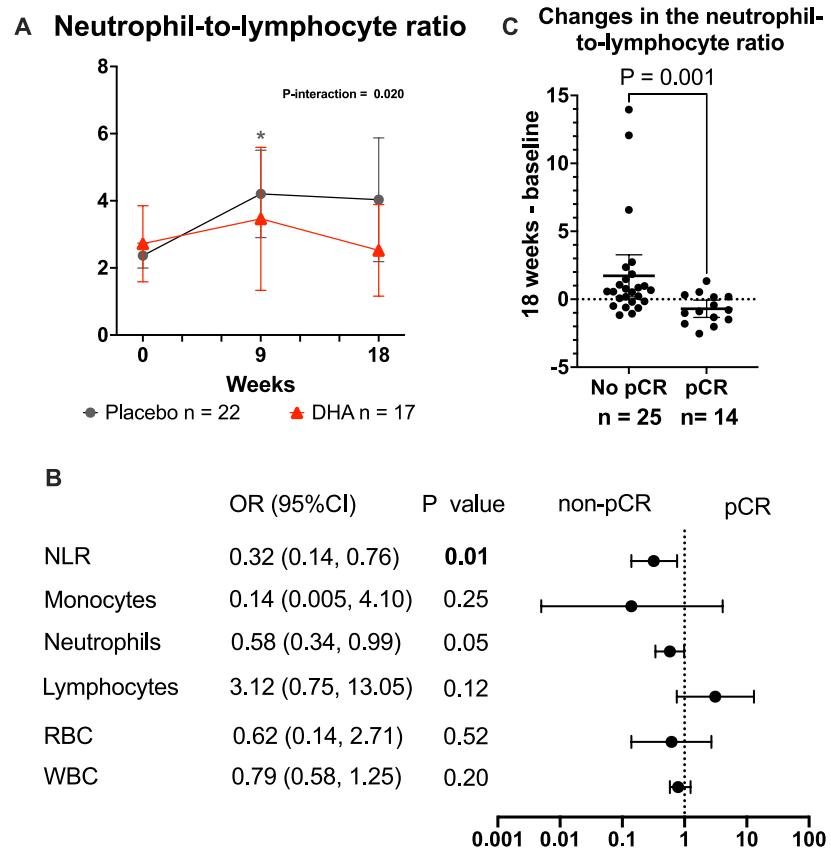

Fig. 1 Change in the concentration of blood cells during treatment in the DHA-WIN trial. **A** Values are represented as mean and 95% of the confidence interval. Generalized estimating equations were performed with the main effects and interaction between time and groups of intervention. The neutrophils to lymphocytes ratio was stratified by placebo and DHA groups. *denotes the value is different from the baseline within the group based on post hoc Bonferroni adjustment ($P < 0.05$). **B-C** Changes in the concentration of blood cell concentrations during neoadjuvant treatment categorized by therapeutic response (pCR). The concentration of blood at 18 weeks was subtracted from values at baseline to determine the change during treatment. **B** Forest plot of univariate logistic regression analysis ($n = 25$ non- pCR and $n = 14$ pCR). **C** Mann–Whitney test comparing changes in the neutrophils-to-lymphocytes ratio between participants who achieved pCR and patients who did not achieve pCR at the end of neoadjuvant chemotherapy (bars represent median and range). DHA, docosahexaenoic acid; RBC, red blood cells; WBC, white blood cells; pCR, pathological complete response

with pCR (OR = 0.13, 95% CI [0.32, 0.49], $P = 0.003$). Therefore, we performed a multivariate logistic regression analysis, adding estrogen receptor status and NLR to the model. We found that in this model, the associations remained significant after controlling for estrogen receptor status (OR = 0.11, 95% CI [0.02, 0.066], $P = 0.016$) and NLR (OR = 0.38, 95% CI [0.15, 0.93], $P = 0.034$).

Phenotype of peripheral blood immune cells

The proportion and number of cells for all markers evaluated by flow cytometry analyses can be found in Supplementary Tables 3 and 4. The proportion of total T cells ($CD3^+$) significantly decreased in the placebo group at 15 weeks of chemotherapy compared to baseline, while this cell population did not significantly change in the DHA group (P-interaction = 0.029) (Fig. 2A). DHA supplementation did not significantly impact the proportion and number of different subtypes of T cells (Supplementary Table 3). Moreover, only time effects, not intervention, were observed in changes in T cell phenotypes during chemotherapy treatment. The proportion of T helper (Th) cells ($CD3^+CD4^+$) decreased at 9 and 15 weeks of treatment (Fig. 2B), while the proportion of T cytotoxic cells (CD3 + CD8 +), Th type 1 (Th1, $CD3^+CD4^+CD183^+CD196^+$), and T regulatory cells (Tregs, $CD3^+CD4^+CD127^-CD25^+$) increased during chemotherapy treatment, regardless of treatment group (Fig. 2C-E). As a result of alterations in the proportion of $CD4^+$ and $CD8^+$ cells, the $CD4^+/CD8^+$ ratio decreased at 9 and 15 weeks of therapy compared to baseline (Fig. 2F).

