Postprint: Effects of Different Neoadjuvant Chemotherapy Regimens on Immune Markers and Tumor Microenvironment in HER2-Positive Breast Cancer Patients

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Abstract

Background Breast cancer is a common malignant tumor in clinical practice that severely impacts women' s health. Although current targeted therapy regimens for breast cancer are relatively well-developed, the difference in clinical efficacy between dual-target and single-target therapy remains unclear. Objective To investigate the effects of different neoadjuvant chemotherapy regimens on immune indicators and the tumor microenvironment in patients with human epidermal growth factor receptor 2 (HER2)-positive breast cancer. Methods

A total of 92 HER2-positive breast cancer patients admitted to the Second People' s Hospital of Changzhou Affiliated to Nanjing Medical University from September 2017 to September 2021 were enrolled and randomly divided into a study group (treated with trastuzumab + pertuzumab + docetaxel, n=46) and a control group (treated with trastuzumab + docetaxel, n=46). The clinical effective rate, disease control rate, inflammatory factor levels, changes in immunological indicators, and other parameters were compared between the two groups. Results After treatment, the clinical effective rate and disease control rate in the study group were higher than those in the control group (P<0.05). The levels of peripheral blood CD3+, CD4+, and CD4+/CD8+ in the study group were higher than those in the control group, while the CD8+ level was lower than that in the control group (P<0.05). The levels of tumor necrosis factor-α, interferon-γ, interleukin-6, and interleukin-8 in the study group were lower than those in the control group after treatment (P<0.05). The percentages of programmed death-ligand 1 (PD-L1) positive cells $ $25% and programmed cell death protein 1 (PD-1) positive cells $ $65% in the study group were higher than those in the control group (P<0.05), while the percentage of forkhead box protein P3 (FoxP3) positive cells $ $0.45% was lower than that in the control group (P<0.05). Conclusion Neoadjuvant chemotherapy with trastuzumab

+ pertuzumab + docetaxel can effectively improve immune indicators and the tumor microenvironment in HER2-positive breast cancer patients.

Full Text

Effects of Different Neoadjuvant Chemotherapy Regimens on Immune Indicators and Tumor Microenvironment in HER-2-positive Breast Cancer Patients

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Abstract

Background: Breast cancer is a common malignant tumor that seriously affects women' s health. Although the current targeted therapy system for breast cancer is well established, the difference in clinical efficacy between double-target therapy and single-target therapy remains unclear.

Objective: To explore the effects of different neoadjuvant chemotherapy regimens on immune indicators and tumor microenvironment in HER2-positive breast cancer patients.

Methods: A total of 92 HER-2-positive breast cancer patients admitted to Changzhou No.2 People' s Hospital Affiliated to Nanjing Medical University from September 2017 to September 2021 were collected and randomly divided into a study group (trastuzumab + pertuzumab + docetaxel therapy, n=46) and a control group (trastuzumab + docetaxel therapy, n=46). The clinical effective rate and disease control rate, changes in inflammatory factor levels, and immunological indicators were compared between the two groups.

Results: After treatment, the clinical effective rate and disease control rate in the study group were higher than those in the control group (P<0.05). The peripheral blood CD3+, CD4+, and CD4+/CD8+ levels were higher in the study group than in the control group (P<0.05), while CD8+ levels were lower (P<0.05). The levels of TNF-α, IFN-γ, IL-6, and IL-8 in the study group were lower than those in the control group (P<0.05). The percentages of patients with PD-L1-positive cells $ $25% and PD-1-positive cells $ $65% were higher in the study group than in the control group (P<0.05), while the percentage of patients with FoxP3-positive cells $ $0.45% was lower (P<0.05).

Conclusion: The neoadjuvant chemotherapy regimen of trastuzumab + pertuzumab + docetaxel can effectively improve immune indicators and tumor microenvironment in HER-2-positive breast cancer patients.

Keywords: Breast neoplasms; HER-2 positive; Antineoplastic combined chemotherapy protocols; Molecular targeted therapy; Double-target therapy; Tumor microenvironment; Immune indicators

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1.4.4 Serum Immune Indicators Detection

Peripheral blood samples were collected from patients in a fasting state before treatment and 2 weeks after treatment. Flow cytometry and corresponding kits were used to detect T lymphocyte subsets (CD3+, CD4+, CD8+, CD4+/CD8+) in peripheral blood. Serum was separated from the samples, and enzyme-linked immunosorbent assay was employed to measure the expression levels of inflammatory factors including interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ).

