Assessment of the relationships between Ki67 expression and neoadjuvant treatment response and prognosis in breast cancer using two types of response evaluation systems

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Research Article

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Abstract

Background: Although it is not a good prognostic marker for all breast cancers, pathological complete response (pCR) is an endpoint in evaluating neoadjuvant chemotherapy (NAC) efficacy. Ki67, a proliferation marker, has a complex role as a predictive marker in determining the NAC response. This study aimed to investigate the relationship between pretreatment and posttreatment Ki67 levels, posttreatment Ki67 change and treatment response using the Miller–Payne (MP) and Residual Cancer Burden (RCB) response assessment systems.

Methods: A total of 178 invasive breast carcinoma patients who underwent NAC were included in the study. Ki67 levels were evaluated by immunohistochemical method in trucut biopsy and surgical excision specimens. Treatment response in the surgical excision specimen was classified according to both MP and RCB classifications. We investigated the relationships between pretreatment Ki67 level, posttreatment Ki67 level and posttreatment change in Ki67 with NAC response and survival. Additionally, the cut-off value of the pretreatment Ki67 level for pCR and nonpathological response (pNR) was investigated.

Results. The pretreatment Ki67 level was significantly higher in the pCR group than in the partial response (pPR) and pNR groups ($p<0.001$) in both the MP and RCB systems. The posttreatment Ki67 level was significantly higher in the pNR group than in the pPR group ($p<0.001$) in both systems. There was a negative correlation between pretreatment Ki67 and disease-free survival (DFS) in the luminal B HER2-negative subtype ($r=-0.377$, $p=0.036$) and a significant negative correlation between posttreatment Ki67 and overall survival (OS) in the HER2-positive subtype ($r=-0.544$, $p= 0.0209$). A significant correlation was found between the posttreatment Ki67 change and the degree of response in the luminal B HER2-positive and HER2-positive subtypes ($p<0.05$). The Ki67 cut-off value was 37.5 for pCR in both the MP and RCB classification systems (95% CI 0.673-0.833 and 95% CI 0.66-0.827) ($p<0.001$). There was a significant moderate agreement between the MP and RCB systems ($p<0.001$).

Conclusions: Pre- and posttreatment Ki67 levels may be used to assess treatment response in various molecular subtypes of breast cancer. In this study, the cut-off value of Ki67 for pCR was 37%. More aggressive treatments may be considered in luminal B HER2-negative tumours with high pretreatment Ki67 levels and tumours that do not show a Ki67 decrease.

Introduction

Breast cancer is the most common cancer in women worldwide [1]. Neoadjuvant chemotherapy (NAC) is applied as a standard treatment to reduce the tumour size and perform a more limited surgery in high-risk operable patients, such as inoperable patients [2]. NAC response is a prognostic determinant in the long term, and it is also useful in assessing the effectiveness of treatment in the short term. It allows the detection of tumours unresponsive to treatment at an early stage and the discontinuation of ineffective treatment and/or the addition of other options [3]. While pathological complete response (pCR) is the end

point in the assessment of treatment efficacy and is generally reported to be correlated with favourable survival, it is not associated with a good prognosis in all breast cancers [4, 5]. pCR is detected in only 10–15% of patients receiving NAC [6]. Assessment of residual disease after NAC is important to identify patients who should receive additional adjuvant therapy, as well as to identify the subgroup with a good prognosis among patients with this residual disease [7]. There are many grading systems that evaluate the pathological response, and these systems evaluate only the breast or the breast and axilla together [8, 9]. The prognostic importance of pCR should be controverted due to the difference in both evaluation methods and the different prognoses of different subgroups [10].

As in all cancers, proliferation is an important characteristic of the tumour in breast cancer [11, 12]. Ki67 is a well-known proliferation marker used to assess cell proliferation. Ki67 assessment is most commonly performed by detecting Ki67 antigen, which is expressed in all phases of the cell cycle except G0, by an immunohistochemical method with Ki67 monoclonal antibody [12, 13]. Its prognostic and predictive value has been investigated in many studies [11, 12, 14–16]. Although it has been criticized for its low reproducibility, it has been shown in many studies that Ki67 is a prognostic and predictive marker [17–21]. It is used as a useful clinical marker for predicting subtype classification, prognosis, and therapeutic response in breast cancer [19, 22]. It has been reported to be an independent prognostic and predictive marker in operable patients [23, 24]. The role of the Ki67 level in NAC is complex [20, 25, 27, 28]. It has been reported that pretreatment Ki67 levels correlate with treatment response, and tumours with higher Ki67 levels respond better to treatment [25–28]. In one study, it was reported that the Ki67 level was higher in patients who progressed during NAC than in those who responded [29]. Although it has been investigated in many studies, there is no definite threshold value that will determine which patients will achieve pCR and which will pathological nonrespons (pNR) to treatment [25, 27, 30]. Although many studies have shown that the pretreatment Ki67 level is prognostic, some studies have reported that evaluating Ki67 after NAC will provide more accurate prognostic information [20, 28, 31–33].

