ORIGINAL RESEARCH

Elevated Sera IL-6 and NK-T Cells Associated With Increased Pathological Complete Response in HER2-positive Breast Cancer With Carboplatin-based Neoadjuvant Therapy

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ABSTRACT

Context • Neoadjuvant therapy is the primary treatment for stage II to III breast cancer (BC). The heterogeneity of BC challenges the identification of effective neoadjuvant regimens and of the related sensitive populations.

Objective • The study intended to explore the predictive role of inflammatory cytokines, immune-cell subsets, and tumor-infiltrating lymphocytes (TILs) for the accomplishment of the pathological complete response (pCR) after a neoadjuvant regimen.

Design • The research team conducted a phase II, single-armed, open-label trial.

Setting • The study took place at the Fourth Hospital of Hebei Medical University in Shijiazhuang, Hebei, China.

Participants • Participants were 42 patients at the hospital receiving treatment for human epidermal growth factor receptor 2 (HER2)-positive breast cancer (BC) between November 2018 and October 2021.

Intervention • Participants received neoadjuvant therapy of six cycles of docetaxel, carboplatin, and trastuzumab (TCbH).

Outcome Measures • The research team: (1) measured 13 cytokines and immune-cell populations in peripheral blood prior to neoadjuvant therapy administration; (2) measured TILs in tumor tissues; (3) analyzed correlations among biomarkers and pCR.

Results • Of the 42 participants, 18 achieved pCR (42.9%) after the neoadjuvant therapy, with 37 having an overall response rate (ORR) of 88.1%. All participants experienced at least one short-term adverse event. The most common toxicity was leukopenia, with 33 participants (78.6%), while no cardiovascular dysfunction occurred. Compared with the non-pCR group, the pCR group had higher serum levels of tumor necrosis factor alpha (TNF-α), with $P = .013$; interleukin 6 (IL-6), with $P = .025$; and IL-18, with $P = .0004$. Univariate analysis showed that IL-6 (OR, 3.429; 95% CI,1.838-6.396; $P = .0001$) had a significant correlation with pCR. Participants in the pCR group had a higher level of natural killer T (NK-T) cells ($P = .009$) and a lower ratio of cluster of differentiation 4 (CD4):CD8 ($P = .0014$) before neoadjuvant therapy. Univariate analysis linked a high population of NK-T cells (OR, 0.204; 95% CI,0.052-0.808; $P = .018$), a low CD4:CD8 ratio (OR, 10.500; 95% CI, 2.475-44.545; $P = .001$), and TILs expression (OR, 0.192; 95% CI, 0.051-0.731; $P = .013$) to pCR.

Conclusions • Immunological factors, including IL-6, NK-T cells, CD4+ T versus CD8+ T ratio, and TILs expression were significant predictors for response to TCbH neoadjuvant therapy with carboplatin (*Altern Ther Health Med.* 2023;29(3):246-253).

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Neoadjuvant therapy is the primary treatment for stage II to III breast cancer (BC).[1,2] Two studies found that individuals with human epidermal growth factor receptor 2 (HER-2)-positive disease who had a pathological complete response (pCR) under neoadjuvant therapy could have a better event-free survival and overall survival.[3,4]

A pCR indicates the lack all signs of cancer in tissue samples removed during surgery or in a biopsy after radiation or chemotherapy. Due to pCR's correlation with a favorable prognosis, clinicians use it as the primary goal of neoadjuvant therapy for some cancers, including HER2-positive BC. More important, a significant number of patients with residual tumors who can't reach pCR are at high risk for disease recurrence and metastasis.[5]

The heterogeneity of BC challenges the identification of effective neoadjuvant regimens and of the related sensitive populations. For that reason, the ability to identify prospective patients for a particular neoadjuvant approach is an urgent need.

