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ORIGINAL RESEARCH

Moderate physical activity during neoadjuvant chemotherapy in breast cancer patients: effect on cancer-related inflammation and pathological complete response—the Neo-Runner study

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Background: Physical activity (PA) reduces the risk of developing breast cancer (BC) and mortality rate in BC patients starting PA after diagnosis. Immunomodulation is considered responsible for these effects. However, limited data exist on the immunomodulation induced by moderate PA (mPA) during neoadjuvant chemotherapy (NACT). We have investigated the longitudinal change of cytokines during NACT alone or combined with mPA.

Materials and methods: Twenty-three cytokines were analyzed in BC patients at consecutive timepoints: at baseline (T0), before starting mPA (T1), before surgery (T2), and after surgery (T3). mPA consisted of 3-weekly brisk-walking sessions for 9-10 consecutive weeks.

Results: Ninety-two patients were assessed: 21 patients refused mPA (untrained) and 71 agreed (trained). At T1, NACT induced significant up-regulation of interleukin (IL)-5, IL-6, IL-15, chemokine ligand (CCL)-2, interferon-γ, and C-X-C motif ligand (CXCL)-10 and reduction of expression of IL-13 and CCL-22. At T2, NACT and mPA induced up-regulation of IL-21, CCL-2, and tumor necrosis factor-α and reduction of expression of IL-8, IL-15, vascular endothelial growth factor, and soluble interleukin 6 receptor. Only CXCL-10 increased in untrained patients. A cytokine score (CS) was created to analyze, all together, the changes between T1 and T2. At T2 the CS decreased in trained and increased in untrained patients. We clustered the patients using cytokines and predictive factors and identified two clusters. The cluster A, encompassing 90% of trained patients, showed more pathological complete response (pCR) compared to the cluster B: 78% versus 22%, respectively.

Conclusions: mPA interacts with NACT inducing CS reduction in trained patients not observed in untrained patients, suggesting a reduction of inflammation, notwithstanding chemotherapy. This effect may contribute to the higher rate of pCR observed in the cluster A, including most trained patients.

Key words: moderate physical activity, breast cancer, cytokines, neoadjuvant chemotherapy, immune system, pathological complete response

INTRODUCTION

Breast cancer (BC) is the most common cancer among women worldwide. Mortality has declined steadily since the peak in 1989. In contrast, incidence has increased by 0.5% per year between 2010 and 2019.[1]

Several lifestyle factors have been associated with the increased incidence, including lack of physical activity (PA).

Regular engagement in PA has many benefits and contributes to the prevention of various diseases, including BC. Physically active women have up to 20% reduced risk of developing BC, supporting the importance of PA.[2]

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Furthermore, PA after BC diagnosis correlates with reduced tumor mortality rate in many studies.[3,4]

The effect of PA has been related to immune modulation[5] and modification of the tumor microenvironment (TME).[6] Consequently, there is interest in studying exercise as a candidate to complement anticancer treatment.[7] Data from preclinical studies are encouraging,[8] but need to be confirmed in clinical trials.

So far, exploratory research in humans has focused mainly on colon cancer and BC.[9,10] However, there is a knowledge gap regarding biological activity, adequate markers of response as well as dose and scheduling of PA.[11] The knowledge developed to date is considered adequate to move towards early clinical trials with particular interest in window of opportunity studies.[11]

Research in BC has studied the impact of different moderate or strength intensity PA in the preoperative or post-operative setting, and during chemotherapy and/or radiotherapy.[12]

However, limited information exists on the effect of PA on circulating cytokines during neoadjuvant chemotherapy (NACT). Obtaining data on circulating cytokines during PA and NACT could help recognize TME modifications, as it has been known since a long time that cytokines reflect the TME.[13] Hence, we conducted a study to explore the impact of mPA on circulating cytokines in BC patients undergoing NACT. We used moderate PA (mPA) because it can potentially be extended to a larger number of patients compared to other types of PA.

MATERIALS AND METHODS

This is a translational, prospective, observational, exploratory study approved by the S. Croce e Carle Hospital ethical committee (Study ONCO-331-2020). The study was carried out at S. Croce e Carle Hospital Cuneo, Italy and at Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico Milan, Italy.

