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High FOXP3+ and Low KI-67 Expression as Risk Factors for Poor Response to Neoadjuvant Chemotherapy in Triple-Negative Breast Cancer (TNBC)

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ABSTRACT

Background: Triple-negative breast cancer (TNBC) is an aggressive subtype with limited treatment options and poor prognosis. Neoadjuvant chemotherapy (NAC) is standard for TNBC, though response rates vary widely. This study investigates high FOXP3+ and low Ki-67 expressions as potential predictors of poor NAC response in TNBC. ***Methods:*** This case-control study included 70 TNBC patients who received NAC. Based on NAC response, patients were categorized as cases (poor response) or controls (good response) per RECIST 1.1 criteria. FOXP3 and Ki-67 levels were assessed via immunohistochemistry, with FOXP3 classified as high (>2) or low (≤2) and Ki-67 as high (>20%) or low (≤20%). Associations between marker levels and NAC response were analyzed using chi-square and Fisher's exact tests, with significance at $p < 0.05$. ***Results:*** High FOXP3+ expression was significantly associated with poor NAC response (OR=4.231, p=0.004), indicating a fourfold increased risk. However, low Ki-67 expression was not a significant predictor of NAC response (p=0.710). These findings highlight FOXP3+ as a relevant factor in NAC outcomes, while Ki-67 lacks independent predictive value in TNBC. ***Conclusion:*** High FOXP3+ expression is a significant predictor of poor NAC response in TNBC, likely due to its immunosuppressive effects. FOXP3+ could serve as a biomarker for refining NAC strategies in TNBC, while Ki-67 appears less predictive.

Keywords: cancer immunology; immune biomarkers; regulatory T cells; tumor microenvironment.

INTRODUCTION

Triple-negative breast cancer (TNBC) is an aggressive and heterogeneous malignancy lacking expression of progesterone, estrogen, and HER2 receptors, comprising 10-20% of all breast cancer cases (Lebert, 2018; Mahmood, 2018). TNBC is associated with a poor prognosis, higher prevalence in younger and non-Hispanic Black women, and is characterized by aggressive, high-grade tumors (Scott, 2019). Neoadjuvant chemotherapy is the primary treatment for TNBC; however, response rates vary widely, influenced by factors including tumor microenvironment and immune profile (Nedeljkovic, 2019).

The immune microenvironment, particularly T-cell infiltration, has been shown to be a predictor of chemotherapy response. While CD8+ T cells are linked to improved outcomes, regulatory T cells (FOXP3+) are often associated with poorer chemotherapy responses in TNBC due to their immunosuppressive role (Silva, 2021). Additionally, Ki-67, a widely used biomarker for tumor proliferation, is associated with higher pathologic complete response rates when elevated.

Given these observations, this study aims to explore high FOXP3+ and low Ki-67 expressions as risk factors for poor neoadjuvant chemotherapy response in TNBC patients. These findings may contribute to the field of tumor immunology and assist clinicians in making holistic treatment decisions to improve patient outcomes.

METHODS

This case-control study explored the association between FOXP3 and Ki-67 expression levels and the response to neoadjuvant chemotherapy in patients with triple-negative breast cancer (TNBC), classifying patients as cases or controls based on chemotherapy response according to RECIST 1.1 criteria. Patients with progressive or stable disease were considered cases, while those with partial or complete responses were designated as controls. Conducted over a period from August to September 2023, the study utilized secondary data from a tertiary oncology referral center. Purposive sampling was employed, focusing on TNBC patients who had completed a minimum of three cycles of neoadjuvant chemotherapy with immunohistochemical (IHC) evaluation.

Inclusion criteria required patients to have complete medical records detailing demographic and clinical information, including data on age, tumor size, and immunological markers. Exclusion criteria applied to patients with incomplete records or who had not completed the minimum chemotherapy requirement.

The primary data points collected included FOXP3 and Ki-67 expression levels, tumor size, and patient age. FOXP3 expression was measured based on T regulatory cell infiltration, documented as either high or low. Ki-67 expression, indicative of cellular proliferation, was classified as high if ≥20% and low if <20%, in line with standard pathological thresholds. Tumor size, categorized clinically as T2 (2-5 cm), T3 (>5 cm), or T4 (with chest wall or skin involvement), and age (grouped as <40 or ≥40 years) were recorded to explore their influence on chemotherapy response. Ethical approval was obtained prior to accessing patient records, and data were systematically gathered from pathology reports and clinical records.