Bianchini and Gianni found that the immune system controls heterogeneous tumors as well as the effects of HER2-targeted therapy.[6] Horii et al and Hamilton et al found that both innate and adaptive immunity are important in HER2-targeted therapy.[7,8]

Denkert et al found that patients with a high, immune-associated gene expression and elevated tumor-infiltrating lymphocytes (TILs) before neoadjuvant treatment had a higher likelihood of achieving pCR than those with a low expression and infiltration.[9] Hamilton et al found an association between a high level of TILs and better clinical outcomes in HER2-positive BC.[10]

Howell et al's clinical trial found different pCR rates in HER2-positive BC for different neoadjuvant options.[11] The TRYPHAENA study evaluated the cardiac tolerability of neoadjuvant pertuzumab and trastuzumab in a neoadjuvant therapy for HER2-positive BC.[12] Those researchers found that the pCR rate was upregulated in the anthracycline-free (docetaxel + carboplatin + Trastuzumab) group, for 66.2% of the group's participants, than in the other two anthracycline-containing groups, at 61.6% and 57.3% of participants, with lower rates of symptomatic left ventricular systolic dysfunction.

For participants who had stage II-III, HER2-positive disease, the TRAIN-2 trial revealed similar pCR rates for neoadjuvant regimens with or without anthracyclines, at 67% and 68%, respectively.[13] Those researchers found that the anthracycline group was related to a high risk of febrile neutropenia and showed decline in the left ventricular ejection fraction.

Docetaxel, Carboplatin, and Trastuzumab (TCbH)

TCbH is a useful treatment option for HER2-positive BC that has less cardiotoxicity than other neoadjuvant regimens.[14]

Docetaxel. Docetaxel is designed to promote apoptosis of cancer cells by interfering with the mitotic process of cells and hindering the replication of the genetic material deoxyribonucleic acid. It is effective in advanced breast cancer, ovarian cancer, non-small cell lung cancer and other cancers. The most common side effects of docetaxel are nausea and vomiting, anorexia, physical weakness, and even bone marrow suppression.[15,16]

Carboplatin. Carboplatin is a broad-spectrum antitumor agent with similar chemistry to cisplatin,[17] Studies have shown that the use of Carboplatin in the chemotherapy of breast cancer can improve the pCR of patients to 45.7%, with significant results.[18]

Trastuzumab. Trastuzumab is a monoclonal antibody that targets HER2 and may enhance clinical outcomes for patients with the HER2-positive subtype.[19,20] For patients with HER-2-overexpressing diseases, neoadjuvant therapy containing trastuzumab is the backbone of treatment.[21]

Combining chemotherapy with trastuzumab during neoadjuvant treatment boosts anticancer immunity and upregulates the antitumor activity of agents. pCR and better clinical outcomes.[22,23] Zardavas et al found a correlation between the therapeutic effects of trastuzumab and the heterogeneous status of the individual immune system.[24]

Current Study

The studies focusing on the correlation between efficacy and immune system status during neoadjuvant therapy are contradictory.

The current study intended to explore the predictive role of inflammatory cytokines, immune-cell subsets, and tumor-infiltrating lymphocytes (TILs) for the accomplishment of the pathological complete response (pCR) after a neoadjuvant regimen.

METHODS

Participants

The research team conducted a phase II, single-armed, open-label trial (Clinical trials.gov identifier: NCT03728829). The study took place at the Fourth Hospital of Hebei Medical University in Shijiazhuang, Hebei, China. Potential participants were patients at the hospital receiving treatment for human epidermal growth factor receptor 2 (HER2)-positive breast cancer (BC) between November 2018 and October 2021.

The study included potential participants if they: (1) were women aged 18-70 years with primary BC; (2) had tumors that were HER2 3+ or 2+ and positive using fluorescence in-situ hybridization (FISH); (3) had an performance-status score of 0 or 1 under the guidelines of the Eastern Cooperative Oncology Group (ECOG) - American College of Radiology Imaging Network (ACRIN) Cancer Research Group[25]; (4) had stage II to III cancer based on the 8th Edition of the *American Joint Committee on Cancer (AJCC) Cancer Staging Manual*[26]; (5) had had an evaluation of the estrogen receptor, progesterone receptor, and HER2 status of the primary tumor that followed the American Society of Clinical Oncology (ASCO) guidelines[27]; and (6) had a diagnosis and proposed treatment that our hospital's ethics committee had confirmed and reviewed.