In this study, we aimed to investigate the relationship between pre- and posttreatment Ki67 levels and Miller-Payne (MP) and Residual Cancer Burden (RCB) pathological response degrees and survival in breast cancer and breast cancer molecular subtypes in patients who received NAC. Our second aim was to investigate the compatibility between two different systems, the MP and RCB pathological response assessment systems.

Materials And Methods

One hundred seventy-eight patients who underwent NAC with the diagnosis of invasive breast carcinoma in Ondokuz Mayıs University Faculty of Medicine Hospital between 2013 and 2020 and who had a trucut biopsy pretreatment and a surgical specimen posttreatment were included in the study. The study was approved by the OMU ethics committee (11.03.21/B.30.2.ODM.0.20.08/144-399). NAC was applied according to the protocols discussed on a case-by-case basis for each patient in the multidisciplinary tumour council. All patients underwent physical examination, mammogram and ultrasound. Coils were placed in all patients before NAC.

Pathological Evaluation

Pretreatment biopsies and posttreatment surgical specimens were reviewed. Biopsy samples were immunostained and evaluated for the expression of oestrogen (ER), progesterone (PgR), HER2 and Ki67. Pathological treatment response grading in posttreatment surgical specimens was performed according to the MP and RCB systems [9,34]. The tumour cellularity in core needle biopsy was compared with the tumour cellularity in the surgical excision specimen according to the MP classification and graded on a 5-point scale according to the degree of tumour cell loss. According to this classification, pNR tumours were graded as grade 1, pCR tumours as grade 5, and pathologically partially responding (pPR) tumours as grades 2, 3 and 4. According to the RCB system, the residual tumour remaining in the breast parenchyma and lymph nodes was evaluated by considering the primary tumour size, tumour bed cellularity and axillary lymph node. This system classified pCR cases as RCB-0, cases with minimal residual disease (marked response) as RCB-I and cases with moderate response as RCB-II, and cases with minimal or no response (chemoresistant) as RCB-III [9,34].

ER, PgR, HER2 and Ki67 immunostaining were performed and evaluated in patients with residual tumours. These slides, which were evaluated by the breast pathologists involved in the study, were reviewed. The histological type, histological grade, ER, PgR, and HER2 status and Ki67 level of the tumour in biopsy samples were recorded. Tumours were divided into molecular subgroups as luminal A, luminal B HER2-negative, luminal B HER2-positive, HER2-positive and triple negative according to the ER, PgR, HER2 and Ki67 results [19]. The histological type of the tumour, histological grade, tumour size, lymph node status, Ki67 level in surgical specimens containing the residual tumour, and MP and RCB treatment

response grades in all surgical specimens were recorded. For staging, information on distant metastasis, status of clinical lymph nodes, overall survival (OS) and disease-free survival (DFS) was obtained from medical oncology department records.

Immunohistochemical Study

All immunohistochemical studies were performed with an automatic immunostaining device (Ventana Benchmark XT, Ventana Medical Systems, France and Ventana Benchmark Ultra, Ventana Medical Systems, Tucson, Az, USA) according to the company's protocol. The primary antibodies, anti-oestrogen receptor rabbit monoclonal primary antibody (clone SP1, Ventana) for ER, anti-progesterone receptor rabbit monoclonal primary antibody (clone 1E2, Ventana) for PgR, antiHER-2/neu rabbit monoclonal antibody (clone 4B5, Ventana) for HER2, and anti-Ki 67 rabbit monoclonal primary antibody (clone 30-9, Ventana) for Ki67, were used. All antibodies were ready for use. One percent nuclear staining was considered the cut-off for ER and PgR positivity, and staining less than 1% was considered negative [35]. For ER, staining between 1% and 10% was scored as low positive, and staining between 11% and 100% was scored as positive according to the ASCO/CAP guideline recommendation [35]. However, since there were only 4 patients in the low positive group, the low positive and positive patients were grouped as positive. HER2 expression evaluation was performed according to the ASCO/CAP 2018 guidelines, and HER2 positivity (score 3) was immunohistochemically defined as complete, intense membrane staining in

more than 10% of tumour cells [36]. Silver in situ hybridization (SISH) was performed on an automated stainer (Ventana Benchmark XT, Ventana Medical Systems, France and Ventana Benchmark Ultra, Ventana Medical Systems, Tucson, Az, USA) using the dual SISH probe (INFORM HER2 Dual ISH DNA Probe Cocktail, Ventana) on immunohistochemical 2+ samples. HER2 status was evaluated according to the ASCO/CAP guideline 2018 [36]. Ki67 expression was evaluated by counting 500-1000 cells, depending on tumour cellularity, in at least 3 high-power fields (x40), provided that at least 500 cells were counted. Hot spot areas in tumours with heterogeneous staining were also evaluated and scored. It was scored as the ratio of stained cells to total tumour cells [13].

Statistical Method

The data were analysed with IBM SPSS V23. Compliance with a normal distribution was analysed by the Kolmogorov–Smirnov and Shapiro–Wilk tests. The chi-square test was used to compare categorical variables according to groups. The Mann–Whitney U test was used to compare the data that were not normally distributed according to paired groups. The Kruskal–Wallis test was used to compare the data that were not normally distributed according to groups of three or more. Spearman's rho correlation coefficient was used to analyse the relationship between the quantitative data that were nonnormally distributed. ROC analysis was used to determine the cut-off values of pretreatment Ki67 for pCR and pNR. The analysis results are presented as the mean ± standard deviation and median (minimum-maximum), and categorical data are presented as frequencies (percentages). The level of significance was taken as $p < 0.050$.