There were differences between the groups of intervention in the expression of the costimulatory molecule HLA-DR in monocytes (Fig. 3A). Moreover, the proportion of monocytes expressing HLA-DR

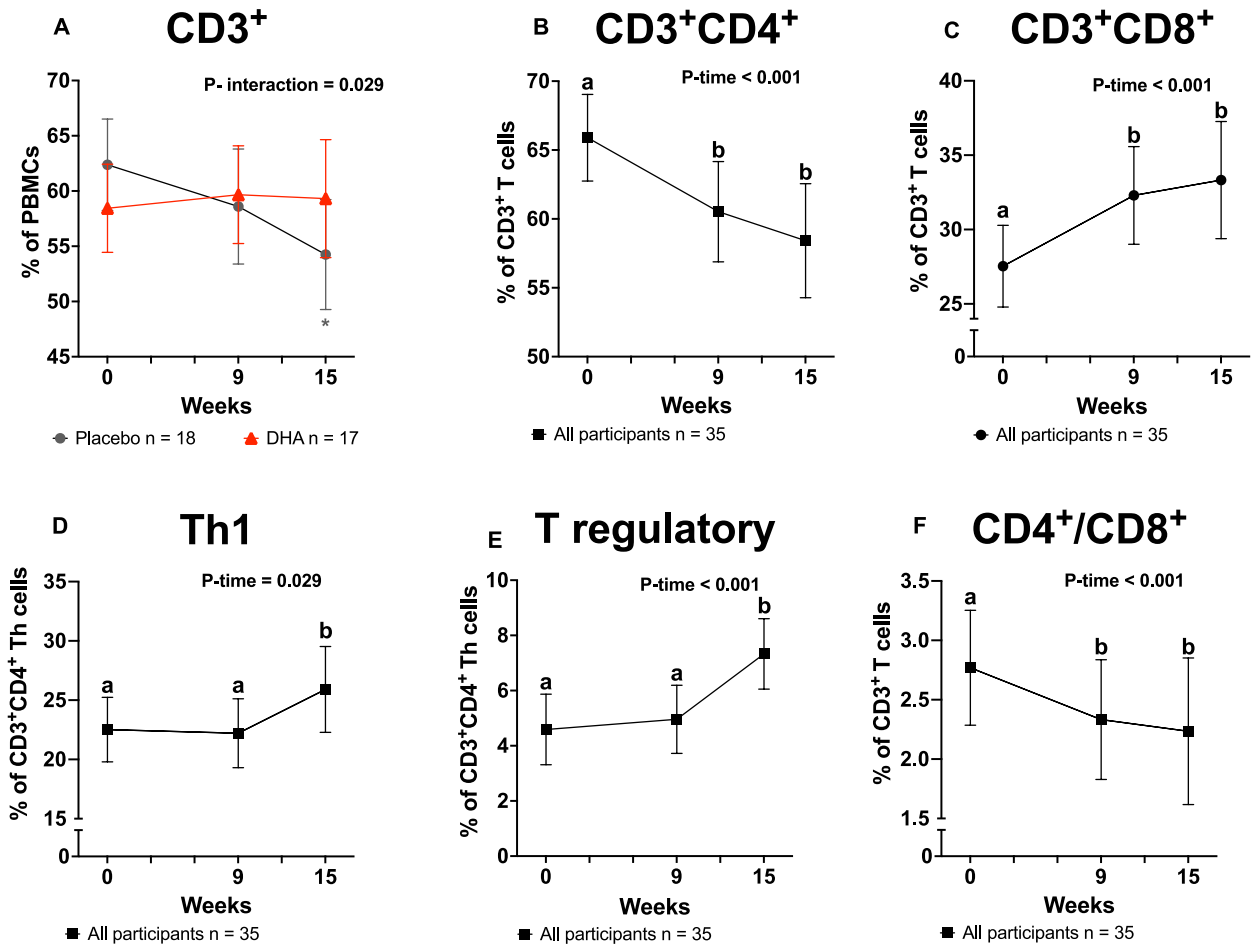

Fig. 2 Changes in the proportion of T cell phenotypes in the PBMCs of participants in the DHA-WIN trial. Values are represented as mean and error bars as the 95% of the confidence interval. Generalized estimating equations were performed for each parameter analyzed with the main effects and interaction between time and groups of intervention. **A** * Indicates the value is different from the baseline within the group based on post hoc Bonferroni adjustment, * $P < 0.05$. **B**-**F** Labelled means without a common letter differ ($P < 0.05$) based on post hoc with Bonferroni adjustment. DHA, docosahexaenoic acid; Th1, T helper type 1

(CD14+HLA-DR+) cells decreased in the placebo group at 9 and 15 weeks of treatment compared to baseline, while the proportion did not significantly change in the DHA-supplemented group (P-interaction = 0.020) (Fig. 3A). Regardless of the intervention, the percentage of total monocytes (CD14+) in the whole blood of participants significantly increased at 9 weeks and this elevation was maintained to 15 weeks of treatment (Fig. 3B).

There were no significant effects of DHA supplementation in the proportion of total natural killer (NK, CD3-CD16+CD56+) cells, NKT (CD3+CD16+CD56+) cells, and $CD11c^+$ cells (marker mainly expressed by dendritic cells) during chemotherapy treatment. Regardless of treatment, the proportion of total NK, NKT, and $CD11c^+$ cells increased during chemotherapy treatment (P-time < 0.05) (Fig. 3C-E). Similarly, the number of NKT and CD11c+cells increased during treatment (P-time < 0.05) (Supplementary Table 4).

Despite the increase in these subsets, the proportion and number of B cells exhibited a dramatic reduction at 9 weeks and 15 weeks of chemotherapy treatment, regardless of treatment group (P-time < 0.001) (Fig. 3F and Supplementary Table 4). B cells also expressed a lower proportion of the costimulatory molecule HLA-DR in both groups, suggesting not only depletion of those cells but also a loss of function (Fig. 3G). Consistent with the depletion and function of B cells, an increase in the proportion and number of B cells expressing CD27+, a marker of mature B cell phenotype at 9 weeks that was maintained at 15 weeks of chemotherapy, suggested a diminished capacity for cell restoration (Fig. 3H).