The study excluded potential participants if they: (1) had metastatic disease, as determined using computed tomography (CT) or magnetic resonance imaging (MRI); (2) had a history of chemotherapy, radiotherapy, or endocrinal therapy; (3) had a left ventricular ejection fraction (LVEF) of <55%, as determined using an echocardiogram (ECHO) or multigated acquisition scan (MUGA), or had significant symptoms or signs of heart failure; or (4) had major organ dysfunction that prohibited them from receiving chemotherapy.

Participants signed written informed consent forms. The Institutional Ethnics Committee of the Fourth Hospital of Hebei Medical University (No. 2018096) approved the study's protocols. This experiment will be conducted in strict compliance with the Declaration of Helsinki.

Procedures

Evaluation of circulating biomarkers. The research team measured levels of 13 cytokines, circulating lymphocytes, immune-cell populations, and TILs in tumor tissue.

At baseline before neoadjuvant treatment, the research team collected participants' peripheral blood samples centrifuged them at 1000×g, and detected the biomarkers using a Cytometric Bead Array kit (Becton Dickinson, Franklin Lakes, New Jersey, USA). The team also assessed participants' immune-cell subpopulations using flow cytometry with equipment from manufacturer (BD Bioscience, Franklin Lakes, New Jersey, USA) and the related antibodies using a kit from (BD Bioscience, Franklin Lakes, New Jersey, USA).

The research team also measured participants' stromal TILs before neoadjuvant treatment using hematoxylin and eosin (H&E) staining from Shanghai Hu Zhen Industrial Co. (Shanghai, China), and tested them following the guidelines of the International TILs Working Group.[28] The team acquired the TILs as a percentage of the immune cells in the tumor's stromal tissue that exhibited mononuclear infiltrate and analyzed the number of TILs continuously.

Intervention: Participants received treatment with neoadjuvant therapy intravenously and primary surgical therapy. The therapy occurred for six cycles, with each cycle lasting 21 days and with participants receiving the therapy on day 1 of treatment and then every three weeks on the first day of each new cycle. They received 75 mg/m^2 of docetaxel, carboplatin at area under the curve [AUC] 6, and 8 mg/kg of trastuzumab in cycle 1 and 6 mg/kg after cycle 1.

Tumor and axillary lymph node status. The research team checked each status three times, before neoadjuvant therapy and at the end of the third and sixth cycles. Participants with confirmed progressive disease (PD) underwent surgery or received another neoadjuvant chemotherapy regimen.

Tumor response. The research team conducted an evaluation of the response, using definitions based on Response Evaluation Criteria in Solid Tumors Committee (RECIST),[29] with pCR = no invasion.

Adverse events (AEs). The research team classified any AEs into grades 1-4 based on the National Cancer Institute's (NCI's) Common Terminology Criteria for Adverse Events (CTCAE), v5.0.[30] The team executed hematological and biochemical assays every week and performed cardiac ultrasonographic examinations and electrocardiograms prior to every cycle.

Outcome measures. The primary endpoint was the correlation of the circulating and tissue biomarkers to clinical outcomes after the neoadjuvant treatment. The secondary endpoints were pCR and safety. For the biomarkers, the research team divided participants into two groups, those who achieved pCR, the pCR group, and those who didn't, then non-pCR group.

Outcome Measures

Efficacy. This analysis included all participants. The research team evaluated the pCR and the overall response rate (ORR). The team also classified participants as having a complete response (CR) ORR, a partial response (PR), stable disease (SD), or progressive disease (PD).