Statistical analyses included descriptive and inferential tests, with categorical variables presented as frequencies and percentages and continuous variables as means and standard deviations. Chi-square tests were used to assess the association between FOXP3 and Ki-67 expression and chemotherapy response. Odds Ratios (OR) with 95% confidence intervals (CI) estimated the strength of associations, with statistical significance established at $p < 0.05$.

RESULTS

This study collected 35 cases (poor response to neoadjuvant chemotherapy [NAC]) and 35 controls (good NAC response) from TNBC patients who received NAC at a tertiary hospital. The mean age of the study sample was 51.11 years (standard deviation [SD] ±11.04), with the youngest being 25 years old and the oldest 77 years. Based on NAC response, 7 samples (10%) achieved pathological complete response (pCR), 28 samples (40%) had a partial response, 23 samples (23%) showed stable disease, and 12 (12%) had a stable disease response. The baseline characteristics of the study sample are presented in Table 1. Statistical analysis showed no differences in age, parity, tumor size, lymph node involvement, or type of chemotherapy between the case and control groups in this study (p>0.05).

TABLE 1: Baseline characteristics of the study.

Characteristics	Total (n=70)	Group		p-value
		Case (n=35)	Control (n=35)	
Age (%)				
≥40 years old	60 (85.7)	28 (80.0)	32 (91.4)	0.172[a]
<40 years old	10 (14.3)	7 (20.0)	3 (8.6)	
Parity (%)				
1-3	48 (78.7)	25 (83.3)	23 (74.2)	0.384[a]
>3	13 (21.3)	5 (16.7)	8 (25.8)	
Tumor size (%)				
T1-T2	7 (10.0)	5 (14.3)	2 (5.7)	0.428[b]
T3-T4	63 (90.0)	30 (85.7)	33 (94.3)	
Lymph node (%)				
N0	5 (7.1)	2 (5.7)	3 (8.6)	1.000[b]
N1-N3	65 (92.9)	33 (94.3)	32 (91.4)	
Histopathology type (%)				
Invasive Ca	61 (87.1)	30 (85.7)	31 (88.6)	1.000[b]
Others	9 (12.9)	5 (14.3)	4 (11.4)	
Chemotherapy (%)				
First line	60 (85.7)	30 (85.7)	30 (85.7)	1.000[a]
Second line	10 (14.3)	5 (14.3)	5 (14.3)	

[a]Chi-square test, [b]Fisher exact test.

The Kolmogorov-Smirnov test indicated that FOXP3 data were not normally distributed ($p<0.001$). The median FOXP3 value was 2, with a minimum of 0 and a maximum of 56. The data were then classified into high (>2) and low (≤2) FOXP3 expression. The majority of samples (54.3%) had high FOXP3 expression. Bivariate analysis with the chi-square test showed that high FOXP3 expression is a significant risk factor for poor NAC response in TNBC patients (OR=4.231; p=0.004). Bivariate test results can be found in Table 2. Furthermore, Ki67 expression was classified as low (≤20%) and high (>20%). The majority of samples (88.6%) exhibited high Ki67 expression. Bivariate analysis with Fisher's exact test indicated that low Ki67 expression is not a risk factor for poor NAC response in TNBC patients (p=0.710). Bivariate test results can be seen in Table 3.

TABEL 2: Bivariate analysis of FOXP3+ on NAC response.

FOXP3+	Total (n=70)	Group		OR	95% CI	p-value
		Case (n=35)	Control (n=35)			
High (>2)	38 (54.3)	25 (71.4)	13 (37.1)	4.231	1.550–11.546	0.004
Low (≤2)	32 (45.7)	10 (28.6)	22 (62.9)			

TABLE 3: Bivariate analysis of Ki67 on NAC response.