Study population

Histologically proven triple-negative (TN), human epidermal growth factor receptor 2-positive (HER2+) and luminal B-like (LUM-B) BC patients suitable for NACT were eligible. Newly diagnosed, previously untreated clinical stage IB to IIIB BC, according to tumor-regional lymph node staging criteria of the American Joint Committee on Cancer seventh edition,[14] adequate hematological, renal, and hepatic function and Eastern Cooperative Oncology Group performance status of 0-1 were required. All patients signed informed consent.

Study design

All enrolled patients underwent blood sample collection for cytokine measurement at prespecified timepoints:

- Baseline (T0)
- Before mPA start (T1)
- Before surgery (T2)
- After surgery (T3)

Figure 1 shows the study design.

Two more timepoints, T4 and T5, were set at 6 and 12 months, post-surgery.

At each timepoint, 12 ml of peripheral blood was collected in EDTA-treated Vacutainer (BD, Franklin Lakes, NJ), centrifuged for 10 min at 340 *g* at room temperature, and plasma stored at −80°C.

Treatment

All participants received NACT according to their tumor subtype.

NACT consisted of intravenous, sequential epirubicin (90 mg/m^2) and cyclophosphamide (600 mg/m^2), every 2/3 weeks for four cycles, followed by weekly paclitaxel (80 mg/m^2) for 12 weeks. Weekly trastuzumab (4 mg/kg loading dose and then 2 mg/kg) was added in HER2+ tumors. Carboplatin (area under the concentration-time curve of 2 mg min/ml, weekly) and pembrolizumab (200 mg every 3 weeks) were administered to TN tumors. However, pembrolizumab was administered only to two patients, as in Italy it has been available since 2022.

Group allocation

All eligible patients were informed about the study and its potential benefits. Patients declining mPA were invited to provide blood samples for comparison with trained patients.

Therefore, participants were allocated to two groups according to their decision to undertake mPA and to allow additional blood test, or to allow additional blood test only.

Moderate physical activity group

Patients joining the training group were invited to undertake mPA session consisting of at least 60 continuative minutes of brisk-walking, three non-consecutive times per week, for 9-10 weeks, starting at day 1 of week 6 of paclitaxel. The last mPA session was expected 24-48 h before surgery.

Patients were not given any further recommendation, other than to avoid excessive activity, using the talk test.[15]

The adherence to the mPA program was self-reported at each follow-up visit.

Untrained group

Patients included in the untrained group received the same treatment and blood samples were collected at the same timepoints as the trained group.

Cytokine measurements

Twenty-three cytokines [interleukins (ILs), chemokines, interferon (IFN) and growth factors] were measured at each timepoint:

- IL-1β, IL-2, IL-12, IL-15, IL-18, IL-21, C-X-C motif ligand (CXCL-10), tumor necrosis factor (TNF)-α, chemokine ligand (CCL)-2, CCL-4, and IFN-γ, considered mainly Th1 cytokines[16];