Adverse events (AEs). The research team examined 11 adverse events: (1) leukopenia, (2) nausea and vomiting, (3) diarrhea, (4) anemia, (5) mucositis, (6) liver dysfunction, (7) peripheral neuropathy, (8) thrombocytopenia, (9) cardiovascular dysfunction, (10) thrombocytopenia, and (11) fatigue. The team divided participants into 2 groups, grade 1-2 and grade 3-4 based on the CTCAE. Grade 1: Mild, asymptomatic or mild, clinically or diagnostically detectable only, no intervention required. grade 2: Moderate, minimal, localized, or non-invasive intervention in age-related instrumental limitations in activities of daily living. grade 3: Severe, but not immediately fatal, requiring hospital intervention or extended hospitalization, disability, spontaneous limitations in activities of daily living. grade 4: Life- threatening; requires urgent intervention. grade 5: death.

Cytokines and immune-cell subsets. For each cytokine and immune-cell subset, the research team performed a univariate and a multivariate analysis to find the correlations between pCR and participants with different cytokine levels and subsets: (1) TNF-α, <3.67 versus ≥3.67 pg/ml; (2) IL-6, <5.52 versus ≥5.52 pg/ml; (3) NK-T, <16% versus ≥16%; and (4) CD 4:CD8 ratio, <3.16 versus ≥3.16. The examined cytokines were IL-1β, TNF-α, IL-6, interferon-gamma (IFN-γ), IL-10, IL-18, IL-33, IL-8, IFN-α, IL-17, IL-23, C-C motif chemokine ligand 2 (CCL2), and IL-12p70. The examined subsets were lymphocytes, CD3+ T cells, B cells, NK cells, NK-T cells, Tregs, CD4+ T cells, CD8+ T cells, CD4:CD8 ratio, CD3+PD1+ T cells, CD4+PD1+ T cells, CD8+PD1+ T cells, CD4+CD8+ T cells, and CD4-CD8-T cells.

BC characteristics and TILs. For each characteristic, the research team performed a univariate and a multivariate analysis to find the correlations between pCR and participants with different characteristics: (1) KI-67, 0-20% versus 30-100%; (2) TILS, (0-9% versus 10-100%); (3) tumor grade, IIIB- versus IIB to IIIA; (4) clinical T stage, cT1-2 versus cT3-4;

Figure 1. Flow Diagram

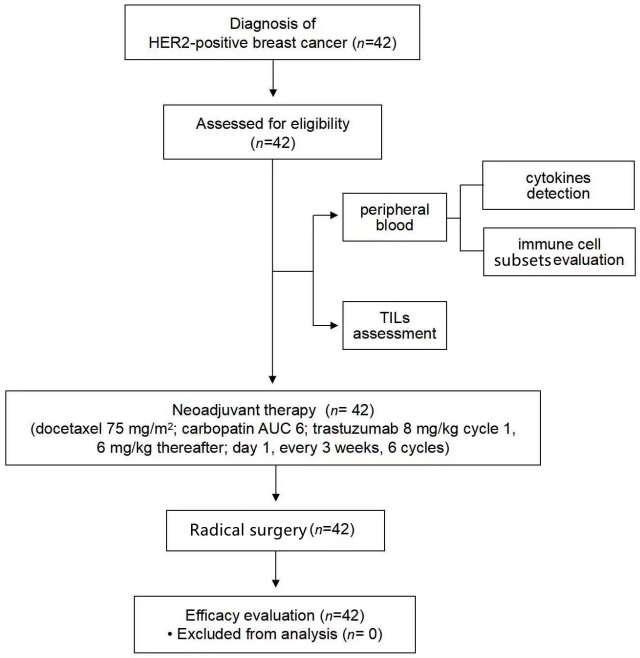


Table 1. Participants Demographic and Clinical Characteristics at Baseline (N = 42)

Characteristics	TCbH n (%)
Age, y	
Median	49
Range	39-69
<50	24 (57.1)
≥50	18 (42.9)
Menopausal	
Premenopausal	23 (54.8)
Premenopausal	19 (45.2)
Clinical Stage	
IIB	6 (14.3)
IIIA	9 (21.4)
IIIB	6 (14.3)
IIIC	21 (50.0)
Tumor Grade	
T1	3 (7.1)
T2	25 (59.5)
T3	4 (9.5)
T4	10 (23.8)
Initial Nodal Status	
N1	11 (26.2)
N2	10 (23.8)
N3	21 (50.0)
Hormone Receptor Status	
+	21 (50.0)
-	21 (50.0)
TILs	
0-9%	21 (50.0)
11-59%	21 (50.0)
≥60%	0 (0.00)

Abbreviations: TCbH, docetaxel, carboplatin, and trastuzumab; TILs, tumor-infiltrating lymphocytes.