1.4.5 Adverse Reactions

The incidence of adverse reactions including gastrointestinal reactions, rash, liver function injury, and leukopenia were compared between the two groups after treatment.

1.4.6 Quality of Life Assessment

Based on the EORTC QLQ-C30 standard, the emotional, cognitive, pain, fatigue, and respiratory status of patients in both groups were evaluated after two treatment cycles.

1.5 Statistical Analysis

SPSS 22.0 statistical software was used for analysis. Normally distributed measurement data were expressed as ($\bar{x}\pm s$), with inter-group comparisons using independent samples t-test and within-group comparisons before and after treatment using paired t-test. Count data were expressed as [n(%)], with inter-group comparisons using ^{2} test. $P<0.05$ was considered statistically significant.

2.1 Comparison of General Patient Data

There were no statistically significant differences between the two groups in terms of age, BMI, smoking history, menstrual status, TNM stage, lymph node status, or histological grade (P>0.05) .

2.2 Comparison of Treatment Efficacy

After treatment, there were no statistically significant differences in CR, PR, SD, or PD between the two groups (P>0.05). However, the clinical effective rate and disease control rate in the study group were significantly higher than those in the control group (P<0.05) .

2.3 Comparison of Blood Indicators

Before treatment, there were no statistically significant differences between the control and study groups in CD3+, CD4+, CD8+, CD4+/CD8+, IL-6, IL-8, TNF-α, or IFN-γ levels (P>0.05). After treatment, the study group showed higher peripheral blood CD3+, CD4+, and CD4+/CD8+ levels and lower CD8+, IL-6, IL-8, TNF-α, and IFN-γ levels compared to the control group (P<0.05). In both groups, post-treatment CD3+ and CD4+ levels were higher than before treatment, while CD8+, IL-6, IL-8, TNF-α, and IFN-γ levels were lower than before treatment (P<0.05) .

2.4 Comparison of Tumor Microenvironment Indicators

Before treatment, there were no statistically significant differences between the control and study groups in the proportions of patients with PD-L1-positive cells $ $25%, PD-1-positive cells $ $65%, or FoxP3-positive cells $ $0.45% (P>0.05). After treatment, the study group showed higher proportions of patients with PD-L1-positive cells $ $25% and PD-1-positive cells $ $65%, and a lower proportion of patients with FoxP3-positive cells $ $0.45% compared to the control group (P<0.05). In the study group, the proportions of patients with PD-L1-positive cells $ $25% and PD-1-positive cells $ $65% were higher than before treatment, while the proportion of patients with FoxP3-positive cells $ $0.45% was lower than before treatment (P<0.05) .

2.5 Comparison of Adverse Reaction Rates

There were no statistically significant differences between the two groups in the incidence of gastrointestinal adverse reactions, rash, liver function injury, or leukopenia during treatment (P>0.05) .

2.6 Comparison of Quality of Life After Treatment

After treatment, the study group showed higher emotional and cognitive scores and lower pain, dyspnea, and fatigue scores compared to the control group (P<0.05) .

Discussion

Epidemiological surveys both domestically and internationally have found that the incidence of breast cancer is increasing year by year, with a trend toward younger onset. Factors such as genetics, unhealthy lifestyle habits, dietary structure changes, obesity, and psychological stress all contribute to increased incidence. According to GLOBOCAN 2020 statistics, there were 2,261,419 new cases of breast cancer globally in 2020, accounting for 11.7% of all cancers and surpassing lung cancer to become the most common cancer worldwide. In 185 countries globally, breast cancer ranked first in incidence in 159 countries and represents the leading cause of death among female cancer patients.

In recent years, advances in gene sequencing technology and the concept of the tumor microenvironment have provided new perspectives for cancer research. The tumor microenvironment plays a crucial role in tumor initiation and progression. Paget metaphorically described this relationship as "seed (cancer cells) and soil (tumor microenvironment)," known as the "seed and soil theory." The tumor microenvironment is a complex and extensive microscopic biological system composed of immune cells, inflammatory cells, microvessels, cancer-associated fibroblasts, and various cytokines and chemokines. It is closely related to tumor occurrence, growth, and metastasis, with both interdependence and antagonism between components. Many important biological markers are involved in tumor progression, including sustained proliferative signaling, anti-apoptosis, permanent replication, angiogenesis, invasion and metastasis, immune evasion, and phenotypic plasticity. The tumor microenvironment can be considered a medium for these cascade processes, providing crucial links for tumor growth.