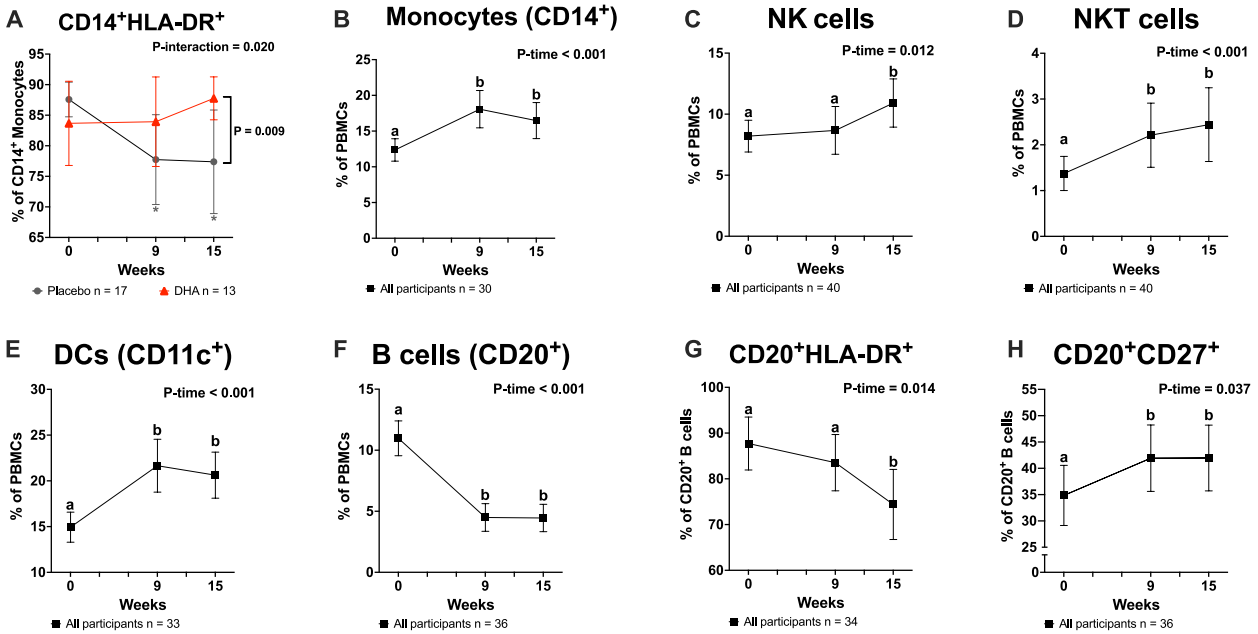

Fig. 3 Changes in the proportion of cell phenotypes in the PBMCs of participants in the DHA-WIN trial. Values are represented as mean and 95% of the confidence interval. Generalized estimating equations were performed for each parameter analyzed with the main effects and interaction between time and groups of intervention. **A** * Indicates the value is different from the baseline within the group based on post hoc Bonferroni adjustment, * $P < 0.05$. *P* values indicate the comparison between groups at the same time point (based on post hoc Bonferroni adjustment). **B**-**H** Labelled means without a common letter differ ($P < 0.05$) based on post hoc with Bonferroni adjustment. PBMCs, peripheral blood mononuclear cells; DHA, docosahexaenoic acid; NK, natural killer; DCs, dendritic cells

Quantification of cytokines in the supernatant of ex vivo stimulated PBMCs

DHA supplementation attenuated changes in the secretion of the cytokines IL-4 (*P*-interaction = 0.006), IL-10 (*P*-interaction = 0.039), and IFN-γ (*P*-interaction = 0.001) during neoadjuvant chemotherapy (Supplementary Table 6). The concentration of IL-4 significantly reduced in the placebo group over the 9 and 15 weeks of therapy, whereas it did not significantly change in the DHA group compared to baseline (Fig. 4A). At 15 weeks of therapy, the concentrations of the cytokines IL-10 and IFN-γ were significantly lower in the placebo group compared to the DHA group (Fig. 4B-C). Furthermore, DHA supplementation significantly increased the secretion of the cytokine IL-17 at both 9 and 15 weeks of treatment (*P*-interaction = 0.008) (Fig. 4D). The concentration of IL-17 was lower in the DHA group compared to the placebo at baseline (128 ± 106 versus 82.3 ± 51.7 pg/mL, *P* = 0.038), but reached similar concentrations in the DHA group at 9 and 15 weeks of therapy (Fig. 4D).

DHA supplementation did not have significant effects on the concentration of the cytokine secretions of IL-2, TNF-α, IL-6, IL-8, TGF- β1, and IL-1β in PBMCs stimulated with PHA (Supplementary Table 5). Moreover, only temporal effects were observed for changes in the secretion of these cytokines, with reduced concentrations of TNF-α and increased concentrations of IL-8 and IL-6 regardless of treatment group (Figs. 4F-H). Although a significant time effect was observed in the changes in the concentration of IL-2 (*P* = 0.005) and a tendency toward decreased concentrations during treatment was noted, post hoc analysis revealed no statistical significance between time points due to the high variability among participants (Fig. 4E).