(5) clinical nodal status, cN1 versus cN2-3; (6) age group, ≥50 versus <50 years; and (7) menopausal status, premenopausal versus postmenopausal. The research team classified the Tils as low (0-10%), intermediate (11-59%), or high (60-100%).

Statistical Analysis

The research team analyzed the data using SPSS 21.0 (IBM, Armonk, New York, USA). The team: (1) used the Chi-square test or Fisher's exact test to measure the changes in variables between baseline and postintervention; (2) performed an efficacy comparison, finding the 95% confidence interval (95% CI) for the proportional difference in each indicator using the Wilson method; (3) used the Cox proportional regression model, with the forward selection procedure, for the identification of independent prognostic factors in multivariate analysis. $P < .05$ was considered to be significant.

RESULTS

Participants

The study included and analyzed the data of 42 participants (Figure 1 and Table 1). Participants' median age was 49, with a range of 39 to 69, and six participants had stage IIB disease (14.3%), 9 stage IIIA (21.4%), 6 stage IIIB (14.3%), and 21 stage IIIC (50.0%), for 36 participants with stage III disease (85.7%).[21] Of the 42 participants, 21 had hormone-receptor-positive status (50%), while the other 21 showed a negative expression (50%).

Efficacy

Table 2 shows the clinical response. All participants completed the six cycles. Of the 42 participants, 8 (19.0%) had a complete response (CR), 29 (69.0%) had a partial response (PR), and five (11.9%) had stable disease (SD). After three cycles, no participants had progressive disease (PD).

After the 6 cycles of neoadjuvant therapy, the overall response rate (ORR) was 88.1%, for 37 out of the 42 participants, and 18 achieved pCR (42.9%).

Table 2. Efficacy (N = 42)

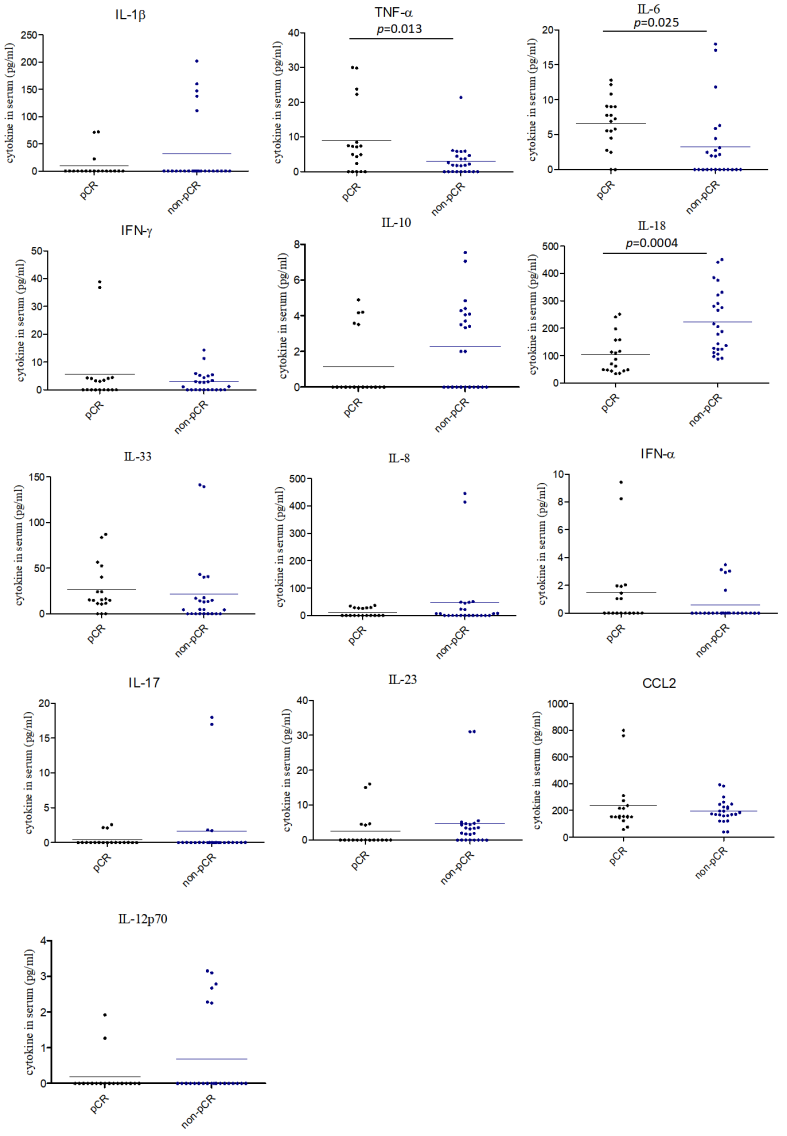
	TCbH n (%)
pCR	18 (42.9)
ORR	37 (88.1)
All	42 (100.0)
CR	8 (19.0)
PR	29 (69.0)
SD	5 (11.9)
PD	0 (0.0)
All	42 (100.0)

