RESEARCH ARTICLE

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Immunoscore *CD3/CD8* and *FOXP3* Expression Correlate to Neoadjuvant Chemotherapy Response in Triple Negative Breast Cancer: A Case-Control Study

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Abstract

Objectives: To explore the significance of diminished CD3/CD8 and CD3/CDR45RO immunoscores, as well as elevated *FOXP3* expression, as potential risk factors for unfavorable responses to neoadjuvant chemotherapy among patients with triple-negative breast cancer (TNBC). **Methods:** A case-control study was conducted across two hospitals (a public and a private facility) from August 1st, 2021, to August 31st, 2022. The study population comprised patients diagnosed with the TNBC subtype, with available paraffin blocks from biopsy procedures. Immunohistochemical staining was performed on specimens for CD3, CD8, CD45RO, and FOXP3 antibodies. **Results:** A total of seventy-two patients were enrolled in the study, among whom seven patients (16.7%) achieved a pathological complete response to neoadjuvant chemotherapy (NAC). Notably, there existed a significant correlation between CD3/CD8 and CD3/CD45RO immunoscores, as well as *FOXP3* expression, and NAC response ($p<0.05$). Multivariate analysis involving 70 samples from the case-control study revealed that a diminished CD3/CD8 immunoscore (aOR=10.930; 95% CI=1.336–89.420) and heightened *FOXP3* expression (aOR=11.775; 95% CI =2.537-54.656) independently posed as risk factors for unfavorable NAC response ($p<0.05$). However, the CD3/CD45RO immunoscore did not emerge as an independent risk factor for NAC response. **Conclusions:** A reduced CD3/CD8 immunoscore and elevated *FOXP3* expression stand as autonomous risk factors for suboptimal NAC response in patients with TNBC.

Keywords: CD3/CD8- CD3/CD45RO- FOXP3- immunoscore- triple negative breast cancer

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Introduction

Breast cancer is a widespread form of cancer that poses significant health risks and fatalities worldwide. In 2018 alone, it accounted for over 2 million cases globally, resulting in 626,679 deaths [1]. In Indonesia, it stood out as the most prevalent cancer among women, with an incidence rate of 42.1 per 100,000 individuals and a mortality rate of 17 per 100,000 individuals in the same year [2]. One of its subtypes, known as triple-negative breast cancer (TNBC), is characterized by the absence of estrogen receptors, progesterone receptors, and human epidermal growth factor-2 (HER-2) receptors [3, 4]. TNBC represents about 10-15% of all new breast cancer cases and is notably more aggressive with a poorer prognosis compared to other subtypes [5–7]. Its clinical outcomes are influenced by various factors including molecular heterogeneity, such as CD8 infiltrate density, which impacts its response to treatments [8, 9].

The interplay between cancerous tumors and the immune system, particularly within the microenvironment surrounding the tumor, has emerged as a central focus of modern cancer research [10]. The immune composition of this microenvironment significantly impacts the efficacy of chemotherapy. Typically, tumors with high levels of immunogenicity exhibit favorable responses to chemotherapy. This is because when cancer cells are destroyed, they release neoantigens that stimulate immune cells, leading to an anti-cancer immune reaction and ultimately boosting the effectiveness of chemotherapy. It's noteworthy that triple-negative breast cancer (TNBC) stands out as the most immunogenic subtype of breast cancer, characterized by its rapid evolution and adaptability. Consequently, the immune makeup of the tumor microenvironment serves as a predictive indicator for chemotherapy response [11, 12].

Given these considerations, lymphocyte markers present themselves as promising contenders for the development of predictive models in breast cancer. Among the effector lymphocytes, three CD markers

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stand out: CD3 (a general marker for lymphocytes), CD8 (marking cytotoxic T-lymphocytes), and CD45RO (marking memory T-cells). The combinations of CD3/CD8 and CD3/CD45RO are collectively known as the immunoscore (IS). The clinical promise of this approach lies in constructing immunoscores based on these markers, assessed within two regions of the tumor: intra-tumor and tumor periphery [13–15]. Presently, immunoscores are seen as the most feasible model, either as substitutes for or supplements to the traditional tumor-node-metastasis (TNM) staging system [12, 13].