In PBMCs stimulated with LPS, supplementation with DHA induced significant alterations in the secretion of the cytokines TNF-α (*P*-interaction = 0.012) and IFN-γ (*P*-interaction = 0.005) (Supplementary Table 5). The placebo group exhibited a decrease in TNF-α concentration at 15 weeks compared to baseline, while it did not significantly change in the DHA group (Fig. 5A). Consequently, there was a significant difference between the placebo and DHA groups in the concentration of the cytokine TNF-α at 15 weeks of therapy (*P* = 0.008) (Fig. 5A). Similarly, IFN-γ concentration decreased in the placebo group at the end of treatment compared to baseline, while it did not significantly change in the DHA group (Fig. 5B). However, DHA supplementation had no significant impacts on the remaining cytokines assessed, including IL-10, IL-6, IL-8, IL-1β, and TGF-β1compared to the placebo group (Supplementary Table 6). Only temporal effects were observed, with concentrations of IL-10,

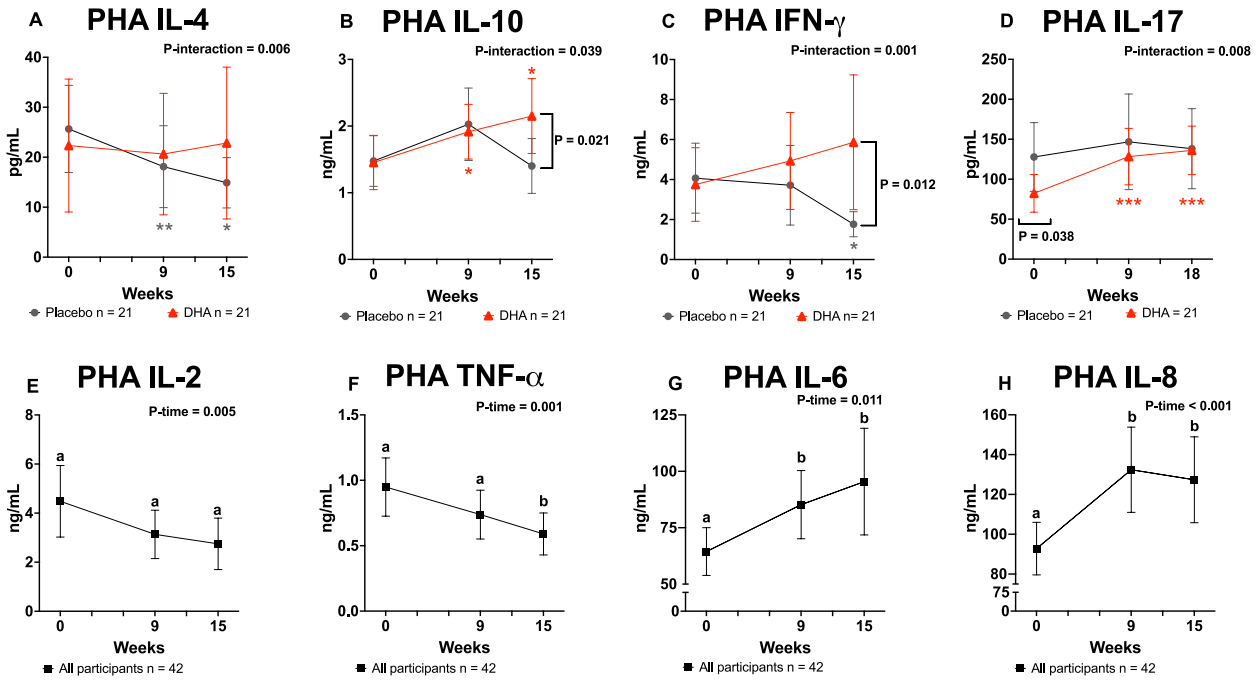

Fig. 4 Cytokine concentration in the supernatant from isolated peripheral blood mononuclear cells after stimulation with PHA for 48 h. Values are represented as mean and 95% of the confidence interval. Generalized estimating equations were performed for each parameter analyzed with the main effects and interaction between time and groups of intervention. **A-D** * Denotes the value is different from the baseline within the group based on post hoc Bonferroni adjustment * $P<0.05$, ** $P<0.01$, *** $P<0.001$. P values indicate the comparison between groups at the same time point (based on post hoc Bonferroni adjustment). **E**–**H** Labelled means without a common letter differ ($P<0.05$) based on post hoc with Bonferroni adjustment. Although a statistically significant time effect was found for the production of IL-2, pairwise comparisons did not result in statistically significant differences (represented by common letters). DHA, docosahexaenoic acid; PHA, phytohemagglutinin; LPS, lipopolysaccharide; IL, interleukin; IFN-γ, interferon-gamma; TNF- α, tumour necrosis factor-alpha

IL-8 and IL-6 increasing at 9 weeks and 15 weeks of treatment compared to baseline with all participant values combined (Figs. 5E-D). Additionally, the concentrations of IL-1β and TGF-β1 remained unchanged, suggesting a lack of treatment or DHA supplementation effects on the secretion of these cytokines by PBMCs (Supplementary Table 5).

To determine whether the differences in cytokine secretion were due to changes in the proportion of immune cells (as determined by the flow cytometry analysis) or to changes in the number of cells placed in culture due to proliferation or cell death, we normalize the results to take into account the number of cells by multiplying the % of the lymphocyte population by 1.0×10^6 (number of total cells added to culture). This number was used to express the cytokine concentration by 10^3 cells (Supplementary Table 6). Our correction resulted in the same trends as using the relative % for the phenotype. The exception being for IL-4, IL-2, TGF- β1 and IL-17 (Supplementary Table 6). Only time effects were observed for the concentration of IL-4 in PHA-stimulated PBMCs after the data was normalized (P-time = 0.001). Changes in the concentration of the cytokine IL-2 over time did not reach significance. TGF-β1 in LPS-stimulated PBMCs was significantly increased during treatment (P-time = 0.048). The significant differences previously observed between the DHA and placebo groups in the secretion of IL-17 after PHA-stimulated at baseline no longer was found, after correcting for the number of cells, however time effects remained.