Ki67	Total (n=70)	Group		OR	95% CI	p-value
		Case (n=35)	Control (n=35)			
Low (≤20%)	8 (11.4)	5 (14.3)	3 (8.6)	1.778	0.391–8.092	0.710
High (>20%)	62 (88.6)	30 (85.7)	32 (91.4)			

DISCUSSION

FOXP3 belongs to the FOX protein family, which plays an essential role in regulating Treg development and function (Domschke, 2016). This study classifies FOXP3 expression into high (>2) and low (≤2) categories based on the median value. Most samples (54.3%) have low FOXP3 expression, while high FOXP3 expression is proven to be a significant risk factor for poor NAC response. Patients with high FOXP3 expression have a 4.231-fold higher risk of a poor NAC response compared to those with low FOXP3 expression.

The relationship between FOXP3 expression and NAC response has been demonstrated in previous studies. Research by Abdelrahman et al. (2021) compared pCR levels in 50 TNBC patients based on FOXP3 expression. Patients without FOXP3 Treg expression (FOXP3-) had significantly higher pCR levels (Abdelrahman et al., 2021). In addition to pre-NAC expression, changes in FOXP3 expression post-NAC have also been reported to be related to NAC response. Breast cancer patients achieving pCR experienced a significant decrease in FOXP3 expression post-NAC compared to non-responders. Lack of FOXP3 infiltration in tumor cells post-NAC is associated with a favorable NAC response (Ladoire et al., 2008).

FOXP3 expression and CD8 T cells also play a crucial role in predicting NAC response and prognosis in TNBC, particularly by evaluating the CD8+/FOXP3+ ratio. A study by Goto et al. (2018) showed a significant decrease in the CD8+/FOXP3 ratio post-NAC. Another study by Miyashita et al. (2015) demonstrated that a high CD8/FOXP3 ratio in residual tumors accurately predicts a better prognosis in TNBC patients post-NAC with non-pCR responses. However, FOXP3 expression alone did not show a significant correlation with TNBC prognosis (Miyashita et al., 2015).

Different findings have also been reported in previous studies. Research by Miyashita et al. (2014) showed that FOXP3+ expression was not a significant predictor of pCR in TNBC patients. This result was non-significant across various assessment locations, including intratumoral, proximal stroma, distal stroma, and total FOXP3+.

Further research in 2015 on 131 TNBC patients showed that 45% experienced an increase in TIL FOXP3 expression post-NAC, but this change was not associated with patient prognosis (Miyashita et al., 2015). A study by Lee et al. (2017) on 44 breast cancer patients reported that FOXP3+ expression was significantly higher in the good NAC response group.

FOXP3+ is also associated with a favorable breast cancer prognosis as FOXP3+ T cells can inhibit tumor-promoting inflammatory responses. FOXP3+ is expressed in various cell populations and functions as both regulatory T cells (Treg) and non-regulatory (non-Treg) cells. This cell type can produce cytokines such as transforming growth factor-beta 1 (TGF-β1) and interleukin-10 from Tregs, as well as interferon-gamma (IFN-γ) and interleukin-17 from non-Tregs. Both subpopulations (Treg and non-Treg) cannot be distinguished solely through immunohistochemical (IHC) staining because both yield positive FOXP3 results, requiring additional marker tests like CD4+ and CD25+ (Semeraro et al., 2016; Kadoya et al., 2022). FOXP3 Tregs have immunosuppressive functions, while FOXP3 non-Tregs lack immunosuppressive functions and are associated with inflammatory cytokine production (Kadoya et al., 2022). This explains the varying research findings regarding the role of FOXP3+ in NAC response and breast cancer prognosis (Lee et al., 2017).

Ki-67, a nuclear protein, is a proliferation marker easily detectable through immunohistochemistry techniques. All cell cycle phases, except the G0 (quiescent) phase, express Ki-67. Breast cancers with high Ki-67 expression generally respond better to chemotherapy but are associated with poor prognosis. This aligns with the paradox seen in TNBC, as TNBC patients generally exhibit higher Ki-67 expression than non-TNBC patients (Elnemr et al., 2016; Wang et al., 2021). However, the cut-offs used to categorize Ki-67 vary widely, ranging from 10% to 50%. This study found that 85.7% of TNBC patients had high Ki-67 expression (>20%). Research by Amin et al. (2022) similarly reported that 81.7% of TNBC patients had Ki-67 expression ≥20%. High Ki-67 expression (≥20%) was also frequently found in residual tumors post-NAC (Toss et al., 2022).

