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Research Article

Effect of Inflammatory Markers on the Pathologic Complete Response in the Neoadjuvan Treatment of HER-2 Positive Local Advanced Breast Cancer

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Abstract

Objectives: This study aimed to retrospectively investigate the rate of pathological complete response (pCR) and markers predicting this response for trastuzumab and pertuzumab-containing regimens compared to trastuzumab-containing regimens for stage II to III HER-2 positive breast cancer (BC).

Methods: In this study, we retrospectively analyzed the data of patients with Her-2 positive advanced BC who were treated with trastuzumab or trastuzumab and pertuzumab in addition to neoadjuvant chemotherapy. Patients were classified according to their pre-treatment clinical stages, hormone receptor status, Her-2 immuno-histochemical score, ki-67 index, neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), and prognostic nutritional index (PNI) ratio.

Results: For NLR, it was 1.9±0.85 in patients with pCR and 2.4±0.83 in patients without pCR. For PLR it was 112±53.3 and 129.4±36.2, respectively. For PNI, it was 51.6±5.8 and 48.1±5.9. Low NLR and PLR as well as high PNI were significantly associated with pCR. In addition, the pCR of patients with hormone receptor negativity and dual Her-2 blockade was statistically significant.

Conclusion: This is the first study in the literature to show that prognostic scores such as NLR, PLR, and PNI have statistically significant benefits on pCR in patients treated with the neoadjuvant dual blockade.

Keywords: Her-2 positive breast cancer, prognostic inflammatory markers, predictivity of pathological complete response

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Human epidermal growth factor 2 (HER-2/neu) gene amplification and receptor overexpression are present in approximately 20% of breast cancers (BCs). These tumors have historically been associated with a more aggressive clinical phenotype, increased metastatic potential, higher recurrence rate, and lower overall survival (OS).[1] Neoadjuvant therapy was previously used to reduce the size and extent of locally advanced tumors in a limited area. However, it has been used more widely in recent years. Neoadjuvant therapies allow rapid evaluation of drug efficacy and may accelerate the development and approval of treatments for early BC, as well as increasing the possibility of tumor control and therapeutic potential in early breast cancer.[2] Two important preliminary clinical studies showing the efficacy and safety of neoadjuvant dual blockade in Her-2 positive advanced BC patients. In the first of these studies, NeoSphere[3], a significant increase in pCR was observed after the dual blockade. In another study, Tryphaena[4], dual

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block treatments with and without anthracycline were combined and statistically significant results were obtained in pCR. As a result of these two phase II neoadjuvant studies, the United States Food and Drug Administration (FDA) has approved pertuzumab and trastuzumab dual-blockade in combination with chemotherapy for the neoadjuvant treatment of early-stage Her-2 positive BC. In this study, we aimed to evaluate the pathological response rates and markers predicting this response between regimens containing a combination of pertuzumab and trastuzumab and regimens containing only trastuzumab for stage II to III Her-2 positive BC. We also investigated the effect of hormone receptor (HR) status, ki-67 index, and inflammatory prognostic markers (IPM) on pCR rates in both groups.

Methods

Patient Population

In this study, the data of patients who were diagnosed between 01.01.2011 and 01.03.2021 with Her-2 positive and clinical stages II-III and who received neoadjuvant therapy were evaluated retrospectively. All patients were treated with anti Her-2-based therapy as neoadjuvant therapy. Thirty-five of these patients were treated with dual blockade with trastuzumab and pertuzumab, and 35 with protocols containing only a trastuzumab regimen. Patients who did not receive an anthracycline regimen and did not complete the targeted therapy were not included in the study due to the standardization of the patients in the study. In total, 10 patients in patients receiving trastuzumab and 3 patients in patients receiving pertuzumab and trastuzumab were not included in the study because they did not meet these criteria. The study was performed according to the institutional ethical standards (Inonu University Medicine High School, Number: 2022/2879 - 11-01-2022). All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

Data Collection

Data collected from patients' files include: age at diagnosis, menopausal status, tumor location, clinical staging based on radiological studies, tumor characteristics including estrogen receptor (ER), progesterone receptor (PR) and Her-2 status, post-treatment Positron Emission Tomography (PET/CT) response, type of surgery and Inflammatory prognostic markers (IPM) calculated by laboratory parameters of the patients. Pathology reports were evaluated to extract post-treatment response information. Clinico-pathological features that predict complete pathological responses were assessed. NLR was calculated as NLR = absolute neutrophil count / absolute lymphocyte count. PLR was calculated as PLR = absolute platelet count / absolute lymphocyte count. PNI was calculated by using the formula (10 × albumin (g / L) + (0.005 × absolute lymphocyte count).