Associations between clinic-pathological factors and changes in immune function

To account for confounding factors and to evaluate the influence of patients' characteristics, we determined whether important clinical-pathological factors were associated with changes in immune function observed during chemotherapy, regardless of group of intervention. Factors analyzed included patient age, BMI, and menopausal status at inclusion, disease stage, tumour stage and HER2. Statistically significant differences were detected between HER2+ and HER2- patients and are depicted in Fig. 6.

Differences in the concentration of RBCs, the proportion of Th17 cells, naïve (CD45RA+) and memory (CD45RO+), CD4+ cells, and the concentration of IL-10 in

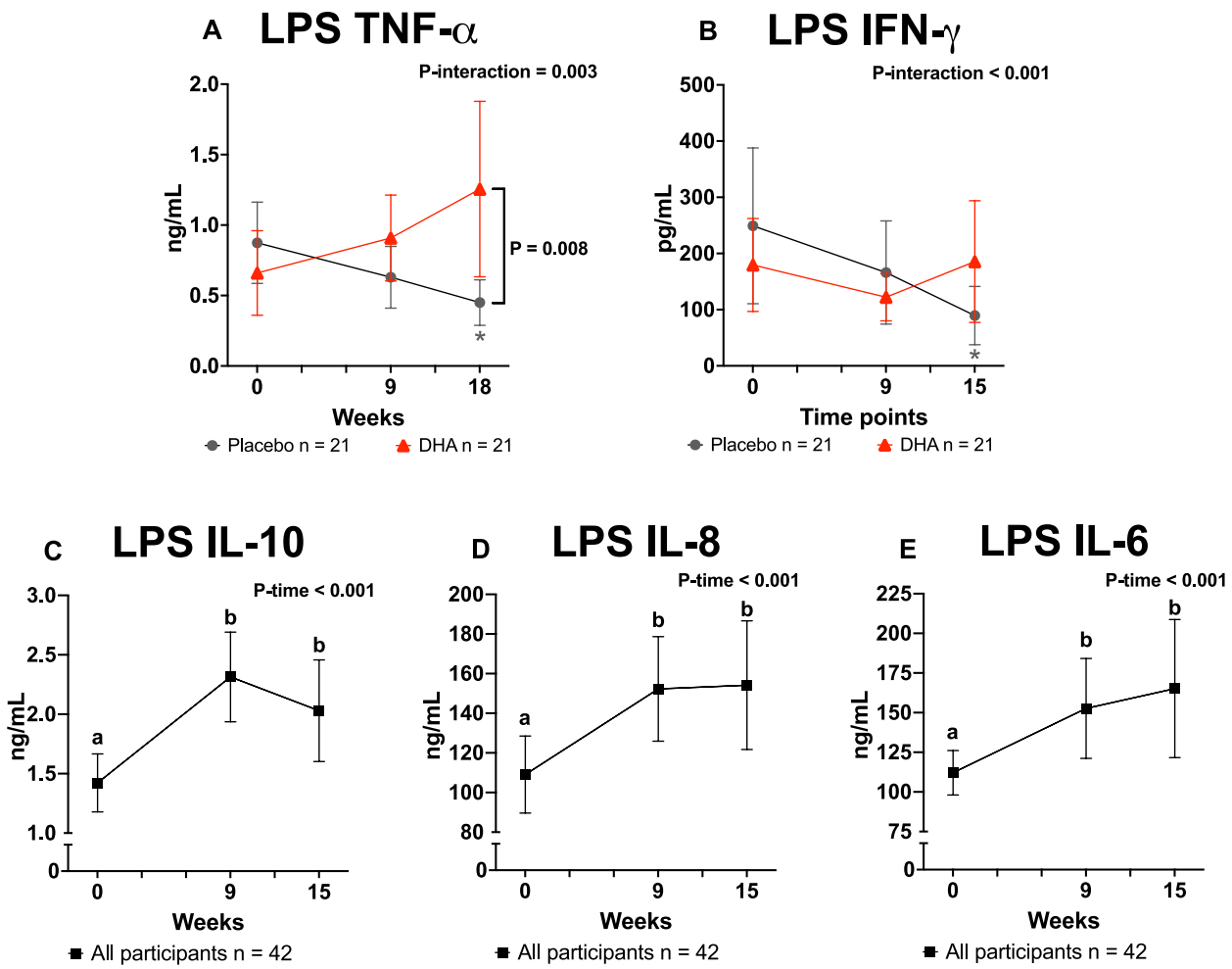

Fig. 5 Changes over time in cytokines concentrations in the supernatant from isolated peripheral blood mononuclear cells after stimulation with LPS for 48 h ($n=49$). Values are represented as mean and 95% of the confidence interval. The P values represent the interaction between the different groups and time points using Generalized estimating equations (GEE). Multiple comparisons with Bonferroni adjustment. **A-B** * Indicates the value is different from the baseline within the group based on post hoc Bonferroni adjustment, * $P<0.05$. *P* values indicate the comparison between groups at the same time point (based on post hoc Bonferroni adjustment). **C-E** Labelled means without a common letter differ ($P<0.05$) based on post hoc with Bonferroni adjustment. PHA, phytohemagglutinin; LPS, lipopolysaccharide; IL, interleukin; IFN-γ, interferon-gamma; TNF- α, tumour necrosis factor-alpha

the supernatant of PBMCs stimulated with LPS were statistically associated with HER2 status after subtraction from the values quantified before initiation of therapy (baseline) (Fig. 6). Specifically, compared to patients with HER2- status, patients with HER2+status had a significantly higher reduction during chemotherapy in the concentration of RBCs ($P=0.002$), in the percentage of Th17 ($P=0.001$) and memory TCD4+cells ($P<0.001$), and in the concentration of the cytokine IL-10 in ex vivo stimulated PBMCs ($P=0.003$) (Fig. 6A-D). In concordance with a higher reduction in the percentage of memory TCD4+cells, the percentage of naïve TCD4+(CD4+CD45RA+) cells increased in the group of patients with HER2+status during the chemotherapy (Fig. 6E).