The immunosuppressive role of the immune system in supporting tumor growth can be observed through the presence of regulatory T cells (Tregs), identified by the expression of forkhead box protein *P3* (*FOXP3*) [16–18]. FOXP3, belonging to the FOX protein family, acts as a key regulator in the formation and functional pathways of Treg cells. Elevated levels of *FOXP3* expression lead to heightened suppression of the immune system, impeding the eradication of cancer cells [19].

The objective of this research was to assess the potential clinical relevance of immunoscores (*CD3/CD8* and *CD3/CD45RO*) and *FOXP3* expression in triple-negative breast cancer (TNBC), recognized as the most immunogenic subtype within this cancer spectrum. The aim was to investigate the contributions of these immunoscores and *FOXP3* expression as potential indicators for predicting suboptimal responses to neoadjuvant chemotherapy among TNBC patients. By doing so, this study endeavors to bridge theoretical and practical voids in the academic and clinical management of TNBC.

Materials and Methods

Study Design

A case-control study was conducted at two healthcare facilities: Prof. dr. I.G.N.G. Ngoerah Public Hospital, serving as a central referral center in Bali, and Prima Medika Denpasar Hospital, a private institution renowned for its cancer care services in Bali. Data collection spanned a period of one year, from August 2021 to August 2022..

Sampling Strategy

The study encompassed all TNBC patients for whom paraffin blocks were available. Inclusion criteria comprised: 1) TNBC patients diagnosed through histopathological and immunohistochemical assessments; 2) recipients of neoadjuvant chemotherapy (NAC); 3) availability of medical records containing demographic, risk factor, physical examination, diagnostic tests, treatment, and treatment response data. Cases included patients exhibiting a poor response, while controls comprised those with a favorable response to treatment. Patients experiencing TNBC relapse, immunodeficiency disorders, and those with ineligible paraffin block readings were excluded. Samples were collected consecutively and matched in a 1:1 ratio based on neoadjuvant chemotherapy regimen and schedule.

Sample Size Calculation

The determination of the minimum sample size in this study was guided by a type I error (alpha) of 0.05 and a power of 0.2. Consequently, a minimum of 35 samples was required for both the case and control groups.

Data Collection and Measurements

Immunohistochemistry Examination

Two independent Anatomical Pathology specialists at the Department of Anatomical Pathology of RSUP Prof. dr. I G.N.G. Ngoerah conducted the examination. The paraffin block was sliced into 3-4 micrometers and placed onto a glass slide. A portion of the tissue underwent deparaffinization in xylene and subsequent hydration in ethanol. Following this, the specimen was subjected to heating at 100 degrees Celsius for 20 minutes in a 0.01 M citrate buffer (pH 6) using an antigen extraction system (Biogenex, USA). Endogenous peroxidase activity was inhibited by treating a portion of the tissue with 0.3 percent hydrogen peroxide for 5 minutes, and nonspecific binding sites were blocked with blocker proteins for 5 minutes. Subsequently, the tissue was incubated with primary antibodies, and the slide was left to incubate overnight in a humid chamber at 40 degrees Celsius. After incubation, the slides were washed with saline buffer (FFB, pH 7.4) and incubated at room temperature for 30 minutes. Following another wash with saline buffer, the specimens were incubated with Novolink polymer for 30 minutes at room temperature. After three additional washes with saline buffer, DAB chromogen (3,3'-diaminobenzidine tetrahydrochloride) was applied for 5-10 minutes to induce staining. The specimen was then stained, dehydrated using ethanol and xylene, and mounted with Di-n-butyl Phthalate in Xylene (DPX). This process was repeated for each marker.

Staining of CD3, CD8 and CD45RO Lymphocyte

Immunohistochemical examination of paraffin block tissue biopsy from TNBC patients during diagnostic use of primary antibodies against CD3, CD8, CD45RO, Novolink Min Polymer Detection System (Novacastra, Leica Biosystem Newcastle Ltd, UK). Observation with Light microscope and 40X magnification. Central tumor (CT) was defined as the area containing stroma and intra-tumoral cells, while invasive margin (IM) was the area spaced 200-500 m between the TIME environment and the normal mucosa selected manually. Density measurement was done by counting the number of cells/mmμ^2 [20].