Results

All 178 patients included in the study were women. The mean age of the patients was 51.0±11.7 years (23–81 years). The mean follow-up period was 31 months (4–84 months). Breast-conserving surgery was performed in 102 (57.3%) patients, and mastectomy was performed in 76 (42.7%) patients. Sentinel lymph node biopsy was performed in 104 patients (58.4%), axillary dissection in 48 (27.0%) patients, and sentinel lymph node biopsy and axillary dissection together in 26 (14.6%) patients. A total of 163 (91.6%) of 178 tumours were invasive ductal carcinoma, 10 (5.6%) were invasive lobular carcinoma, 2 (1.1%) were mixed invasive breast carcinoma (invasive ductal+invasive lobular carcinoma), 2 (1.1%) were mucinous carcinoma, and 1 (0.6%) was metaplastic carcinoma. Three (1.7%) of 178 patients had grade I tumours, 57 (32.0%) had grade II tumours, and 118 (66.3%) had grade III tumours. Seventy-nine (55.6%) patients had lymph node metastasis. Nine patients were in stage I (5.0%), 118 (66.3%) patients were in stage II, 34 patients (19.1%) were in stage III, and 17 patients (9.6%) were in stage IV. A total of 122 (68.5%) of the patients were ER-positive, and 56 (31.5%) were ER-negative. A total of 113 (63.5%) were HER2-negative, and 65 (36.5%) were HER2-positive. Out of 178 patients, 48 (26.9%) had luminal A, 32 (18.0%) had luminal B HER2-negative, 44 (24.7%) had luminal B Her2-positive, 27 (15.2%) had HER2-positive, and 27 (15.2%) had triple-negative breast cancer (Table 1).

pCR was observed in 41 of 178 (23.0%) patients, pPR was observed in 80 (45.0%), and pNR was observed in 57 (32.0%) according to the MP grading system. According to the RCB classification system, 37 out of 178 patients (20.8%) had pCR, 87 (48.9%) showed pPR, and pNR was detected in 54 patients (30.3%). There was a significant moderate aggrement between the MP and RCB systems ($p < 0.001$) (Table 2). The lowest pCR rates in the MP and RCB classification systems were found in the luminal A subtype at 4.2% in both classification systems (Fig. 1). The luminal B HER2-negative subtype was second at 12.9% and 9.7%, respectively. The highest pCR rate was observed in triple-negative tumours (55.6% and 51.9%), and the HER2-positive subtype was in second, 33.3% in both classification systems (Fig. 2). A significant difference was found in the treatment response of the molecular subtypes according to both assessment systems ($p < 0.001$) (Table 3).

There was a significant difference in pretreatment Ki67 values between the MP response groups ($p < 0.001$). The pretreatment Ki67 level was higher in the pCR group than in the pNR and pPR groups (Table 4). A significant positive correlation was found between the MP grade and pretreatment Ki67 values ($r = 0.269$, $p < 0.001$). A significant difference was found between the posttreatment Ki67 values of the pNR group and the pPR groups ($p < 0.001$) (Table 4). A significant negative correlation was found between the MP response degree and posttreatment Ki67 ($r=-0.463$, $p < 0.001$). A significant difference was found between the posttreatment Ki67 change (pretreatment Ki67-posttretment Ki67) and MP response groups ($p < 0.001$) (Table 4). A significant positive correlation was found between the MP response degree and posttreatment Ki67 change ($r = 0.581$, $p < 0.001$).

A significant difference was found between the pretreatment Ki67 values of different RCB response classes ($p < 0.001$). The Ki67 value was higher in the pCR group than in the pNR and pPR groups (Table 4). A significant negative correlation was found between RCB response class and pretreatment Ki67 ($r= -0.266$, $p < 0.001$). A significant difference was found between the posttreatment Ki67 values of the pNR group and the pPR group ($p = 0.018$). The Ki67 value of the pNR group was significantly higher than that of the pPR group (Table 4). A positive correlation was found between the posttreatment Ki67 value and RCB response class ($r = 0.293$, $p < 0.001$). A significant difference was found between the posttreatment Ki67 change and RCB response groups ($p = 0.008$). There was no significant change in Ki67 in the pNR group after treatment, while there was a decrease in Ki67 in the pPR group (Table 4). A significant negative correlation was found between the RCB response class and posttreatment Ki67 change ($r= -0.318$, $p < 0.001$).

OS and DFS were not significantly different in the MP and RCB response groups ($p > 0.05$) (Table 4). In both grading systems, when the nearly complete response groups were combined with the complete response groups and classified as complete response, no correlations were found between the response groups and OS and DFS ($p > 0.05$).