Patients in the trial received two different neoadjuvant chemotherapy regimens depending on the HER2 status. Specifically, HER2+patients received the DCH regimen (docetaxel, carboplatin, and trastuzumab, IV q3week×6 cycles) and HER2-negative patients received FEC-D (fluorouracil, epirubicin, cyclophosphamide IV q3week×3 cycles followed by docetaxel IV q3week×3 cycles). Regardless of the therapy regimen, all patients in the trial received granulocyte colony-stimulating factor (G-CSF, pegfilgrastim, 6 mg/each cycle of chemotherapy) as a prophylactic therapy against chemotherapy-induced neutropenia. Interestingly, comparisons of the immune changes during treatment correlated with HER2 status did not differ between subgroups at baseline

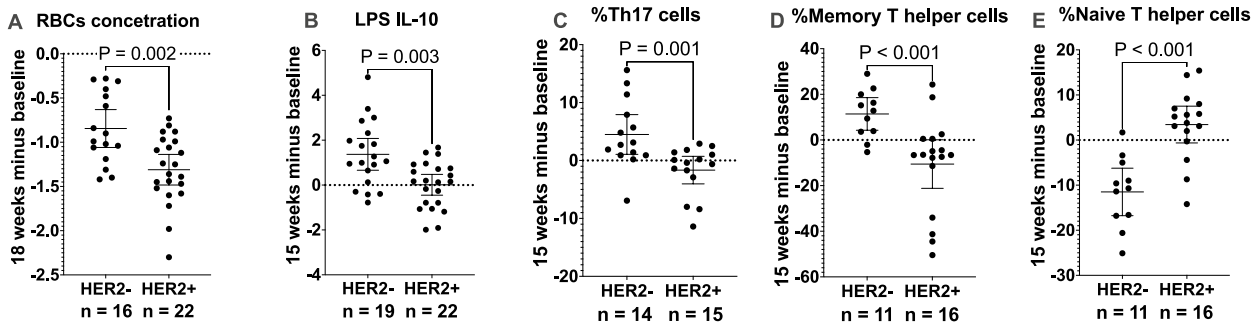

Fig. 6 Human epidermal growth factor receptor 2 (HER2) status and changes in different immune parameters analyzed in the trial. The values at the end of therapy (18 weeks, RBCs concentration) or cycle 6 (15 weeks, LPS IL10, % of $Th17^+$ cells, mature and naïve T helper CD4 cells), were subtracted from values assessed at baseline (before intervention). Groups were stratified based on the expression of HER2 and Mann–Whitney tests were performed to evaluate differences between groups. **A** Changes in red blood cell concentration. **B** Change in the concentration of the cytokine IL-10 in the supernatant of LPS-stimulated PBMCs. **C** Changes in the proportion of $Th17^+$ cells in $CD3^+CD4^+$ from the flow cytometry analysis. **D** Changes in the proportion of memory ($CD45RO^+$) in $CD3^+CD4^+$ from the flow cytometry analysis. **E** Changes in the proportion of naïve ($CD45RA^+$) in $CD3^+CD4^+$ from the flow cytometry analysis; LPS, lipopolysaccharide; IL, interleukin; PBMCs, peripheral blood mononuclear cells

(Supplementary Fig. 2), indicating that chemotherapy, regardless of the regimen, had a significant impact on immune changes.

Discussion

In this study exploring a secondary outcome of the DHA-WIN trial, we found that DHA supplementation during neoadjuvant therapy prevented the increase of the NLR, a clinically relevant biomarker of changes in circulating peripheral blood immune cells [14]. Additionally, we described the protective immune effects of DHA supplementation during the last cycle of chemotherapy. DHA supplementation prevented the reduction in the proportion of T cells (CD3+), monocytes (CD14+HLA-DR+), and in the ability to secrete the cytokines IL-4, IL-10, and IFN-γ upon T cell stimulation, as well as TNF-α and IFN-γ after stimulation with the most abundant antigen on the cell surface of gram-negative bacteria (LPS).

Extensive evidence recognizes the importance of immune responses on the initiation, progression or elimination of tumour cells in breast cancer [24]. Chemotherapy agents can cause immune dysregulation through various mechanisms. These include direct cytotoxic damage to immune cells, suppression of immune cell differentiation, and impairment of immune cell function [25]. All these factors contribute to immune dysfunction [25]. Therefore, it is relevant to investigate strategies to reduce the negative impacts of chemotherapy drugs on immune outcomes.

Effects on the number, phenotype, and function of immune cells in peripheral blood

Depletion of circulating lymphocytes and alterations in other hematologic cell parameters were previously reported in breast cancer patients undergoing chemotherapy [26]. These changes would potentially make these patients more susceptible to infections, limit the efficacy of cytotoxic agents, and decrease overall survival [27]. The literature of the effects of DHA supplementation on the dynamics of blood cell populations and function in breast cancer patients is scarce. In our study, we observed lower NLR in the DHA group, and that higher NLR was associated with lower chances of achieving pCR. Similar to our findings, Ruperto et al. [28] reported a lower NLR ratio in hemodialysis patients after supplementation with DHA (645 mg/day for 8 weeks) and this was associated with better management of anemia.