Treatments

All of the patients in the study were treated with anti-Her-2 therapy in combination with chemotherapy. Some patients received the addition of trastuzumab and pertuzumab to chemotherapy, while other groups of patients received treatment in combination with trastuzumab alone and chemotherapy. All of the patients in the study received Anthracyclines. In the anthracycline preference, only 2 patients received epiribucin therapy, and the remaining patients were treated with 60 mg/m^2 doxorubicin every 3 weeks and cyclophosphamide 600mg/m^2 every 3 weeks. All of the patients in the study received taxane treatment, and the choice of taxane was given as weekly paclitaxel 80 mg/m^2 or 3-week docetaxel 75 mg/m^2 depending on the physician's decision. Trastuzumab was administered at a loading dose of 8 mg/kg followed by 6 mg/kg every 3 weeks and pertuzumab was administered at a loading dose at 840 mg every 3 weeks followed by 420 mg every 3 weeks.

Pathology Evaluation After Neoadjuvan Chemotherapy

Pathology reports were reviewed as documented in the medical records to determine the post-treatment pathological response. pCR definition; It was considered the absence of invasive tumor cells, and both breast and axilla were evaluated separately. The absence of any invasive tumor cells in either tissue area (ypT0/is, N0) was also evaluated.

Statistical Analysis

All data collected in this study were summarized using standard descriptive statistics. Statistical analyzes of factors predicting pCR were performed in both groups. It was planned to find the cut-off value by performing Roc-Curve analysis for IPMs. However, due to the insufficient number of patients, this cut-off value was found to be significant only in PNI. No significant cut-off value was found in NLR and PLR. For this reason, NLR and PLR values were calculated by dividing all patients into two groups as pCR and non-pCR. Student t-test was used to evaluate descriptive statistical methods (mean. standard deviation, frequency) and quantitative data between two groups. Qualitative data was assessed using the Chi-Square test. Mann Whitney U test was used to evaluate for nonparametric data. For statistical methods, $p<0.05$ was considered statistically significant. Statistical analysis was performed using the SPSS-22 program.

Results

A total of 83 patients were evaluated and 70 patients which met the inclusion/exclusion criteria were included in this study. While 35 of these patients received trastuzumab in addition to neoadjuvant chemotherapy, the other 35 received trastuzumab and pertuzumab combined therapy. The clinical and demographic data of these patients are shown in Table 1. The median follow-up period was 1.63 years (0.46-2.50) in the pertuzumab and trastuzumab group, and 3.18 (0.84-10.89) years in the trastuzumab group. The median age at diagnosis of patients treated with dual blockade was 51 (30-71), while the median age of patients treated with only trastuzumab was 44 (28-76). While 17 of the dual blockage group patients were premenopausal, 18 of them were menopausal. This situation was 25 premenopausal and 10 menopausal in the trastuzumab group. When breast cancer was evaluated according to its location, it was seen that there were 18 right and 17 left locations in both groups. In the combined chemotherapy scheme, docetaxel was preferred for all patients in the pertuzumab group, while weekly paclitaxel was preferred in 25 patients and docetaxel for 3 weeks in 10 patients in the trastuzumab group. Patients were also requested to be classified as LVI, PNI and tumor grade. However, since these criteria were missing in pathological evaluations, no meaningful data could be obtained. The patients were also evaluated according to their hormone receptor status and ki-67 index score. According to these evaluations, ER-positive patients in the pertuzumab group were 22, PR-positive patients were 13, and the median ki-67 score was 25% (10-80). In the trastuzumab group, these rates were 23 for ER, 19 for PR, and a median value of 30% (10-75%) for ki-67 score, respectively.