Abbreviations: CR, complete response; ORR, overall response rate; pCR, pathological complete response; PD, progressive disease; PR, partial response; SD, stable disease.

Table 3. Adverse Events (N = 42)

Adverse events	Grade 1-2 n (%)	Grade 3-4 n (%)
Leukopenia	33 (78.6)	1 (2.4)
Nausea/vomiting	31 (73.8)	0 (0.0)
Diarrhea	13 (30.9)	0 (0.0)
Anemia	13 (30.9)	0 (0.0)
Mucositis	12 (28.6)	0 (0.0)
Liver dysfunction	11 (26.2)	0 (0.0)
Peripheral neuropathy	9 (21.4)	0 (0.0)
Thrombocytopenia	6 (14.3)	1 (2.4)
Cardiovascular dysfunction	0 (0.0)	0 (0.0)
Thrombocytopenia	11 (26.2)	0 (0.0)
Fatigue	2 (4.8)	0 (0.0)

Figure 2. Inflammatory Cytokine Expression at Baseline Before Neoadjuvant Therapy



*$P < .05$, indicating that the pCR group, as compared with the non-pCR group, expressed significantly higher levels of TNF-α, IL-6, and IL-18

Adverse Events

All participants experienced at least one short-term AE (Table 3). The most common toxicity was leukopenia, which 33 participants experienced (78.6%). Among the 42 participants, no cardiovascular dysfunction occurred, and no deaths occurred due to the AEs or PD during the study.

Peripheral Blood Cytokines

Figure 2 shows that the pCR group, as compared with the non-pCR group, expressed significantly higher levels of TNF-α ($P = .013$), IL-6 ($P = .025$), and IL-18 ($P = .0004$) at baseline before treatment. The pCR group had a higher expression of IL-33 than the non-pCR group, but no significant difference existed between that group and the non-pCR group.

Participants with an elevated level of IL-1β and IL-10 in peripheral blood before the neoadjuvant therapy showed a tendency toward the non-pCR outcome, but the difference wasn't significant. No differences occurred between the groups for the other cytokines.

Table 4 shows that among the 13 cytokines, univariate analysis found a significant correlation between pCR and high IL-6 levels (OR, 3.429; 95% CI, 1.838-6.396; $P = .0001$).