Despite their essential role as the first line of defense against pathogens, neutrophils can contribute to an immunosuppressive phenotype in the context of cancer chemotherapy. This occurs through the uncontrolled activation of these cells by the release of damage-associated molecular patterns (DAMPs), cytokines, and debris from the tumour [29]. The administration of G-CSF can further exacerbate the functional impairment of neutrophils [30, 31]. Neutrophils can produce cytokines and growth factors that support immune exhaustion, immune evasion, and tumour growth [32]. Conversely, lymphocytes are associated with protection of the host's immune system and elimination of tumour cells [33]. Therefore, an elevated NLR was found to be associated with a poor prognosis in breast cancer [34], and was suggested to reflect an inflammatory status. DHA affects membrane properties by altering lipid raft organization and regulates the production of specialized pro-resolving mediators (SPMs) [35, 36]. A plausible hypothesis for the observed maintenance of NLR in the DHA-supplemented group is that DHA, through the release of SPMs, might

facilitate the clearance of inflammatory cells, debris, and cytokines coming from the tumor. This mechanism could modulate the neutrophilic response and help prevent secondary, unfavorable immunosuppression. However, since neutrophil function was not assessed in this study, future research should incorporate assays to explore the functional aspects of neutrophils during breast cancer therapy and evaluate the impact of DHA supplementation on these cells.

Consistent with our findings, chemotherapy is reported to decrease the proportion of T helper cells (CD4+) and dramatically reduce B cell populations in breast cancer patients [37]. Additionally, systemic treatment with doxorubicin induces the recruitment of myeloid-derived suppressor cells (MDSCs) and further promotes T cell dysfunction in sites of metastasis in an animal model of TNBC [11]. Similarly, we observed a decrease in the proportion of CD14+HLA-DR+at the last cycle of chemotherapy. These cells are also known as myeloid-derived suppressor cells (MDSCs) and higher levels are found to be associated with metastasis in breast cancer patients [38]. In our study, despite the lack of effects of supplementation on the majority of immune phenotypes analysed, there was a positive effect of DHA supplementation in maintaining the proportion of CD14+HLA-DR+cells and CD3+T cells. The capacity of n-3 long-chain polyunsaturated fatty acids to enhance monocyte function was previously described. Souza et al. report increased bacterial phagocytosis (of fluorescently labeled *E coli*) in monocytes from the peripheral blood of healthy volunteers supplemented with marine oil (5 mg/kg of 17-HAD and 3 mg/kg of 18-HEPE, precursors of SPMs), as identified by flow cytometry analysis [39].

Evidence from studies on the effects of DHA supplementation on immune cells in breast cancer patients is still inconclusive. One of the few mechanistic studies evaluating the role of DHA on immune function in cancer reported that administration of DHA (2 mg/kg) reduced the expression of the programmed death (PD)-ligand 1 in cancer cells in a model of lung carcinoma [40]. While our study did not assess the phenotype of cancer cells, we did observe that the proportion of CD8+cells expressing PD-1 remained unchanged. Additionally, we found an increased expression of cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) on CD3+T cells. Both PD-1 and CTLA-4 are well-known inhibitory markers of T cell function [41].

One of the significant challenges in the success of immune checkpoint inhibitors in cancer therapy is the emergence of compensatory mechanisms. Studies have shown that additional blockade of Tregs can further enhance the beneficial effects of PD-1/PD-L1 axis inhibition [42]. Tregs are also recognized as key players in tumor progression and are generally associated with poorer prognosis in breast cancer therapy [43]. Our findings show an increase in the proportion of Tregs during neoadjuvant chemotherapy. This observation aligns with a recent study showing that breast cancer patients have elevated levels of Tregs after neoadjuvant therapy, with higher proportions correlating with higher tumor stage [44]. Contradictory to these findings, other studies demonstrate the immunostimulatory effects of neoadjuvant chemotherapy in the reduction of the proportion of Tregs during the conventional regimens of FEC-D and DCH [45]. Therefore, future studies should consider the heterogeneity of different regimes of treatment and the contribution of important clinic-pathological factors to better understand the impacts of neoadjuvant chemotherapy on different subsets of immune cells.

Effects on the immune response of peripheral blood mononuclear cells

The maintenance of the immune response is essential for an anti-tumour response in the host and for the resolution of infections. Reduced immunological response to vaccines and higher incidence of infections are reported in cancer patients during cytotoxic chemotherapy [46]. In concordance with these observations, we found a reduction in secretion of cytokines from PBMCs following PHA or LPS challenge at the last chemotherapy cycle (15 weeks) in the placebo group. These findings suggest that neoadjuvant chemotherapy negatively impacts the immune response, in addition to its direct cytotoxic effects on immune cells.

DHA supplementation attenuated the reduction of TNF-α and IFN-γ without affecting the secretion of other cytokines after LPS challenge. The effects of DHA in enhancing B cell function and resulting in increased ex vivo cytokine secretion without changes in major histocompatibility complex expression (MHC), were previously reported an in vivo study [47]. Despite not observing an effect of DHA on the dynamics of B cells in our study, the higher expression of HLA-DR by monocytes can partially explain the higher secretion of TNF-α observed at the last cycle of chemotherapy in the DHA group. However, we did not evaluate the specific phenotype of cells after mitogen stimulation. Therefore, future studies are necessary to confirm this hypothesis.

Among important cells of the adaptive immune system, Th1 and Th2 cells are major regulators of the immune response. Th2 cells are characterized by the secretion of IL-4 and IL-10 cytokines, while Th1 cells are the main producers of IFN-γ [48]. Interestingly, our results show that DHA supplementation attenuated the reduction of these cytokines during the last cycle of chemotherapy

(15 weeks). However, no effects were observed in the proportion of different subtypes of CD4 + T helper cells.