When evaluated based on molecular subtypes, according to both systems (MP and RCB), no significant correlation was found between the pretreatment Ki67 value and treatment response ($p > 0.05$) (Table 5). In both classification systems, the posttreatment Ki67 value of the pNR group was significantly higher

than that of the pPR group in the luminal B HER2-positive molecular subtype (p = 0.041) (Table 5). While no significant change was observed in the Ki67 value after treatment in the pNR group in the luminal B Her2-positive subtype, a significant decrease was found in the pPR group in both classification systems (p = 0.011) (Table 5). The posttreatment Ki67 value increased in the pNR group in the HER2-positive subtype, while it decreased in the pPR group in both classification systems (p = 0.025) (Table 5).

A significant difference was found between the pretreatment and posttreatment median Ki67 values according to the molecular subtypes (p < 0.001, p < 0.001) (Table 6). The luminal A subtype had the lowest pretreatment ki67 value, while the triple-negative subtype had the highest pretreatment Ki67 value (Table 6). The triple-negative subtype had a significantly higher posttreatment Ki67 value than the other subtypes (p < 0.001) (Table 6). There was a significant difference between molecular subtypes in Ki67 change after treatment (p = 0.007). The luminal B HER2-positive subtype was the subtype with the highest decrease in Ki67, whereas in the triple-negative subtype, no difference between pre- and posttreatment Ki67 values was observed (Table 6).

There was no significant difference between the molecular subtypes in terms of DFS and OS (p > 0.05) (Table 6). A significant positive correlation was found between the posttreatment Ki67 value and OS in the luminal A molecular subtype (r = 0.303; p = 0.048). A significant negative correlation was found between the posttreatment Ki67 value and overall survival in the HER2-positive subtype (r=-0.544, p = 0.020). A significant negative correlation was found between pretreatment Ki67 and disease-free survival in the luminal B HER2-negative subtype (r=-0.377, p = 0.036). No significant correlations were found between posttreatment Ki67 change and OS and DFS (p = 0.680 and p = 0.657, respectively). No correlations were found between pretreatment Ki67, posttreatment Ki67 and OS and DFS in groups that did not respond completely to treatment (pNR + pPR) (MP and RCB) (p > 0.05).

A significant correlation was found between the pretreatment Ki67 value and the grade of biopsy (pretreatment) (p < 0.001), but there was no significant correlation between pretreatment Ki67 value and the presence of lymph node metastasis, HER2 status, and stage (p = 0.062, p = 0.933, p = 0.458). A significant positive correlation was found between posttreatment tumour size and posttreatment Ki67 in both MP and RCB systems (r = 0.222, p = 0.01; r = 0.218, p = 0.011).

ROC curve analysis was performed to define a cut-off value of Ki67 for pCR. The area under the curve (AUC) was 0.753 when the cut-off was 37.5 for Ki67 according to the complete response status in the MP system (95% CI 0.673–0.833) (Fig. 3a). The value obtained was significant (p < 0.001). The sensitivity was 73.2%, and the specificity was 59.9%. When the value of cut-off was taken as 37.5 for Ki67, the area under the curve (AUC) was 0.744 according to the RCB complete response group (95% CI 0.66–0.827). The value obtained was significant (p < 0.001). The sensitivity was 73%, and the specificity was 58.9% (Fig. 3b). In both MP and RCB systems, a Ki67 cut-off value to identify pNR cases was not detected (respectively, p = 0,125, p = 0,059).

Discussion

Since NAC chemotherapy is currently an accepted treatment for breast cancer, including early-stage breast cancer, markers with prognostic and predictive value on the basis of NAC have been resarched [25, 37]. The importance of the Ki67 expression level as a prognostic factor in breast cancer has been demonstrated in many studies [20, 38].

pCR is an end point of NAC and is used as an independent prognostic marker [39].

However, there are also differences between studies [39–41]. The strongest correlation between pCR and survival was reported in aggressive tumours [39, 40]. The results are inconsistent in the luminal subtypes, while pCR is correlated with prolonged survival, especially in the triple-negative and HER2-positive subtypes [13, 41]. Von Minckwitz et al. [4] reported that pCR was significantly correlated with prolonged DFS in luminal B HER2-negative, HER2-positive and triple-negative tumours, but no such correlation was found in luminal B HER2-positive and luminal A tumours. One of the reasons for the differences among studies may be the use of different criteria for pCR. In some studies, only the response of the breast is taken into account, while in others, the lymph node response is also taken into account along with the breast response. There should be no residual tumour in breast and in lymph node for pCR in the RCB classification. In contrast, the lymph node is not considered in evaluation in the MP grading system [9, 34]. Hence, it is crucial to optimize the whole process as a multidisciplinary approach. However, the histopathological evaluation method is not usually stated in the literature [41, 42]. Luminal A tumours, which have low pCR rates, have a good prognosis and low chemosensitivity [43]. It has been shown that a pCR is not prognostic in ER-positive tumours [44, 45].