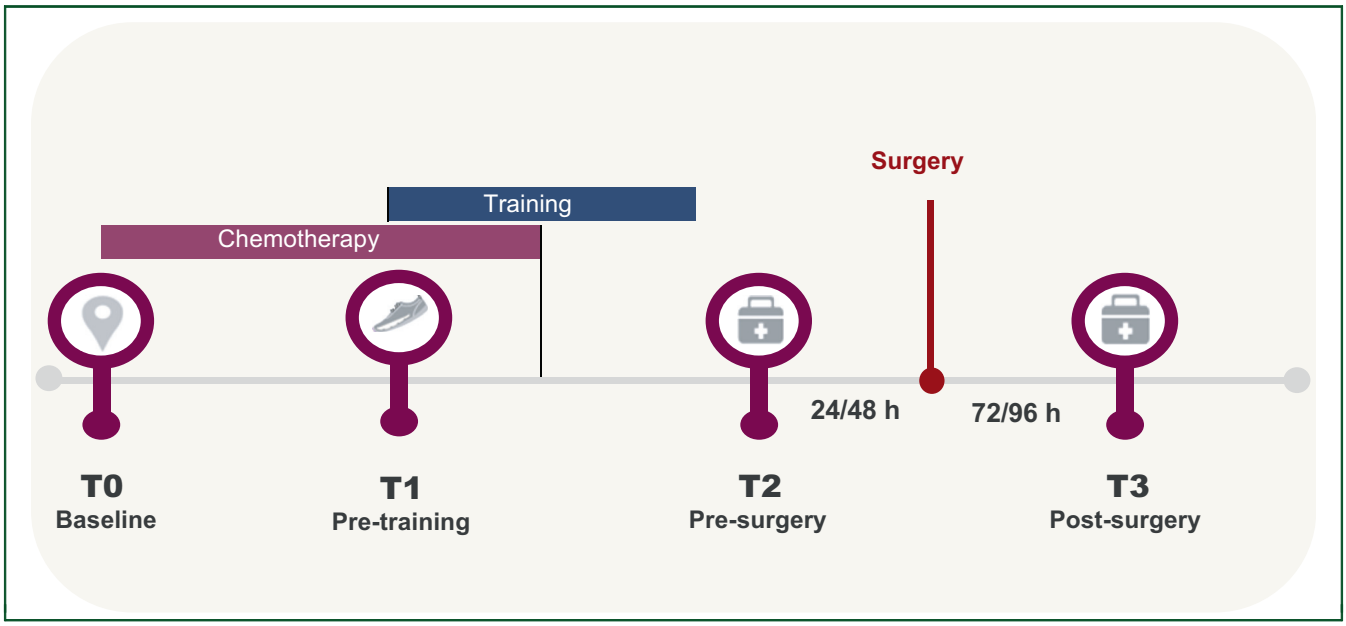

Figure 1. **Study design and timeline of blood samples collection.**

- IL-4, IL-5, IL-10, IL-13, and CCL-22 considered mainly Th2 cytokines[16];
- IL-6 and IL-8, associated with poor survival in many solid tumors[17,18];
- IL-7 important factor for immune cells development[19];
- Transforming growth factor (TGF)-β and vascular endothelial growth factor (VEGF) considered major immunosuppressive factors[20,21];
- IL-17 for its role in foster tumor progression[22];
- Soluble interleukin 6 receptor (sIL-6R) for its role in chronic inflammation and cancer.[23]

Plasma analysis (pg/ml) utilized Ella Simple Plex system[24] (ProteinSimple™, San Jose, CA). IL-21 was analyzed using the enzyme-linked immunosorbent assay method[25] (R&D System® Minneapolis, MN). Samples were centrally assessed at S. Croce e Carle Teaching Hospital.

Statistical analysis

The primary objective was to identify and quantify the cytokines that changed during mPA and NACT (comparison T1-T2).

Secondary objectives were to identify the following:

- The correlation between cytokine changes (T1-T2) and the pathological complete response (pCR) defined as no residual invasive tumor in the pathological specimen
- The changes of circulating cytokines during chemotherapy (comparison T0-T1)
- The changes of circulating cytokines before and after surgery (comparison T2-T3)
- A descriptive comparison between trained and untrained patients at the same timepoints

The sample size was calculated using the effect size method considering a Choen's d of 0.68 as observed for a candidate, IL-6, between the trained and untrained group.[26,27] Considering a power of 0.8 and a probability level of 0.05, 71 patients were required.

Comparison among the median values of each variable at each timepoint was analyzed in both groups using the Mann—Whitney *U* test; for comparison in normal distribution, Student's *t*-test was employed. Differences between T2 and T1 were computed as dependent variables and confounding factors were employed as independent variables (IV) in analysis of covariance (ANCOVA) analysis. The Wilcoxon signed-rank test was carried out for paired samples, within the same group. Differences in categorical variables were analyzed with the χ^2 test or Fisher's exact test.

Normalization of variables was realized with z-score.

Hierarchical clustering on principal components (HCPC) was computed to apply a dimension reduction and cluster our population regarding all variables.

The Mann—Whitney *U* test, Student's *t*-test, Wilcoxon signed-rank test, and χ^2 test or Fisher's exact test were carried out with GraphPad v.5 (GraphPad Software, Boston, MA).

ANCOVA was assessed using SPSS V.24 (IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY). FactoMiner and Factoextra packages were used for HCPC analysis in R 4.3.0.