Immunoscore Quantification

The Immunoscore CD3/CD8 and CD3/CD45RO represent population quantification scores of CD3 and CD8 lymphocytes, as well as CD3 and CD45RO, respectively, at both the invasive margin (IM) and the tumor center (CT). The quantification entails determining the number of stained cells, followed by establishing a cut-off value through statistical analysis to classify them into high and low groups (refer to Figure 2). Initially, a cross-sectional study was conducted to determine the cut-off point for CD3, CD8, and CD45RO T lymphocyte densities. Subsequently, lymphocyte densities were divided into two categories: 1) low (≤ cut-off value), and 2) high

(>cut-off value). These categories of CD3, CD8, and CD45RO densities at both CT and IM were utilized to calculate CD3/CD8 immunoscores and CD3/CD45RO immunoscores. The resulting immunoscores were then categorized as low (score 0.1) and high (score 2, 3, 4), drawing from the findings of prior research conducted by Anitei et al. [32].

Figures 3 and 4 illustrate the density evaluation of CD3-positive cells under 40x magnification, with red arrows indicating stained cells. These figures demonstrate the histological differences in CD3-positive cell infiltration, which were quantified to determine the immunoscores used in the survival analyses.

Quantification of FOXP3 Expression

Evaluation of *FOXP3* expression was conducted in "hotspot" regions on the T cell surface utilizing primary antibodies targeting *FOXP3*, employing the Novolink Min Polymer Detection system (Novacastra, Leica Biosystem Newcastle Ltd, UK). Subsequently, *FOXP3* expressions were categorized into high (≥1.5) and low (<1.5) expression groups, delineated by cut-off values derived from the ROC curve (refer to Figure 5).

Neoadjuvant Chemotherapy Response Assessment

Neoadjuvant chemotherapy response was evaluated by surgical oncology specialists based on imaging criteria and physical examination and classified based on RECIST Version 1.1 guideline as follows:

Complete response

loss of all target lesions or lymph nodes reduced to <10 mm on the short axis

Partial response

The longest diameter of the target lesion is reduced >30%

Progressive disease

The longest diameter of the target lesion increased by >20 with an absolute increase of 5 mm in new lesions≥;

Stable disease

beyond the above three criteria

Complete and partial response will be classified as good response, while stable and progressive disease will be classified as poor response. Classification of neoadjuvant chemotherapy line was in accordance with the NCCN Clinical Practice Guidelines in Oncology, Breast Cancer, Version 3.2021, classified as first-line and not first-line neoadjuvant chemotherapy. The NAC schedule was the time interval for patients to get the next NAC since the last NAC, normally within 3 weeks. On time NAC schedule was achieved if the time interval was no more than 1 week.

Statistical Analysis

Categorical variables were depicted using frequency and percentage. Continuous variables were presented as mean and standard deviation (SD) for normally distributed data, or as median and range (minimum-maximum values) for non-normally distributed data, as determined by the Kolmogorov-Smirnov test. Receiver operating characteristic (ROC) curves were generated to establish the cut-off points for *CD3*, *CD8*, and *CD45RO* densities in both the tumor core (CT) and invasive margin (IM), as well as for *FOXP3* expression.

In this case-control study, cases were defined as patients with a poor response to neoadjuvant chemotherapy, and controls were defined as those with a favorable response, based on specific clinical criteria. Bivariate analysis was conducted using the Chi-Square test or Fisher's Exact test. The strength of association for risk factors was expressed as odds ratios (OR) with corresponding 95% confidence intervals (CI). Covariates with a p-value <0.25 in the bivariate analysis were included in the multivariate analysis, where logistic regression was applied to model the likelihood of a poor response (case) versus a favorable response (control). A multiple logistic regression analysis was then conducted in the multivariate phase to determine adjusted odds ratios (aOR) and adjusted p-values. Statistical significance was defined as $p<0.05$.

Results

During the study period, seventy-eight patients with triple-negative breast cancer (TNBC) were initially identified. However, six samples were excluded due to defective or unreadable paraffin blocks, leaving a total of 72 samples for analysis. The mean age of the participants was 50.86 years (±10.99), with ages ranging from 25 to 77 years. Some variables had missing data: 10 samples (13.9%) lacked parity information, 6 samples (8.3%) lacked tumor-infiltrating lymphocyte data, and 11 samples (15.3%) lacked lymphovascular invasion data. For the purposes of this case-control analysis, the authors selected 36 patients who met specific criteria for a poor response to neoadjuvant chemotherapy and 36 patients who met criteria for a favorable response. Within the total cohort, 37 patients (51.4%) exhibited a favorable response, while 35 patients (48.6%) demonstrated a poor response. Additionally, 7 patients (16.7%) achieved a pathological complete response (pCR). The area under the curve (AUC), sensitivity (SEN), and specificity (SPE) values for each cutoff are presented in Table 1.