While the MP grading system evaluates treatment response according to the reduction in cancer cellularity, the RCB classification evaluates tumour response based on the tumour diameter, tumour cellularity, and axillary lymph node status [9, 34]. For this reason, the compatibility of the two evaluation systems was investigated [46, 47]. Romero et al. [46] found a high correlation between the MP grading system and the RCB classification in their study, where they compared the two treatment response assessment systems. They also found a correlation between the MP and RCB systems and survival [46]. We found a moderate correlation between the MP and RCB response assessment systems in this study. There is some difference between the response groups since there are differences in the evaluation criteria of the two systems. In this study, no correlation was found between the degrees of treatment response and survival in either system. Similarly, no correlation was found between MP grade and RCB score and survival in a study comparing treatment response systems [48]. Choi et al. [49] compared 7 different treatment response assessment systems and found all systems to be correlated with OS and DFS in their study. When evaluated in molecular subtypes, they found that only the RCB system was correlated with OS and DFS in all subtypes. This finding suggests that evaluation of breast and lymph nodes together is better correlated with the clinical course [47, 49]. All systems were significantly correlated with OS and DFS in the triple-negative group. We found no correlations between treatment response and OS and DFS in either system.

Data regarding the importance of the Ki67 index in predicting pCR are controversial [25, 26]. It has been reported that tumours with a high pretreatment Ki67 index have a higher pCR rate [27, 28]. Balmativola et al. [25] found that the pretreatment Ki67 index was higher in the pCR + pPR group than in the pNR group in their study, in which they grouped treatment response as pNR and pCR + pPR. They found that the cut-off for the Ki67 index, which distinguishes pNR patients from pPR and pCR patients, was 18% [25]. It was reported that patients with a Ki67 index of less than 25% were nonresponders to treatment and that DFS was longer in tumours with a Ki67 index of less than 12% in a study showing that the Ki67 index is correlated with pCR [30]. Acs et al. [27] reported that the Ki67 index and subtype were significantly correlated with pathological response, and NAC was more effective in tumours with a Ki67 index of at least 20%. In their study, the cut-off value for Ki67 was found to be 15% when OS was taken into account, but no Ki67 cut-off correlated with DFS was found [27]. The pCR rates were 4.2%, 12.8%, and 29% in a study grouping the Ki67 index as low (≤15%), moderate (15.1–35%) and high (>35%), respectively [37]. Evaluation of all the patients in this study revealed that in the hormone receptor-positive group, OS and DFS were longer in tumours with a low Ki67 index. However, it has been reported that such a correlation was not observed in triple-negative tumours [37].

Zhao et al. [50] reported that a high Ki67 index was a strong predictor of pCR in their study of HER2-positive tumours. The Ki67 index was correlated with the degree of pathological response in this study. In our study, the pretreatment Ki67 value was found to be higher in the pCR group than in the pPR and pNR groups in both the MP and RCB classification systems. We identified a Ki67 cut-off for pCR of 37%. We could not find a Ki67 value that distinguished pNR patients from pPR and pCR patients.

The treatment responses of the molecular subtypes were correlated with those in the literature [25]. In our study, triple-negative tumours had the highest pCR rates. HER2-positive tumours ranked number two. The lowest pCR rate was in luminal A tumours, while the luminal B HER2-negative subtype was ranked number 2. Luminal A tumours, which had the lowest pCR rate in our study, had the lowest pretreatment Ki67 index values. The triple-negative molecular subtype, which had the highest pCR rate, was the group with the highest pretreatment Ki67 index values. No correlation was found between MP and RCB grades and pretreatment Ki67 values in the molecular subtypes in our study. Luminal B HER2-negative tumours are tumours of intermediate category, and endocrine therapy is recommended for all these patients and cytotoxic therapy for the majority of these patients [19]. Multigene analyses are performed on adjuvant and neoadjuvant grounds to distinguish patients who are likely to benefit from chemotherapy [51, 52]. The evaluation of the cell cycle-specific antigen Ki67 by immunohistochemical methods in the evaluation of the proliferative activity of tumours has been widely studied. It has been shown that patients with luminal B/HER2-negative tumours with a high proliferation index benefit from chemotherapy [21, 37, 53]. In our study, we could not find a correlation between the pretreatment Ki67 proliferation index and treatment response in the luminal B HER2-negative subtype. However, we found a negative correlation between pretreatment Ki67 and disease-free survival in the luminal B HER2-negative subtype. Zong et al. [54] also reported that the DFS of luminal B HER2-negative tumours with a high Ki67 index was shorter than that of the group with a low Ki67 index. They also reported that they did not observe a correlation

between OS and the Ki67 index, similar to our study [54]. Ki67 should be considered for the selection of more aggressive treatment modalities in luminal B HER2-negative tumours [54].