To our knowledge, this is the first study to demonstrate the protective effects of DHA supplementation on immune responses of breast cancer patients. These differences in immune responses are supported by previous reports of interventions with omega-3 fatty acids that reduced the severity of infections and side effects in cancer patients undergoing chemotherapy [49, 50].

Immunostimulatory properties of neoadjuvant chemotherapy

Despite the immunosuppressive effects of chemotherapy, its ability to also induce immune responses also requires consideration. Our study revealed an increase in the proportion of CD8 + T cells and Th1 cells during chemotherapy. Additionally, there was an increase in the proportion of NK cells and dendritic cells. The stimulatory effects of breast cancer neoadjuvant chemotherapy in increasing the proportion CD8 + T cells and NK cells were previously described [51, 52]. The chemotherapy drug combination of epirubicin, docetaxel, and fluorouracil can induce cell death in immune cells, with the release of neoantigens that in turn can activate a CD8 + T cell response [53]. Classically, CD8 + T cells promote killing through direct ligation of death receptors such as Fas (CD95) or by releasing granules containing perforins and granzymes, including LAMP-1 (lysosomal associated membrane protein 1, CD107a) [54]. Although our study did not observe any changes in FAS expression, we found an increase in LAMP-1 expression on CD8 + T cells during chemotherapy. These findings support the stimulatory effects of neoadjuvant chemotherapy on CD8 + T cells.

Additionally, we found that PBMCs from patients with HER2 + status who received the DCH regimen exhibited the most significant reductions in RBC concentration, the proportion of Th17 cells, and LPS-stimulated IL-10 secretion. This group also showed a more pronounced decrease in the proportion of memory CD4 + cells, with a corresponding increase in naïve CD4 + cell proportion. These differences may partially explain the higher variability observed in some immune markers, and future studies should consider these variations in their design. These distinctions between the DCH and FEC-D regimens have not been previously reported. Therefore, future studies are necessary to confirm these findings and to better predict which patients would benefit the most of clinical interventions with the goal of reducing immune dysfunction in patients during breast cancer neoadjuvant therapy.

Strengths and limitations

Our study has several limitations. Firstly, since our study was designed to investigate a secondary outcome from the main study, it was not sufficiently powered to analyze all reported immune parameters. This limitation contributed to our decision not to conduct multivariable analysis on the majority of parameters analyzed. Furthermore, we did not assess the phenotype of immune cells post-stimulation with mitogens which imitated our adjustments, nor did we isolate specific immune cells to assess their response. Lastly, we did not assess the dose of chemotherapy agents used during treatment, which could have limited our interpretation of clinical associations.

Our study also had a number of strengths. The main study was the first to use a high dose of DHA during neoadjuvant breast cancer therapy and this secondary study was the first to evaluate immune changes over the course of therapy. Additionally, despite the limitations of sample size, our statistical analysis was mindful of possible confounding factors. Lastly, we performed an extensive analysis of different immune cell phenotypes. However, future studies with larger sample sizes are necessary to confirm these findings, to investigate clinical immune outcomes, and to elucidate the mechanisms by which DHA may protect the immune system during neoadjuvant chemotherapy. Additionally, further studies are needed to determine the effects of DHA on the tumor immune microenvironment.

Conclusion

In conclusion, our study is the first to demonstrate that supplementation with 4.4 g/day of DHA during six cycles of neoadjuvant chemotherapy can attenuate changes in systemic inflammatory biomarkers, specifically the NLR. It also prevents the reduction in HLA-DR expressing monocytes and maintains the production of TNF-α by LPS-activated monocytes and B cells, and Th1 and Th2 cytokine production in PBMCs. Furthermore, our findings highlight the complex balance between the immunostimulatory and immunosuppressive effects of neoadjuvant chemotherapy, emphasizing its impact on immune responses and immune cell depletion. This research provides evidence of the potential benefits of DHA supplementation in preserving immune function during neoadjuvant breast cancer therapy. These findings are relevant for early breast cancer therapy, as improving our understanding of chemotherapy's effects on the immune system may help guide future clinical guidelines. The significance of these results is highlighted by the evolving clinical landscape, where new protocols integrating immunotherapy with traditional chemotherapy are being adopted in breast cancer treatment.

Abbreviations

DHA	Docosahexaenoic acid
DHA-WIN	Docosahexaenoic acid for women with breast cancer in the neoadjuvant setting
NLR	Neutrophil-to-lymphocyte ratio
pCR	Pathological complete response
TNBC	Triple negative breast cancer
HER2	Human epidermal growth factor receptor 2
PDXs	Patient-derived xenografts
PHA	Phytohemagglutinin
LPS	Lipopolysaccharide
WBCs	White blood cells
RBCs	Red blood cells
IFN	Interferon
TNF	Tumour necrosis factor
IL	Interleukin
DCH	Docetaxel, carboplatin, and trastuzumab
FEC-D	Fluorouracil, epirubicin, cyclophosphamide

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13058-025-02048-z.

Supplementary Material 1.

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Institutional review board
The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Health Research Board of Alberta – Cancer Committee (HREBA.CC-18–0381).

Authors' contributions
Conceptualization – J.M., M.N., S. Goruk., K.C., A.J., G.B., D.G.H, and C.J.F.; data analysis and statistical modelling – J.M., S. Ghosh., and C.J.F.; methodology – J.M., M.N., S. Goruk, and D.P. Recruitment and coordination of clinical staff – A.J; Supervision – C.J.F. Writing of original draft – J.M. and C.J.F.; Review of original draft and edits – all authors. All authors have read and approved the final and published version of the manuscript.

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Data availability
Data available by request, subject to approval.

Declarations

Ethics approval and consent to participate
Informed consent was obtained from all subjects involved in the study.

Competing interests
The authors declare no competing interests.

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