Subsequently, 36 cases (indicative of poor NAC response) and 36 controls (demonstrating good NAC response) were consecutively selected (see Figure 1). Low *CD3/CD8* and *CD3/CD45RO* immunoscores, along with high *FOXP3* expression, were found to be linked with an unfavorable NAC response through bivariate analyses ($p<0.05$). There were no discernible differences observed in age, menopausal status, parity, tumor size, lymph node involvement, histopathology, grade, tumor-infiltrating lymphocytes (TIL), lymphovascular invasion (LVI), and *Ki-67* expression between the case and control groups ($p>0.05$) (see Table 2). All variables demonstrating a p-value <0.25 were included in the multivariate analysis. These variables comprised age, LVI, CD3/CD8 immunoscore, CD3/CD45RO immunoscore, and *FOXP3* expression (refer to Table 3).

Table 1. Cut off value of CD3, CD8 and CD45RO Density and *FOXP3* Expression

Variable	Cut-off	SEN	SPE	AUC	P value	95% CI
CD3 CT	35:05:00	64:09:00	65:07:00	0.510416667	0.001	0.619 – 0.851
CD3 IM	37:05:00	62:02:00	65:07:00	0.44375	0.042	0.510 – 0.769
CD8 CT	23	70:03:00	71:04:00	0.514583333	0.000	0.624 – 0.857
CD8 IM	21	73	65:01:00	0.500694444	0.001	0.603 – 0.838
CD45RO CT	22:05	67:06:00	71:04:00	0.504166667	0.001	0.609 – 0.844
CD45RO IM	30:05:00	51:04:00	51:04:00	0.411805556	0.122	0.457 – 0.728
FOXP3	1:05	62:02:00	71:04:00	0.223611111	0.01	0.198 – 0.447

A multivariate analysis unveiled that both the CD3/CD8 immunoscore and *FOXP3* expression stood out as significant and independent predictors for NAC response. Specifically, the low CD3/CD8 immunoscore group exhibited a staggering 10,930-fold increase in the risk of experiencing a poor NAC response in comparison to the high CD3/CD8 immunoscore groups. Conversely, the high *FOXP3* expression group demonstrated an 11,775-fold higher risk of encountering a poor NAC response when juxtaposed with the low *FOXP3* expression group (refer to Table 3). Although the CD3/CD45RO immunoscores yielded adjusted odds ratios (aOR) with values exceeding 1, indicative of their role as risk factors for neoadjuvant chemotherapy response, these associations did not reach statistical significance.

Discussion

Tumor-infiltrating immune cells play dual roles in tumor progression and metastasis by both targeting and eliminating tumors and facilitating tumor cells' evasion of the immune system [21]. The presence of T cell CD3 is associated with microinvasive status, while the presence of T cell CD8 at the tumor core signifies its pivotal role in immune response and disease prognosis. Both CD3 and CD8 T cells exhibit comparable staining patterns and antigen stability. The density of CD3 and CD8 T cells shows an inverse relationship with tumor proliferation stages (I-IV) [22, 23].

In our investigation, we identified a noteworthy

Table 2. Baseline Characteristics of Sample

Variable	Case		Control		OR	95% CI	P value
	N	%	N	%			
Menopausal state							
Pre menopause	20	57:01:00	22	62:09:00	0.5472	0.302-2.054	0.626[a]
Post menopause	15	42:09:00	13	37:01:00			
Parity							0.384[a]
1 - 3	25	83:03:00	23	74:02:00	1.739	0.497-6.086	
≥4	5	16:07	8	25:08:00			
Tumor size							0.428[b]
T1-T2	5	14:03	2	5:07	2.750	0.496-15.246	
T3-T4	30	85:07:00	33	94:03:00			
Lymph node involvement							1.000[b]
N0	2	5:07	3	8:06	0.4486	0.101-4.128	
N1-3	33	94:03:00	32	91:04:00			
Histopathology							1.000[b]
Invasive Ca. NST	30	85:07:00	31	88:06:00	0.5375	0.190-3.163	
Others	5	14:03	4	11:04			
Grade							0.607[a]
1 - 2	12	34:03:00	10	28:06:00	1.304	0.474-3.590	
3	23	65:07:00	25	71:04:00			
Tumor Infiltrating Lymphocyte (TIL)							0.427[b]
Positive	31	88:06:00	28	80:00:00	2.214	0.376 - 13.034	
Negative	2	5:07	4	11:04			
Ki67 expression							0.710[b]
>20%	30	85:07:00	32	91:04:00	0.39097	0.124-2.560	
≤20%	5	14:03	3	8:06			