Information including the pre-treatment TNM staging, post-treatment PET/CT response evaluation, the type of surgery performed, the pathological responses after surgery, and the recurrence status in the follow-up are shown in Table 2. In the pertuzumab group, 15 of the patients were in Stage-2,

Table 1. Patient Demographics and Clinical Characteristics

	Pertuzumab + Trastuzumab n=35 (%)	Trastuzumab n=35 (%)
Age, Years		
Median	51	44
Range	30-71	28-76
Menopause Status		
Premenopausal	17 (48,5)	25 (71,4)
Postmenopausal	18 (51,5)	10 (29,6)
Primary Mass Location		
Right	18 (51,5)	18 (51,5)
Left	17 (48,5)	17 (48,5)
Chemotherapy Type		
Docetaxel	35 (100)	25 (71,4)
Paclitaxel	0 (0)	10 (29,6)
Hormone Receptor Status		
ER Positive	22 (62,85)	23 (65,7)
PR Positive	13 (37,15)	19 (54,3)
ER and PR Positive	13 (37,15)	11 (31,4)
Ki-67 Index		
Median	25	30
Range	10-80	10-75

Table 2. Clinical Staging and Surgical Characteristics of Patients

	Pertuzumab + Trastuzumab (n=35)	Trastuzumab (n=35)
T Stage		
T1	3	5
T2	26	21
T3	3	8
T4	3	1
Primary Surgical Resection		
N0	1	1
N1	17	14
N2	10	8
N3	7	12
TNM Stages		
Stage-2	15	12
Stage-3	20	23
PET/CT Response		
Complete Response	30	14
Partial Response	5	9
No Evaluation	-	12
Type of Surgery		
BCS	2	3
MRM	33	32
Axillary Surgery Type		
SLND	3	-
ALND	32	35
Complete Response (pCR)		
Breast	27	13
Axilla	28	16
Breast and Axilla	24	12
Follow-up (Year)		
Median	1.63	3.18
Min-Max	0.46-2.5	0.84-10.89
Recurrence Status		
Yes	1	10
No	34	25
Dead		
Yes	0	6
No	35	29

while 20 of them were in the Stage-3 disease group. In the trastuzumab group, it was 12 and 23, respectively. According to PET/CT results after neoadjuvant therapy in the pertuzumab group, 30 of the patients had metabolic complete response, while this rate was seen in only 14 patients in the trastuzumab group. However, post-treatment PET/CT response evaluation of 12 patients was not performed in the trastuzumab group. Surgical treatment was applied to these patients without evaluation of radiological response. In the group that received neoadjuvant pertuzumab treatment, 33 of the patients had MRM, while only 2 of them were treated with BCS. Similarly, while ALND was performed in 32 patients, sentinel lymph node biopsy was performed in only 3 patients. In the trastuzumab group, MRM was performed in 32 patients and BCS was performed in 3 patients. However, it was seen that all patients in this group underwent ALND as a standard. Postoperatively, pathologies were observed for pertuzumab group patients, with pCR detected in the breast in 27 patients, pCR in the axilla in 28 patients, and pCR in both breast and axilla in 24 patients. For patients receiving trastuzumab treatment, respectively; It was detected as 13, 16 and 12. The number of patients who received RT in the adjuvant period after surgery was 21 in the pertuzumab group and 30 in the trastuzumab group. While recurrence was detected in 1 patient who received pertuzumab during the follow-up period, 10 of the patients who received trastuzumab had a recurrence. No patient died yet in the pertuzumab group, but 6 patients died in the trastuzumab group. However, the median follow-up times of both groups were different.

The pre-treatment NLR, PLR and PNI values of the patients are shown in Table 3. The median values for these inflammatory prognostic markers in the pertuzumab group are respectively; 1.97±0.91 for NLR; 111.90±47.71 for PLR; It was determined as 51.80±5.36 for PNI. In the trastuzumab group, respectively; 2.35±0.78 for NLR; 126.35±43.63 for PLR; For PNI, it was determined as 48.57±6.59.

Subgroup evaluations of the patients included in this study were performed according to whether they had pCR or not. When the patients' age, menopause status, gender, tumor locations, clinical stages and ki-67 index were evaluated, no difference was found in terms of pCR. While there were 22 premenopausal patients who achieved a pathological complete response, there were 20 patients without pCR. In menopausal patients, there were 13 patients who achieved pCR, while there were 12 patients who did not have pCR. Similarly, 19 patients with right breast cancer achieved pCR, while 16 patients did not. 17 patients with left breast cancer achieved pCR, while 17 patients did not. The mean of the ki-67 index of the patients who achieved a pathological complete response was 25%, while the mean of the patients who did not reach the pCR was found to be 30%. All these results are not statistically significant ($p>0.05$). However; Patients receiving pertuzumab and trastuzumab combined therapy as neoadjuvant therapy had a higher pCR rate than patients receiving trastuzumab alone. This difference was statistically significant ($p<0.05$). Similarly, the pCR of patients with negative ER and/or PR was statistically significantly higher than patients with positive ER and/or PR ($p<0.05$).