The evaluation of residual disease after NAC is important not only to identify patients who should receive additional adjuvant therapy but also to identify the subgroup with a good prognosis among patients with this residual disease [7, 13]. It has been reported that posttreatment Ki67 expression levels are associated with prognosis [3, 7, 13, 32, 55]. Especially in tumours where the prognostic value of pCR is limited, a prognostic predictor such as Ki67 in residual tumours has gained importance. It has been reported that patients with low Ki67 levels in residual tumours after NAC have a similar prognosis to patients with pCR(5). Nishimura et al. [30] reported in their study that there was a significant decrease in the Ki67 value after neoadjuvant treatment and that patients evaluated as clinical complete response had a lower Ki67 value after treatment. Assersohn et al. [56] reported that the level of Ki67 at excision is correlated with prognosis in tumours that do not respond completely. In our study, when we evaluated the Ki67 value without division by molecular subtypes, the posttreatment Ki67 index was found to be higher in the pNR group than in the pPR group. When we evaluated the molecular subtypes separately, the posttreatment Ki67 index of the pNR group was found to be higher than that of the pPR group in the luminal B HER2-positive molecular subtype. Von Minckwitz et al. [5] reported that high posttreatment Ki67 levels were correlated with relapse and death. They reported that the posttreatment Ki67 level was more prognostic than the pretreatment Ki67 level and the change in the Ki67 level after NAC in their study. They found that posttreatment and pretreatment Ki67 levels were correlated with residual tumour size [5]. In our study, a negative correlation was found between the posttreatment Ki67 index and OS only in the HER2-positive subtype. Similarly, in our study, the residual tumour size was correlated with the posttreatment Ki67 level. However, unlike that in their study, there was a negative correlation between the residual tumour size and the pretreatment Ki67 level in our study. This result is different from theirs and is compatible with the correlation between the pretreatment Ki67 level and the response we found in our study. Interestingly, a weak positive correlation was found between the posttreatment Ki67 index and OS in the luminal A subtype in our study.

It has been reported that DFS and OS are longer in patients with changed Ki67 expression than in patients with unchanged Ki67 expression [7, 57]. It has also been reported that the proliferative activity of tumours that respond to treatment is higher and that the proliferative activity decreases after treatment [58]. That study reported that the DFS was longer in tumours whose proliferative activity decreased by more than 30% after NAC [58]. It was reported that the change in the Ki67 level was prognostic in the luminal B, triple-negative and HER2-positive subtypes in the study of Matsubura et al. [57]. In accordance with the literature, while there was no change in the posttreatment Ki67 index in the pNR group, the Ki67 index was decreased in the pPR group in our study. However, no correlations were found between the Ki67 change and OS and DFS. In this study, the greatest decrease in the Ki67 index was observed in the luminal B HER2-positive subtype. A decrease was observed in the pPR group, while no change was observed in the pNR group in the luminal B HER2-positive subtype. While the posttreatment Ki67 increased in the pNR group of the HER2-positive subtype, it decreased in the pPR group. The Ki67 index showed no change in triple-negative tumours, in which we observed the highest response rate, in the pNR and pPR groups in our

study. Diaz-Botero et al. [7] also reported that no decrease in the Ki67 index was observed in the pPR group in the triple-negative and HER2-positive subtypes with high pCR response rates [7]. Triple-negative breast cancer is an aggressive subtype of breast cancer with higher recurrence and metastasis rates than other subtypes [59]. In this group with an aggressive course, NAC allows to evaluate the chemotherapy response of the tumour before surgery. Triple-negative tumours, for which pCR has been achieved after NAC, have been reported to have a better prognosis than those without pCR [2]. In our study, while we found a correlation between the posttreatment Ki67 index and OS for only the HER2-positive subtype, we did not find a correlation in the triple-negative subtype. Although our study could not show its relationship with survival, different treatment options should be considered for triple-negative tumours that do not show a posttreatment Ki67 level decrease.

This study has some limitations. Although the number of our patients was not small, the decreased number of patients when divided into five molecular subtypes limited the evaluations between of molecular subtypes. In addition, our follow-up periods may have been insufficient for prognostic evaluation due to late recurrence of luminal tumours.

Conclusion

In our study, in which we assessed two different response evaluation systems, the relationship between pre-NAC and post-NAC Ki67 levels and treatment response was shown in both systems. The Ki67 index should be used as a marker to predict treatment response. The negative correlation we found between the pre-NAC Ki67 level and disease-free survival in the luminal B HER2-negative subtype shows that the pre-NAC Ki67 level should be considered in the treatment plan for this subtype. In the triple-negative subtype, the Ki67 level did not decrease after NAC, and we found a negative correlation between the post-NAC Ki67 level and OS in the HER2-positive subtype, suggesting that the Ki67 level should be evaluated after treatment and that more aggressive treatments should be given to patients with these subtypes.

Declarations

Consent for publication

Not applicable.

Competing interests

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Authors' contributions

YS was a major contributor in writing the manuscript. FK participated in the histological/gross evaluations. GD, BK and NO participated in the clinical data collection. GD participated in the study conception. All authors read and approved the final manuscript.

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Tables

Tables 1 to 6 are available in the Supplementary Files section.