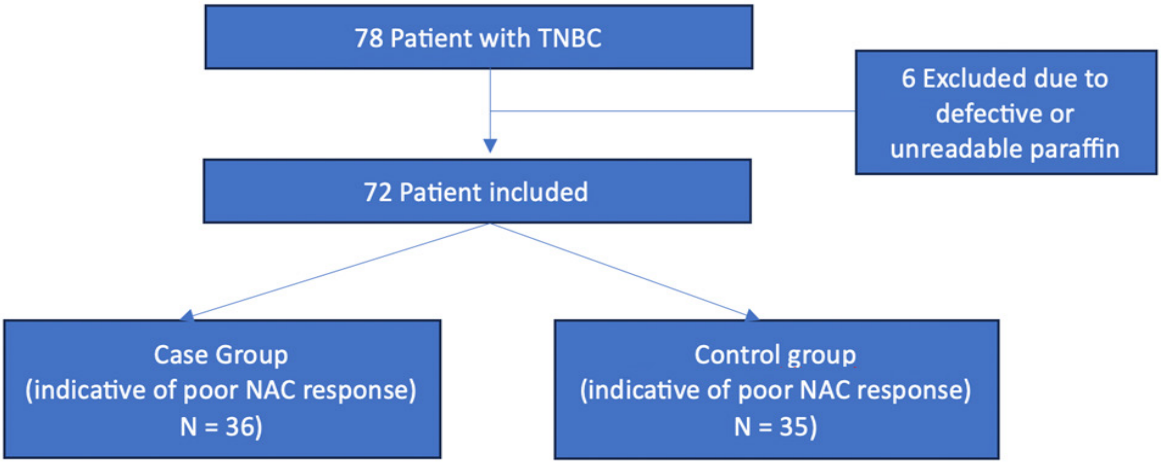

Figure 1. Sample Recruitment Flowchart

association wherein a diminished CD3/CD8 immunoscore emerged as a notable independent predictor of unfavorable response to neoadjuvant chemotherapy (NAC). Notably, prior studies have not explored the correlation between CD3/CD8 immunoscores and NAC response in triple-negative breast cancer (TNBC) patients. Existing literature indicates that reduced intra-tumor *CD8* expression is linked to a decreased likelihood of achieving pathological complete response (pCR), Although contradictory findings exist [24–29]. Previous inquiries predominantly delved into the association of CD3/CD8 immunoscores with cancer prognosis, particularly in colorectal cancer. These immunoscores are stratified into low (I0-I2) and high (I3-I4) categories, with findings suggesting that patients with low scores in advanced stages (III-IV) tend to have inferior prognoses compared to those with high scores [30, 31]. Moreover, CD3/CD8 immunoscores have demonstrated superiority over tumor-node-metastasis (TNM) staging in predicting recurrence and survival [31, 32]. Highlighted the significance of immunoscores as risk determinants for colorectal cancer, particularly when evaluated at metastatic sites rather than primary tumors, underscoring the necessity for further exploration into the impact of assessment locations on chemotherapy response.

CD8+ T lymphocytes represent a critical component of the immune system, functioning as cytotoxic cells that eliminate tumor cells by initiating cytolysis through the formation of the B-perforin granzyme complex. These cells play a pivotal role in the initial phase of the immunoediting cycle, where cancer cells must evade their destructive effects to survive and spread [33–35]. Notably, *CD8* expression is significantly elevated in triple-negative breast cancer (TNBC) compared to other subtypes of breast cancer. Elevated levels of CD8 are associated with reduced tumor cell proliferation, decreased aggressiveness, and enhanced survival rates. Moreover, heightened *CD8* expression correlates with a