Inflammatory prognostic markers and pCR were also evaluated, which is the main subject of this study. It was planned to use Roc Curve analysis to find a certain cut-off value for these markers. However, due to the insufficient number of samples, the cut-off value could only be calculated for PNI with Roc Curve analysis. A cut-off value of 50.8 was obtained for PNI using Roc Curve analysis. Patients below and above this value were divided into two groups. Patients above 50.8 clearly showed a contribution of pCR in both treatment groups. This Roc Curve Analysis is shown in Figure 1. The cut-off value was determined by calculating the mean values for both NLR and PLR values. The patients were divided into two parts, with and without pCR. In these patients, the rate of NLR was found to be 1.9±0.85 in those with pCR, while it was 2.4±0.83 in patients without pCR. The difference between both arms was statistically significant ($p<0.05$). A similar situation was seen in the PLR ratio. While the PLR of patients with pCR was 112±53.3, this rate was 129.4±36.2 in patients without a complete response. This detected difference was also statistically significant ($p<0.05$). Considering the PLR rate, patients with and without complete response were also evaluated. While the PLR of patients with complete response was 51.6±5.8, the PLR of patients without complete response was 48.1±5.9. This difference between the patients was also statistically significant.

Table 3. Inflammatory Prognostic Markers and Pathological Response

	Pathological Complete Response	Not Pathological Complete Response	p
General Population (n=70)	36	34	-
NLR	1.9±0.85	2.4±0.83	0.01
PLR	123.8±53.3	138.5±36.2	0.03
PNI	51.6±5.8	48.1±5.9	0.019

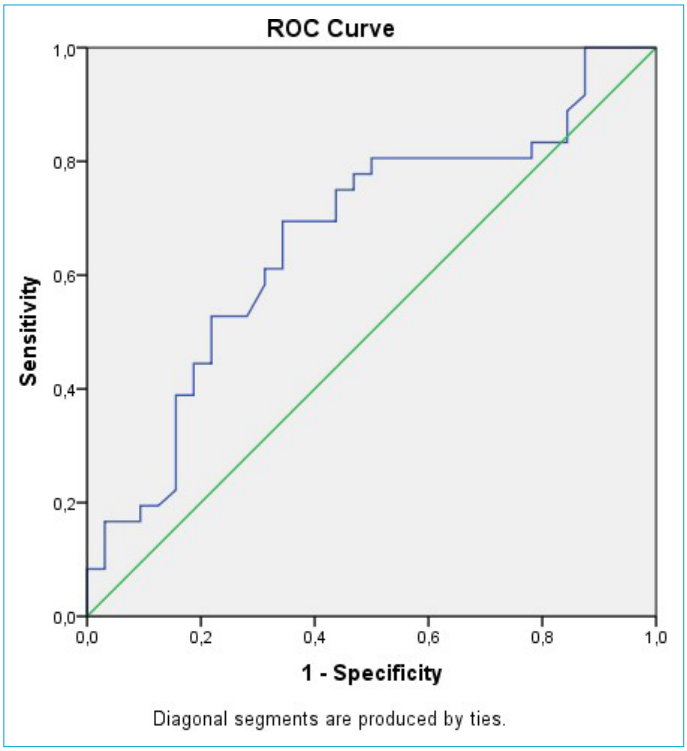

Figure 1. Roc Curve Analysis of PNI.