Immune-cell Subsets

Table 4 also shows that the univariate analysis found significant correlations between pCR and a high population of NK-T cells (OR, 0.204; 95% CI, 0.052-0.808; $P = .018$) as well as a low ratio of CD4+ T to CD8+ T (OR, 10.500; 95% CI, 2.475-44.545; $P = .001$). No tendencies existed for a correlation between total lymphocytes, CD3+ T cells, B cells, NK cells, CD4+ T cells, CD8+ T cells, Treg, CD3+PD1+ T cells, CD4+PD1+ T cells, CD8+PD1+ T cells, CD4+CD8+ T cells, or CD4-CD8- T cells and pCR or non-pCR.

The pCR group displayed a significantly higher level of NK-T cells ($P = .009$) before treatment than the non-pCR group did (Figure 3). The ratio of CD4+ T to CD8+ T was significantly higher ($P = .0014$) in the non-pCR group before neoadjuvant therapy than that of the pCR group.

TILs Assessment

Table 5 shows that only two TILs levels existed: 21 (50%) participants displayed low TILs (0-10%), while the other 21 (50%) showed intermediate TILs levels (11-59%). No high TILs levels (60-100%) occurred.

Univariate analysis confirmed a significant correlation between TILs expression and pCR (OR, 0.192; 95% CI,

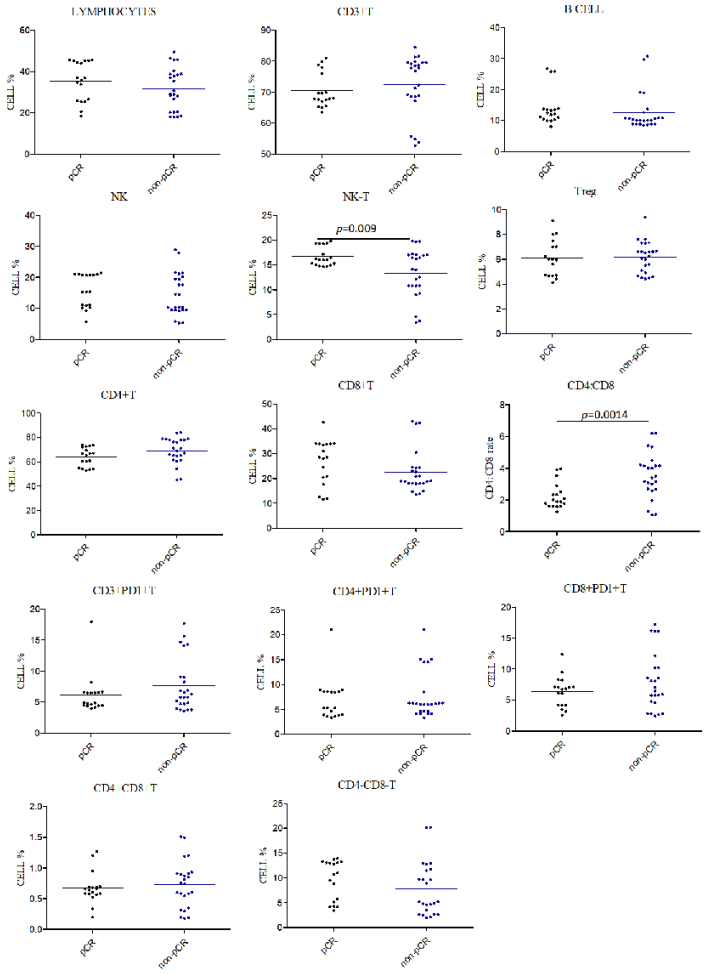
Table 4. Univariate and Multivariate Analysis of Correlations Between Different Cytokines and Immune-cell Subsets and pCR (N = 42). For each cytokine and subset, the research team performed a univariate and a multivariate analysis to find the correlations between pCR and participants with different cytokine levels and subsets: (1) TNF-α, <3.67 versus ≥3.67 pg/ml; (2) IL-6, <5.52 versus ≥5.52 pg/ml; (3) NK-T, <16% versus ≥16%; and (4) CD 4:CD8 ratio, <3.16 versus ≥3.16.