In all tests, $P < 0.05$ was regarded as significant. The Benjamini—Hochberg (B-H) procedure was applied to decrease false discovery rate at 5%.[28]

RESULTS

From June 2020 to February 2023, 99 patients have been enrolled. Seven patients withdrew their consent before mPA. Nine-two patients completed the study. Seventy-one patients joined the trained and 21 the untrained group

Table 1. Patients' characteristics

Variables	Patients, $n = 92$	Trained, $n = 71$	Untrained, $n = 21$
Age, years (median, IQR)	54 (46-62)	53 (45-62)	56 (51-64)
BMI, kg/m^2 (median, IQR)	24.6 (21.5-27.8)	24.5 (21.5-27.2)	25.4 (21.3-31.5)
LDH (median U/l, IQR)	199 U/l (178-222)	197 U/l (175-219)	202 U/l (174-230)
Subtype *n* (%)			
LUM-B	32 (34.8)	21 (29.6)	11 (52.4)
HER2+	40 (43.5)	35 (49.6)	5 (23.8)
TN	20 (21.7)	15 (21.1)	5 (23.8)

BMI, body mass index; HER2+, human epidermal growth factor receptor 2-positive; IQR, interquartile range; LDH, lactate dehydrogenase; LUM-B, luminal-B; *n*, number; TN, triple-negative.

(Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2024.103665).

The main patients' characteristics are reported in Table 1.

Longitudinal cytokine analysis

Analysis T0-T1

Considering the whole population, we found that IL-5, IL-6, IL-15, CCL-2, IFN-γ, and CXCL-10 increased and IL-13 and CCL-22 decreased (Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2024.103665).

Analysis T1-T2

In trained patients, we found that IL-21, CCL-22, and TNF-α increased while IL-8, IL-15, VEGF, and sIL-6R decreased significantly (Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2024.103665). In the untrained group only CXCL-10 increased significantly (Figure 2).

After the ANCOVA analysis, the difference of the level of expression of both IL-21 and VEGF remained significant ($P = 0.044$ and $P = 0.011$, respectively) (Supplementary Table S3, available at https://doi.org/10.1016/j.esmoop.2024.103665).

Analysis T2-T3

We showed that the cytokine profile is unchanged in untrained patients, while IL-6 increases and CXCL-10