Discussion

As a result of recent clinical studies, increased pathological complete response (pCR) with neoadjuvant therapy in breast cancer has shown a statistically significant contribution to survival. In a published article, patients who achieved pCR with neoadjuvant therapy had significantly higher overall survival (OS). In the subgroup analysis of the same study, the addition of trastuzumab to neoadjuvant therapy in Her-2 positive patients caused a significant increase in pCR.[5] With the results of this and similar data, the addition of trastuzumab to neoadjuvant chemotherapy in Her-2 positive breast cancer patients has become a standard practice all over the world. In later studies, the idea of using Her-2 dual blockade in neoadjuvant therapy was developed. In a study conducted for this purpose, it was shown that adding lapatinib to neoadjuvant trastuzumab treatment contributed positively to pCR.[6] However, in similar study designs were performed later, this treatment could not take its place in routine practice because this difference was not statistically significant. Subsequently, studies of pertuzumab, a new Her-2-targeted monoclonal antibody, were conducted. These studies are respectively; NeoSphere, TRYPHAEA and BERENICE.[3,4,7] And as a result of these studies, the standard of neoadjuvant treatment for Her-2 positive breast cancer patients has been the combination of dual Her-2 blockade and chemotherapy. The pCR responses may differ from each other depending on factors such as the agents used in these studies and the patient population. Their treatment is shaped according to escalation or de-escalation pCR. Because, in the study of Kathrine [8], it is seen that TDM-1 molecule used for 1 year in patients who could not reach pCR with neoadjuvant therapy increases the duration of Disease-Free Survival (DFS) according to the 3-year results of the study and is recommended in the guidelines in this indication. Considering all these studies, providing a higher pCR is the main target of future studies. For this, studies to be created with more specific and more individualized treatment models will guide future treatments. Although Her-2 targeted therapies are called individualized treatment agents, there is a lack of efficacy in patients for whom pCR can not be reached. This group of patients should be identified before treatment and treated by providing escalation or de-escalation with the most appropriate treatment regimen. This 'more individualized' treatment plan is seen in recent studies in hormone receptor- positive, Her-2 negative patients. In the study, the genetic risk status obtained by genomic profiling was determined. It has been shown that adjuvant chemotherapy does not contribute to overall survival in patients with high clinical risk and low genetic risk.[9]

In previous studies, there is information that inflammatory markers can predict response in different cancer types and different treatments. Conflicting positive and negative results in these studies have been reported in the literature. Based on data from three major studies in patients with locally advanced breast Her-2 positive breast cancer, the neoadjuvant standard therapy was dual blockade of trastuzumab and pertuzumab added to chemotherapy. [3,4,7] In the literature review, inflammatory prognostic markers NLR, monocyte-lymphocyte-ratio (MLR), Pan-Immune-Inflammatory Ratio (PIV) were studied in patients treated with taxane, trastuzumab and pertuzumab as first-line treatment agents in advanced stage patients. At the end of the study, higher levels of these inflammatory markers were associated with worse PFS and OS.[10] In a similar study design, absolute lymphocyte count (ALC) and PFS were compared. In this study, the cut-off value for ALC was accepted as 1500/µL. It has been shown that it makes a statistically significant difference in PFS for 1500/µL and above values.[11]

There is only one study evaluating pCR with inflammatory markers after neoadjuvant systemic therapy in patients with locally advanced Her-2 positive breast cancer. In this study, a comparison was performed based on the 1.97 cut-off value of NLR. According to this determined value, it was observed that there was a statistically significant increase in pCR.[12]

This study that we have completed has shown that inflammatory prognostic markers can predict pCR. The main goal of recent neoadjuvant studies is to increase pCR. Because long-term follow-up data show that the survival of patients who reach pCR with neoadjuvant therapy is better than patients who cannot reach pCR. The goal of achieving a complete response determines the escalation and de-escalation strategy of neoadjuvant therapy. The main goal of future clinical studies should be to develop a scoring system that includes inflammatory prognostic markers before treatment. Thus, we can identify patient groups that require more intensive or less treatment.

This study, which we have completed, is the first study in the literature with multiple inflammatory prognostic markers in Her-2 positive locally advanced patients. It will be important in terms of guiding future biomarker studies. However, this study also has weaknesses. In particular, the small number of patients and the fact that it was performed by evaluating only clinicopathological features are its weaknesses. And also, survival analyzes could not be performed due to the short follow-up. We think that it is important to increase the number of patients in future studies and to create a combined scoring system by including genetic analyzes.

Conclusion

The pCR can be increased using certain threshold values for NLR, PLR and PNI. Moreover, in future studies, the predictive power can be increased by combining these markers with genomic tests. The importance of this study; It is the first study in its field with multiple prognostic markers and it guides future studies.

Disclosures

Ethics Committee Approval: This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University Inonu (Date 11-01-2022/No 2022/2879).

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – A.G.; Design – A.G.; Supervision – A.G.; Materials – A.G.; Data collection &/or processing – A.G.; Analysis and/or interpretation – H.H.; Literature search – A.G.; Writing – A.G.; Critical review – H.H.

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