Figures

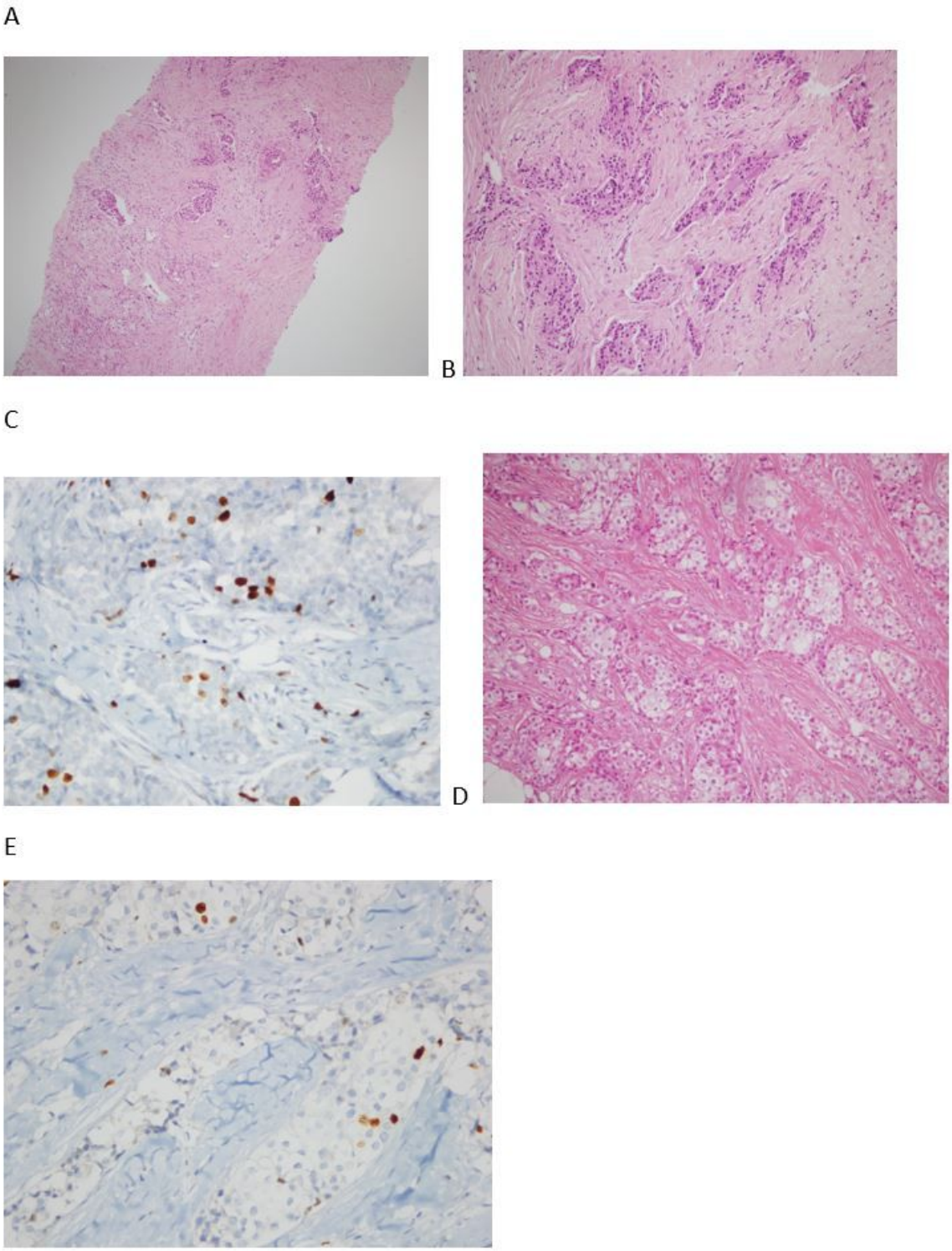
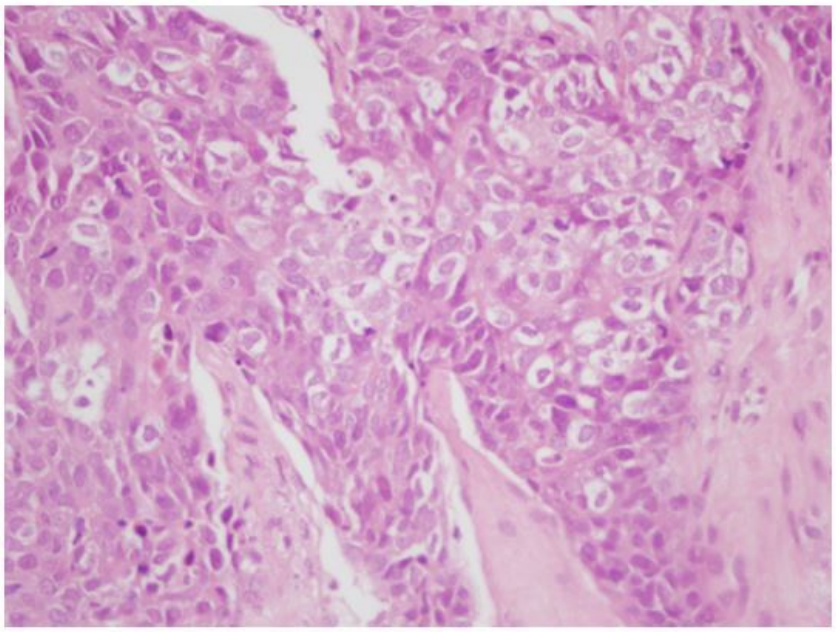


Figure 1

Partially responding (Miller -Payne grade2, RCB- II) Luminal A invasive ductal carcinoma, pretreatment biopsy H-Ex100 (A); H-Ex200 (B); Ki67X400 (C) (Ki67 level was evaluated as %10) and residual disease after neoadjuvant cheomotherapy H-Ex200(D) and Ki67x400 (Ki67 level was evaluated as %5).