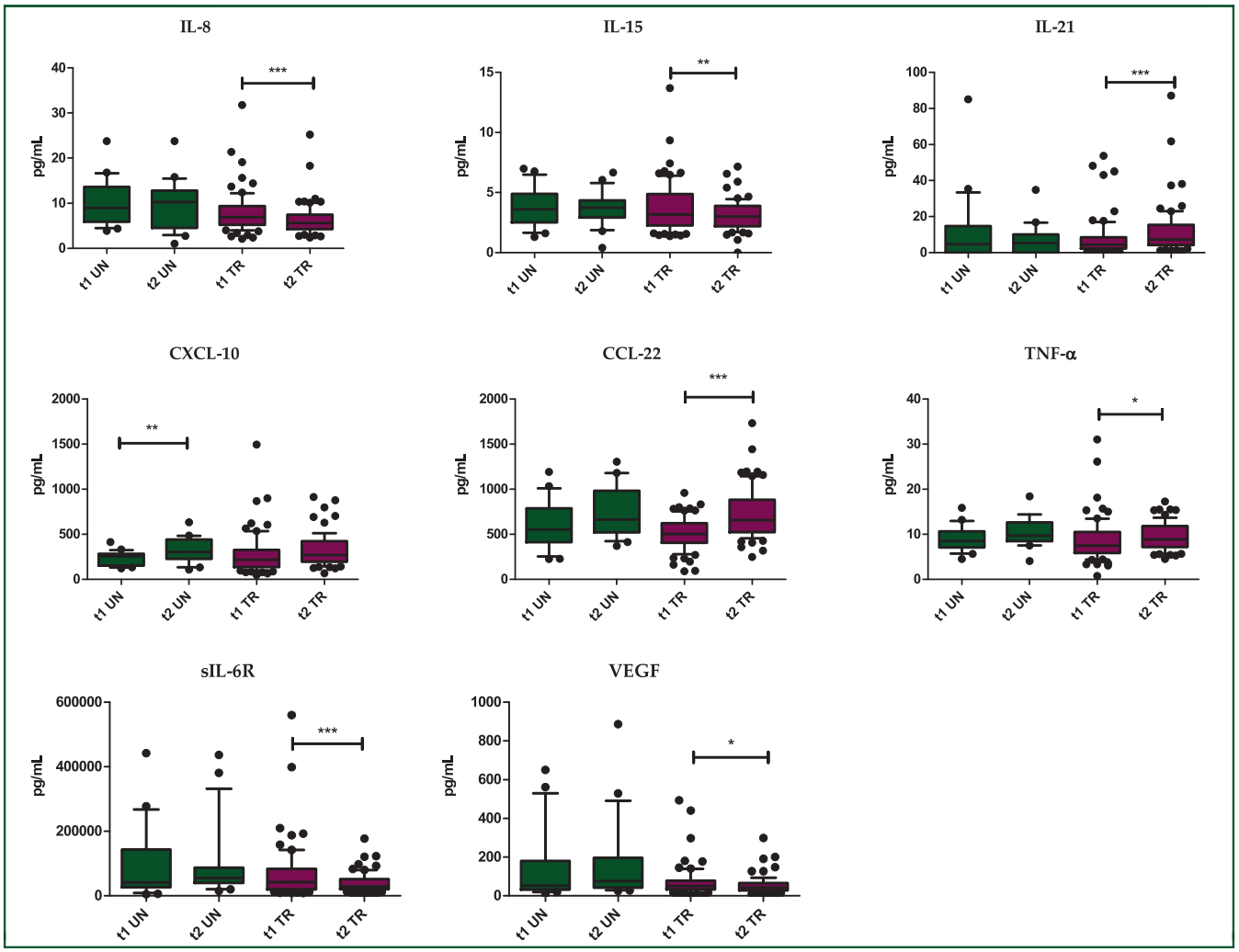

Figure 2. Distribution of cytokines in the trained (TR) and untrained (UN) group at T1 and T2. *$P < 0.05$, ** $P < 0.01$, ***$P < 0.001$. The difference among the means of these eight variables was tested using analysis of covariance multivariate analysis while controlling for several covariates (age, BMI, and tumor subtype). Levene's test and normality check were carried out and the assumptions were met.

BMI, body mass index; CCL, chemokine ligand; CXCL, C-X-C motif ligand; IL, interleukin; sIL-6R, soluble interleukin 6 receptor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

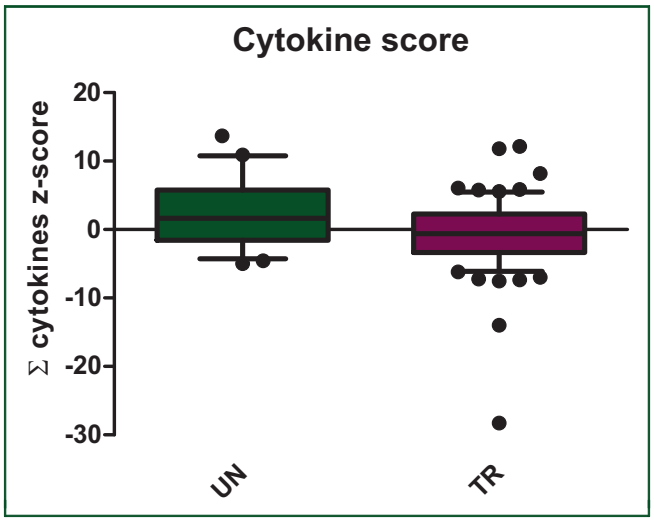

Figure 3. Cytokine score (T2-T1) for the 16 cytokines selected both in the trained (TR) and untrained (UN) group. *$P < 0.05$ (descriptive).

decreases in trained patients (Supplementary Table S4, available at https://doi.org/10.1016/j.esmoop.2024.103665).

Considering that the final effect of each cytokine is context dependent, we gathered in a score the changes of cytokine values that presented at least a trend towards statistical significance ($P < 0.2$)[29] between T1 and T2, to strengthen their context-dependent effect. We identified 16 cytokines: IL-4, IL-6, IL-15, VEGF, IFN-γ, TNF-α, CCL-22, TGF-β, IL-21, IL-8, IL-7, sIL-6R, CXCL-10, IL-18, IL-10, and IL-17.

We also computed the delta from T2 and T1 for each cytokine value and for each patient, calculating a z-score. The patients' z-scores were added to each other obtaining a 'cytokine score' (CS), as shown in Figure 3. The median of the CSs was higher in the untrained group compared to the trained group ($P = 0.0143$, descriptive only). These data suggest an opposite trend between trained and untrained patients.

The same method was used to analyze the longitudinal changes between T2 and T3; however, no difference has been recorded.