The median Ki-67 expression in TNBC patients was also reported to be significantly higher than in non-TNBC patients (Yeh et al., 2017).

This study found no differences in Ki-67 expression between the poor and good NAC response groups, and Ki-67 expression was not a significant NAC response risk factor. Previous studies on the role of Ki-67 in NAC response have reported varying results. Some previous studies support this study's findings (Kim et al., 2015). Research by Kim et al. (2015) showed that the significant relationship between pCR and Ki-67 expression in breast cancer patients is not independent but should consider p53 expression. The lowest pCR rate (5.2%) was observed in patients with low Ki-67 and p53 expression, while the highest pCR rate (25.8%) was observed in patients with high Ki-67 and p53 expression. However, this relationship was not significant in the group with only high Ki-67 expression. Elnemr et al. (2016) reported similar findings, showing that high Ki-67 expression (>14%) in breast cancer patients generally correlated significantly with higher pCR rates, though this was not significant in the TNBC subgroup. This underscores that, despite high Ki-67 expression in TNBC, this proliferative index alone cannot predict chemosensitivity in this subtype. Furthermore, Ki-67 expression was not associated with TNBC patient prognosis (Miyashita et al., 2015).

Different results have been reported in previous studies (Kubouchi et al., 2020; Keam et al., 2011; Zhang et al., 2014; Li et al., 2010; Guestini et al., 2018). Research by Kubouchi et al. (2020) on 51 stage I and II TNBC patients with apocrine types reported significantly better antitumor effects from NAC in patients with high Ki-67 levels (≥50%). Low Ki-67 levels (<50%) in apocrine and special TNBC subtypes are crucial and effective in avoiding unnecessary NAC. Using the same cut-off (≥50%), Li et al. (2010) also reported that high Ki-67 expression was a significant predictor of pCR. Keam et al. (2011) similarly reported that high Ki-67 expression (≥10%) in 105 TNBC patients correlated with higher pCR rates in NAC compared to the low Ki-67 group. A meta-analysis by Zhang et al. (2014) of five articles reported that the pCR rate in TNBC with high Ki-67 expression was 3.36 times higher than in TNBC with low Ki-67 expression.

Ki-67 expression significantly changes post-NAC, indicating that NAC affects cancer cell proliferation. A reduction in Ki-67 expression post-NAC correlates with improved clinical prognosis. Lack of Ki-67 reduction post-NAC significantly correlates with lower recurrence-free survival (RFS) and overall survival (OS) in TNBC patients (Wang et al., 2021). This finding is also supported by previous studies by Wang et al. (2016), reporting that Ki-67 expression correlates with NAC response in TNBC patients given paclitaxel plus carboplatin regimens. Significant reductions in Ki-67 post-NAC were more common in good responders. Significant Ki-67 reductions post-NAC also significantly indicate a better prognosis (Wang et al., 2016).

This study has several limitations that could impact the findings. First, the chosen Ki-67 cut-off of 20% is lower than that in some prior studies, which might have limited the biomarker's utility in predicting NAC response. Future studies may consider using higher cut-offs or continuous variable analyses to assess Ki-67's predictive value with greater precision. Additionally, FOXP3 staining was restricted to "hotspot" regions, which may not fully capture the heterogeneity of FOXP3 expression across stromal and intratumoral areas. A more comprehensive analysis incorporating multiple regions of the tumor microenvironment would provide better insights into FOXP3's role. Finally, the CD8+/FOXP3+ ratio warrants further investigation as an integrative biomarker to better predict TNBC response to NAC, especially in highly proliferative subtypes with complex immune landscapes.

CONCLUSION

This study identifies high FOXP3+ expression as a significant risk factor for poor response to neoadjuvant chemotherapy (NAC) in triple-negative breast cancer (TNBC), indicating the impact of immune suppression on chemotherapy outcomes. In contrast, low Ki-67 expression was not a predictor of NAC response, suggesting limited utility as a standalone marker in TNBC. These findings underscore FOXP3+ as a potential biomarker for refining NAC strategies in TNBC.

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