A



B

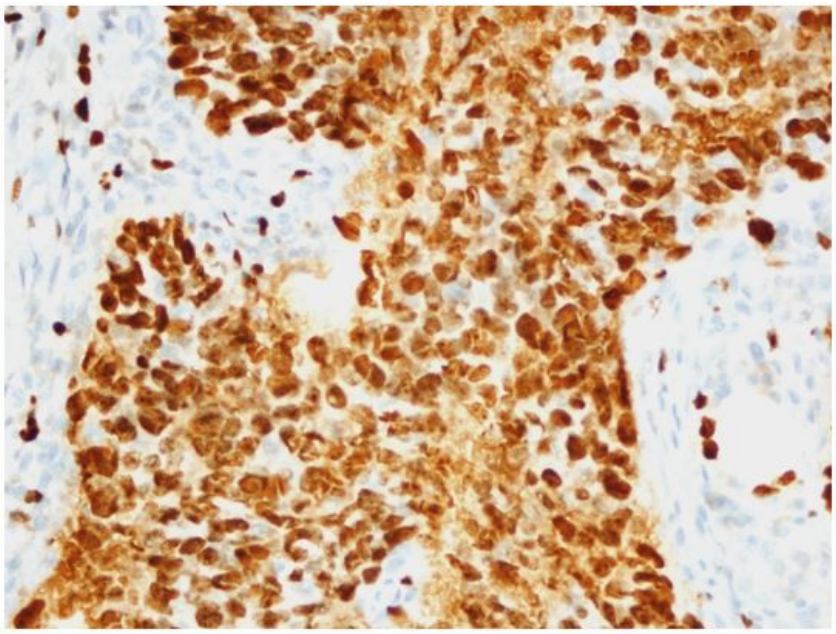


Figure 2

Pathological complete responding tripple negative invasive ductal carcinoma, preteratment biopsy H-Ex400 (A) ; Ki67x400 (B) (Ki67 level was evaluated as %90).

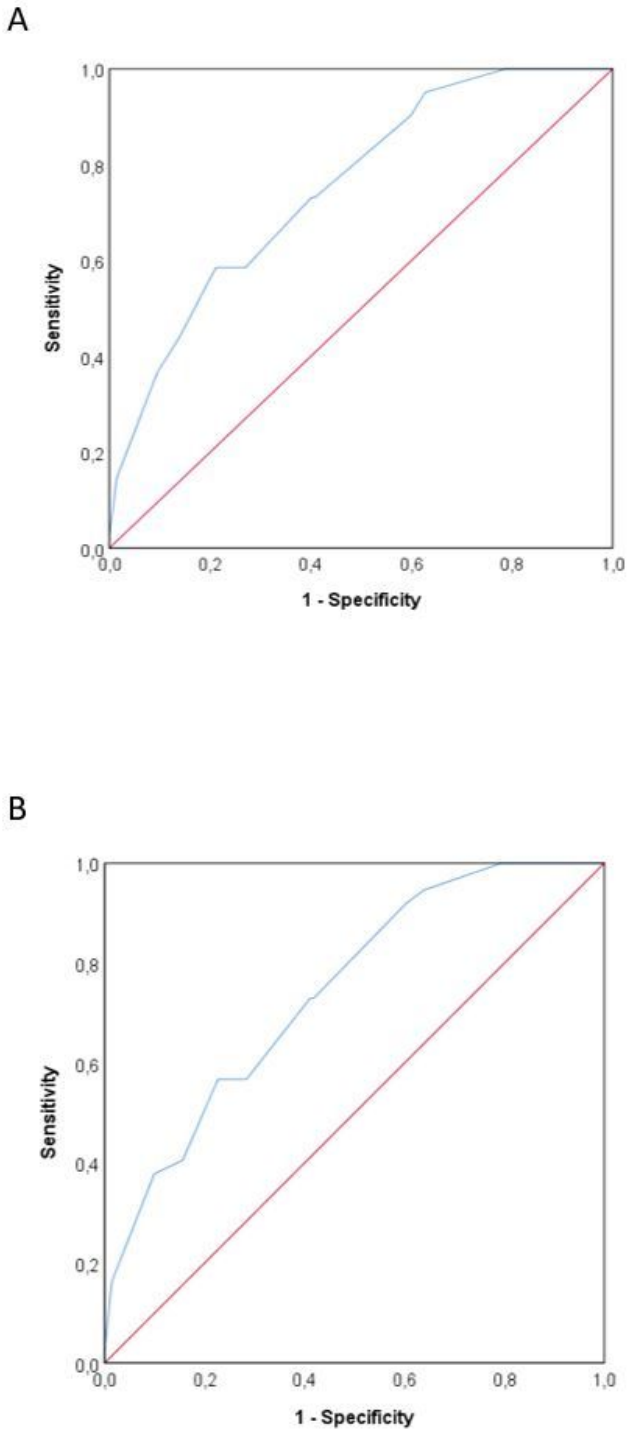


Figure 3

ROC curves of Ki-67 cut-off values for pCR. Miller-Payne (A) and RCB (B).

Supplementary Files

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