In addition, a subgroup analysis was carried out employing CS in a generalized linear model, using trained status as a fixed factor, BC subtype as the covariate, and the interaction between status and subtype. Trained status demonstrated a significant effect on CS also considering its interaction with subtype (Supplementary Table S5, available at https://doi.org/10.1016/j.esmoop.2024.103665).

Interestingly, the distribution of CS among BC subtypes, in the trained and untrained groups, pointed out HER2+ subtype as the only one significantly correlated with the mPA effect, thus suggesting a lowering of inflammation (Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2024.103665).

Comparison between timepoints

At T0, T1, T2, and T3 a significantly larger amount of IL-4 and IL-17 was observed in untrained patients in comparison to trained patients (Supplementary Tables S6- S9, available at https://doi.org/10.1016/j.esmoop.2024.103665), suggesting a significant difference between the groups (descriptive only). In addition, at T2, untrained patients showed a higher level of IL-6, sIL-6R, IL-7, and VEGF compared to trained patients (Supplementary Figure S3, available at https://doi.org/10.1016/j.esmoop.2024.103665).

Table 2. Patients allocation between clusters according to TR and UN status

	Clusters		Total
	A	B	
Status			
TR			
Count	54*	17*	71
% within status	76.1%	23.9%	100.0%
% within clusters	90.0%	53.1%	77.2%
% of total	58.7%	18.5%	77.2%
UN			
Count	6*	15*	21
% within status	28.6%	71.4%	100.0%
% within clusters	10.0%	46.9%	22.8%
% of total	6.5%	16.3%	22.8%
Total			
Count	60	32	92
% within status	65.2%	34.8%	100.0%
% within clusters	100.0%	100.0%	100.0%
% of total	65.2%	34.8%	100.0%

'Count' is the number of patients; 'within status' described the percentage of patients, considering the whole population; 'within clusters' described the percentage of patients considering the total patients grouped by the reference cluster.
TR, trained; UN untrained.
*$P < 0.05$ (descriptive).

Response

Overall, pCR was observed in 36 out of 92 patients (39%): 30 out of 71 (42%) trained and 6 out of 21 (29%) untrained, respectively.

We carried out a HCPC clustering method over principal component analysis applying all cytokines analyzed, age, body mass index, and lactate dehydrogenase (LDH) (Supplementary Figure S4, available at https://doi.org/10.1016/j.esmoop.2024.103665). Two main clusters were highlighted. Cluster A, the largest, accounting for 60 patients, included a significantly higher percentage of trained patients (54 out of 71; 76.1%, $P < 0.001$) compared to cluster B, (Table 2).

In addition, patients in cluster A achieved a significantly higher percentage of pCR (28 out of 36 patients, 77.8%, $P = 0.043$) compared to patients in cluster B (Table 3). It is worth noting that cluster A encompasses 90% of trained patients (54 out of 60 patients) and 47% of them (28 out of 60 patients) obtained pCR. It is noteworthy that, no significant difference was observed among BC subtypes within both clusters (Supplementary Table S10, available at https://doi.org/10.1016/j.esmoop.2024.103665): considering HER 2+ BC patients, 27 and 13 patients were represented in cluster A and cluster B, respectively, and within each cluster the percentage is quite similar: 45% in cluster A and 41% in cluster B. A similar distribution was observed for

Table 3. pCR distribution between clusters

	Clusters		Total
	A	B	
pCR			
Yes			
Count	28*	8*	36
% within pCR	77.8%	22.2%	100.0%
% within clusters	46.7%	25.0%	39.1%
% of total	30.4%	8.7%	39.1%
No			
Count	32*	24*	56
% within pCR	57.1%	42.9%	100.0%
% within clusters	53.3%	75.0%	60.9%
% of total	34.8%	26.1%	60.9%
Total			
Count	60	32	92
% within pCR	65.2%	34.8%	100.0%
% within clusters	100.0%	100.0%	100.0%
% of total	65.2%	34.8%	100.0%

'Count' is the number of patients; 'within pCR' described the percentage of pCR considering the total of pCR; 'within clusters' described the percentage of pCR considering the total patients grouped by the reference cluster.
pCR, pathological complete response.
*$P < 0.05$ (descriptive).

LUM-B (33% in cluster A and 38% in cluster B) and TN subtypes (22% in both clusters).