Chronic inflammation and immune response are important molecular biological foundations for tumor initiation and development. The molecular mechanisms by which chronic inflammation contributes to tumor formation remain unclear, but related studies suggest that long-term inflammatory stimulation may increase the probability of cellular carcinogenesis. For example, the proportion of patients with chronic hepatitis and cirrhosis who develop liver cancer is much higher than in the general population, and the risk of colorectal cancer in patients with ulcerative colitis is 2-7 times higher than in the general population. Immune response plays a key role in tumor cell occurrence and clearance, particularly T lymphocyte-mediated cellular immunity.

As a highly heterogeneous malignant tumor, breast cancer treatment protocols are primarily determined based on cancer cell immunohistochemistry results. HER-2-positive breast cancer represents a histologically aggressive subtype with poor prognosis, rapid tumor growth, high malignancy, and susceptibility to recurrence and metastasis. With advances in radiotherapy and chemotherapy technology, breast cancer has become one of the cancers with relatively high treatment efficacy and survival rates. Double-target therapy represents the

development trend in neoadjuvant treatment for breast cancer and has been confirmed in multiple previous clinical studies.

Trastuzumab, the first classic anti-HER-2 drug approved, primarily works by inhibiting the HER-2 homodimer signaling pathway to suppress tumor cell growth. However, tumor cells are highly adaptable; when their own signaling pathways are inhibited, they can activate alternative pathways to support cancer cell growth. Pertuzumab compensates for this limitation of trastuzumab. The combination of both drugs can simultaneously inhibit the formation of homodimers and heterodimers, blocking signal transduction at the source. Additionally, the combination can mediate antibody-dependent cell-mediated cytotoxicity (ADCC), better facilitating immune cell killing of cancer cells and achieving a synergistic effect where 1+1>2.

Research shows that IL-6 is a pleiotropic cytokine closely related to cell growth, apoptosis, and proliferation. IL-8 has tumorigenic and pro-angiogenic effects. TNF-α is produced by macrophages and monocytes; under physiological conditions it plays a positive role in immune surveillance and tumor suppression, but in pathological states of overproduction it can promote tumor occurrence and metastasis. IFN is a cytokine with immunomodulatory and antiviral effects, among which IFN-γ can inhibit tumor cell growth. The results of this study show that treatment with trastuzumab + pertuzumab + docetaxel can improve patients' inflammatory factor levels of IL-6, IL-8, TNF-α, and IFN-γ, suggesting that this treatment regimen can effectively reduce inflammatory response and thereby decrease the promoting effect of the inflammatory microenvironment on tumor cells.

Malignant tumor patients often experience immune dysfunction, particularly in T lymphocyte-mediated cellular immunity. CD3+ represents total T lymphocytes, CD4+ are helper T lymphocytes with immune surveillance and anti-tumor functions, while CD8+ has cytotoxic and immunosuppressive effects that can inhibit antibody synthesis and secretion. This study found that CD3+ and CD4+ levels in the study group were higher than before treatment, while CD8+ levels were lower than before treatment, indicating that neoadjuvant therapy can effectively improve patients' immune function and facilitate recovery.

Treg cells are a subset of T lymphocytes that maintain immune homeostasis and regulate immune balance, with FoxP3 being the core of their gene expression profile. However, higher FoxP3 expression is associated with worse prognosis in cancer patients. In this study, the proportion of patients with FoxP3-positive cells $ $0.45% in the study group decreased from 72% to 54%, while the percentages of PD-L1- and PD-1-positive cells significantly increased, indicating that neoadjuvant therapy also plays a positive role in promoting immune response and reducing tumor immune evasion.

In summary, double-target therapy combined with chemotherapy can significantly improve immune indicators and tumor microenvironment in HER-2-positive breast cancer patients, exerting positive effects on treatment and prog-

nosis. However, due to the relatively small sample size and lack of long-term follow-up in this study, the results may have certain biases. Future studies should increase sample size, extend follow-up duration, and enhance data authenticity to provide more meaningful clinical references.

Author Contributions

PEI Bei conceived the study; CHENG Lin and XU Lingyun conducted feasibility analysis and research guidance; PEI Bei and CHENG Lin participated in data collection and organization; PEI Bei was responsible for data collection, statistical analysis, and manuscript writing; XU Lingyun provided writing guidance and manuscript revision.

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Note: Figure translations are in progress. See original paper for figures.