	Univariate Analysis			Multivariate Analysis		
Cytokine	**OR**	**95% CI**	***P* value**	**OR**	**95% CI**	***P* value**
TNF-α	0.325	0.088-1.203	.087	0.513	0.223-1.177	.090
IL-6	3.429	1.838-6.396	.0001[a]	2.429	1.376-4.286	.090
Immune-cell subsets	**OR**	**95% CI**	***P* value**	**OR**	**95% CI**	***P* value**
NK-T	0.204	0.052-0.808	.018[a]	0.381	0.151-0.964	.364
CD4:CD8 Ratio	10.500	2.475-44.545	.001[a]	3.111	1.491-6.492	.160

[a]$P < .05$, indicating that significant correlations existed between pCR and high IL-6 levels and between pCR and a high population of NK-T cells and a low CD4:CD8 ratio

Abbreviations: CD, cluster of differentiation; IL-6, interleukin 6; NK-T, natural killer T cells; pCR, pathological complete response; TNF-α, tumor necrosis factor alpha.

Figure 3. Population of Immune-cell Subsets at Baseline Before Neoadjuvant Therapy



*$P < .05$, indicating that the pCR group expressed significantly higher levels of NK-T cells than the non-pCR group did, and the non-pCR group had a significantly higher ratio of CD4+ T to CD8+ T than the pCR group did

Table 5. Univariate and Multivariate Analysis of Correlations Between pCR and Breast Cancer Characteristics and TILs (N=42). For each characteristic, the research team performed a univariate and a multivariate analysis to find the correlations between pCR and participants with different characteristics: (1) KI-67, 0-20% versus 30-100%; (2) TILS, (0-9% versus 10-100%); (3) tumor grade, IIIB- versus IIB to IIIA; (4) clinical T stage, cT1-2 versus cT3-4; (5) clinical nodal status, cN1 versus cN2-3; (6) age group, ≥50 versus <50 years; and (7) menopausal status, premenopausal versus postmenopausal. The research team classified the Tils as low (0-10%), intermediate (11-59%), or high (60-100%).

	Univariate Analysis			Multivariate Analysis		
	OR	95% CI	*P* value	OR	95% CI	*P* value
Ki-67	1.923	0.434-8.522	.385	1.800	1.191-2.721	.257
TILs	0.192	0.051-0.731	.013[a]	not included		
Tumor grade	0.333	0.09-1.231	.094	0.286	0.043-1.889	.112
Clinical T stage	1.000	0.274-3.656	1.000	1.125	0.531-2.384	.751
Clinical nodal status	1.900	0.473-7.625	.362	0.900	0.115-7.031	.920
Age group	1.000	0.295-3.395	1.000	1.000	0.497-2.011	.673
Menopausal status	1.500	0.324-6.942	.603	0.741	0.365-1.504	.402

[a]*P* = .013, indicating that significant correlations existed between pCR and TILs

Abbreviations: pCR, pathological complete response; TIL, tumor-infiltrating lymphocyte

0.051-0.731; *P* = .013). The univariate analysis found no significant correlations between pCR and Ki-67, tumor grade, clinical T stage, clinical nodal status, age, or menopausal status. Multivariate analysis found no correlations between any clinical factor and pCR.

DISCUSSION

The current study found that participants who achieved pCR had significantly higher serum TNF-α, IL-6, and IL-18 levels before treatment as well as a high population of NK-T cells. These findings support the predictive value of those biomarkers for achievement of pCR after a TCbH regimen. The current study found TILs in the tumor tissue of the 42 participants before treatment with TCbH.

The current study had limitations, including the small sample size and the fact that it didn't determine a detailed mechanism. The heterogeneous characteristics of the immune microenvironment might contribute to different outcomes associated with the same treatment. Finding a correlation between a systemic T-cell population and TILs expression in local tissue may provide a research basis for developing therapeutic treatments and determining the immune system's activation mechanism.

CONCLUSIONS

Immunological factors, including IL-6, NK-T cells, CD4+ T versus CD8+ T ratio, and TILs expression were significant predictors for response to TCbH neoadjuvant therapy with carboplatin.

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