DISCUSSION

In this study, we investigated the effect of mPA in BC patients during NACT on a panel of circulating cytokines. The World Health Organization defines PA as 'any bodily movement produced by skeletal muscles that requires energy expenditure'.[30] Physical exercise is a planned, structured, and repetitive activity aimed at improving physical fitness.[31] The terms are often used interchangeably but are different,[32] with mPA being a subset of PA that encompasses various activities (walking, cycling, wheeling, running, sports, active recreation, and play) and is accessible to all skill levels.[33]

Therefore, intuitively, it can be proposed to more patients than physical exercise. The mPA duration and frequency (180 min per week in three sessions) in our study is aligned with the literature.[34] The primary goal of our study was to evaluate the effect of mPA on circulating cytokines during NACT in BC patients. Between T1 and T2, 7 out of 23 cytokines significantly changed during mPA while in untrained patients only CXCL-10 raised significantly.

After ANCOVA analysis only IL-21 and VEGF remained statistically significant between T1 and T2. IL-21 is mainly produced by CD4+ T cells. In particular, IL-21 is known to be able to stimulate the proliferation and function of crucial effector cells such as CD8+ and natural killer cells.[35] VEGF inhibits trafficking, proliferation, and effector functions of cytotoxic T lymphocytes.[36] Overall, the increase of IL-21 and the decrease of VEGF seem to indicate the same phenomenon leading to a better immune response.

Interestingly, mPA is able to reverse the increase of IL-15 and the decrease of CCL-22 observed between T0 and T1. Indeed, IL-15 increased between T0 and T1 while it decreased between T1 and T2; CCL-22 decreased between T0 and T1 and increased between T1 and T2. These statistically significant changes were not observed in untrained patients.

CXCL-10 increased significantly between T0 and T1 in both groups. However, between T1 and T2, the increase remained significant only in untrained patients.

Facing the change of each cytokine based on its dominant pro-tumor or antitumor effect can be misleading. Indeed, cytokines work together in a network and the effect of each cytokine depends on the context.[37]

This aspect explains why markers of chronic inflammation are not yet identified.[38] Indeed, chronic inflammation differs from acute inflammation only in duration and low magnitude.[39] In developing tumors, pro-tumorigenic and anti-tumorigenic immune mechanisms are balanced in the equilibrium phase.

However, if the tumor is not eliminated, the continuous release of inflammatory cytokines switches the environment towards a chronic pro-tumorigenic inflammation, leading to the escape phase.[39]

Most established tumors harbor a profound immune suppressive inflammatory context.[39-41] Therefore, the measurement of a panel of cytokines may be more appropriate to define the inflammatory status of cancer.[37]

We tried to overcome the potentially misleading effect derived from the analysis of each cytokine, by making a 'CS'. The 'CS' takes into account all the cytokines showing major changes between T1 and T2. This score was lower at T2 in the trained compared to the untrained group.

Considering that circulating cytokines may mirror the TME,[13] all the above data suggest a modulation towards a less-inflamed environment. Data from a systematic review and meta-analysis of PA in BC survivors lead to similar conclusions supporting our opinion.[42]

To avoid the confounding effect of cancer subtype, we analyzed the cytokine trajectories for each subtype between T1 and T2, according to the trained and untrained status.

Considering chronic inflammation, the HER2+ population gained the major achievement from mPA. No benefit has been observed in the LUM-B group. TN BC patients displayed an intermediate behavior.

The effect observed in the HER2+ subgroup and, possibly, in the TN subtype, may be related to the highly inflamed tumor immune microenvironment of these tumors,[42-44] making them more prone to react to the effects of mPA. Once again, we underline the descriptive-only value of these comparisons.

At T0, some cytokines were significantly higher in untrained patients compared to trained patients (IL-4 and IL-17), suggesting a baseline difference between the two groups. It is noteworthy that, these cytokines remained higher in the untrained group at each following timepoint. Intriguingly, in the trained group, both IL-4 and IL-17 showed very consistent values across the four timepoints, while in untrained patients they exhibited wide inter-timepoint variations. We could speculate that mPA also

influenced these cytokines, preventing major modification in trained patients. However, we have to keep in mind that the comparison between trained and untrained patients is descriptive only.

Data from observational studies have shown that aerobic PA can reduce chronic inflammation in patients with chronic inflammatory diseases.[45]

Xu et al. highlighted that several studies showed a decrease in inflammatory cytokines along with mPA in BC survivors, recently.[46] In this context, our study is the first suggesting such an effect during NACT.

After surgery (T3), the trained group showed a significant increase of IL-6 and a reduction of CXCL-10. We did not observe significant changes in untrained patients. One might speculate that this observation is in line with the reduction of the pre-existing inflammatory status in trained patients at T2, which would make a post-surgical inflammatory response more easily detectable.

Finally, we applied a dimension reduction approach considering every variable available at the end of PA window (T2). Using an unsupervised clustering method, we identify two macroclusters. Cluster A included both a vast majority of trained patients and a higher pCR rate compared to cluster B. It must be stressed that BC subtypes were well balanced between the two clusters. This is particularly important considering that the HER2+ and LUM-B subtypes were numerically imbalanced between trained and untrained patients. In other words, the vast majority of pCR rate was achieved in trained patients who were mostly represented in cluster A irrespective of BC subtype. Sanft et al.[47] recently studied PA and nutritional intervention effects on chemotherapy dose intensity in BC patients. They found higher pCR rate in a subgroup of patients receiving NACT, PA, and nutrition intervention compared to those receiving usual care. However, pCR was a secondary study objective and, although intriguing, caution is warranted in interpreting the result.

Most clinical studies on circulating cytokines during PA or exercise involve BC survivors, off from chemotherapy and cancer confounding effects.[48] Among studies in BC patients during chemotherapy, few considered NACT, limiting comparison of our results with existing literature.

Malveiro et al. have recently reviewed the effects of exercise training on cancer patients undergoing neoadjuvant treatment[49] and found 27 randomized studies published between 2012 and 2022. Only five studies were specifically addressed to BC patients, and all of them accrued 10-30 patients. Only one investigated the modification of some circulating cytokines.[50] The authors studied 20 patients undergoing either NACT or NACT with supervised physical exercise. The results demonstrated a drop in IL-1β in both trained and untrained patients, an increase of IL-8 in both groups and an increase of IL-2 in trained patients only. In our series, neither IL-1β nor IL-2 significantly changed between T1 and T2 in both trained and untrained patients and solely IL-8 reduction was observed in trained patients. It is difficult to explain these differences, but the two studies are different in many key points, including the type of activity (mPA versus supervised physical exercise), number of trained patients (71 versus 10), timepoints of sample collection, and patients' characteristics.

Our study has some limitations. The study is not randomized and the comparison between trained and untrained patients has only descriptive value. We avoided formal randomization considering the nature of the study. In fact, in a randomized study, all patients must be informed about the possible beneficial effect of mPA. In our opinion, the patients who would have liked to join the study but had fallen in the control group might have started spontaneous PA, making the results of the study misleading.

Secondly, we used a CS to evaluate, all together, the major changes observed between T1 and T2 separately in trained and untrained patients. To the best of our knowledge, this is the first attempt to overcome the confounding effect induced by the context on the role of each cytokine through the cumulative analysis of a cytokine profile. However, it must be taken into account that the use of this score is arbitrary and requires future validation. Therefore, its clinical relevance is still undetermined.

Thirdly, mPA starts during the second part of the therapeutic program not together with NACT. This aspect of the study design may seem arbitrary. However, this choice is due to the different intensity of chemotherapy in the first and second treatment periods, based on the use of anthracycline and paclitaxel, respectively. The use of anthracycline is frequently associated with side-effects that potentially could seriously interfere with actual participation in the mPA program.

CONCLUSIONS

The Neo-Runner study shows that mPA, which can be approached by most patients, induces changes in the expression of many circulating cytokines and therefore can modulate the immune system. The study also shows that this effect is detectable even in BC patients during NACT. This effect may be related to a reduction of chronic inflammation and has been observed notwithstanding the inflammatory effect of chemotherapy. It is known that chemotherapy is a 'double-edged sword', able to fight cancer, while supporting chronic inflammation favoring cancer resistance and tumor progression.[51]

We hypothesize that a reduction of cancer-related chronic inflammation during NACT might improve pCR and, ultimately, ameliorate patients' outcome.

To confirm these data and to verify the clinical benefit of mPA during NACT in BC patients, a randomized multicenter study will be planned.

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DISCLOSURE

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DATA SHARING

Data supporting this study are not publicly available due to ethical reasons. Please contact MP. matteo.babeuf@gmail.com.

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