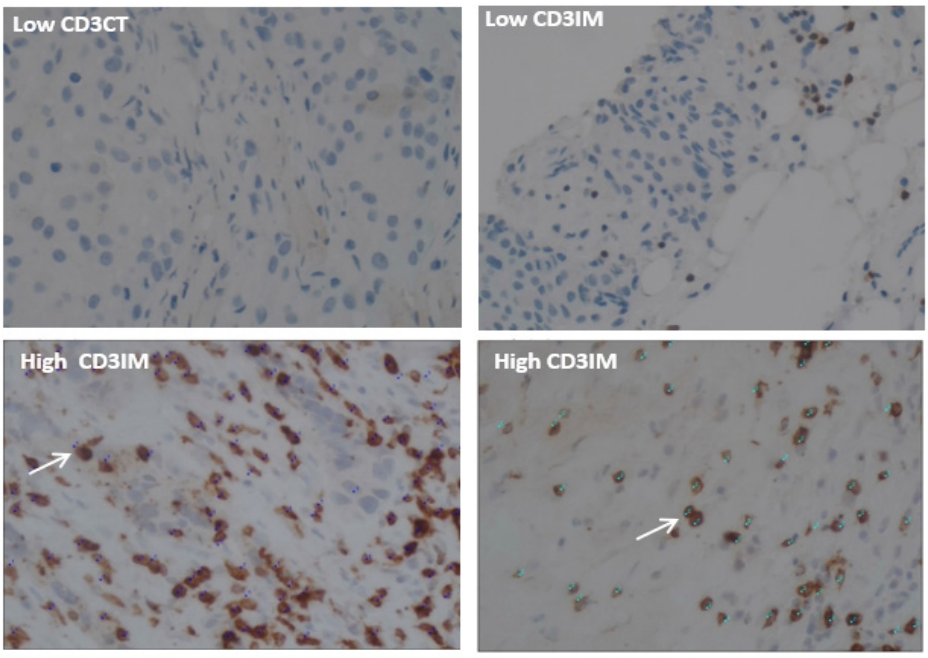

Figure 2. The Immunohistochemical Staining Results of CD3. White arrows indicate positive results

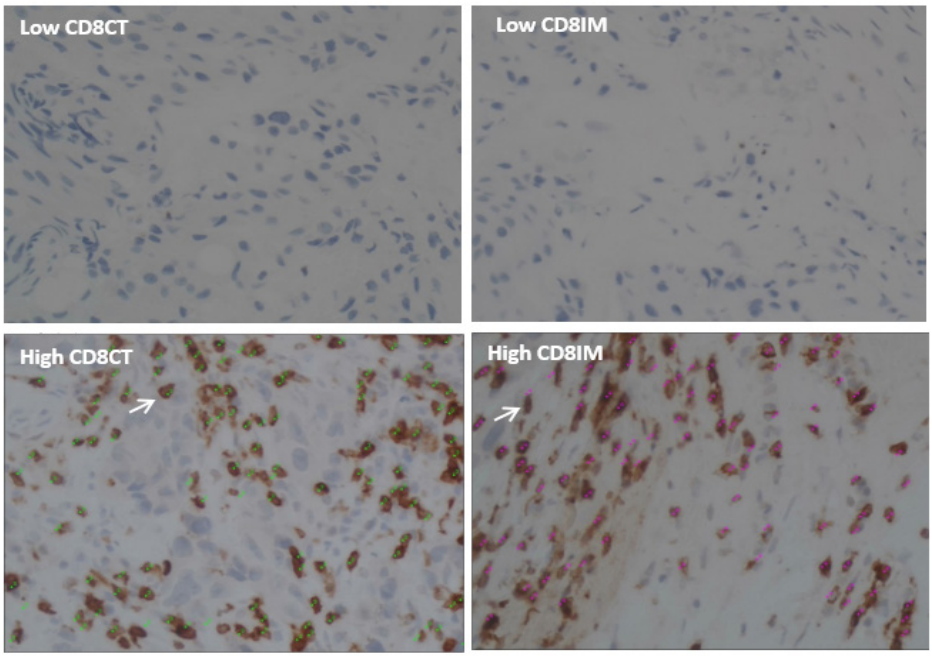

Figure 3. The Immunohistochemical Staining Results of CD8. White arrows indicate positive results

more robust immune response against tumors, increased expression of interferon-α and interferon-γ, and a higher immune cytolytic activity score (CYT) within the tumor microenvironment. Interferon-γ can inhibit the cell cycle, promote apoptosis, suppress angiogenesis, and augment the tumoricidal activity of macrophages. TNBC tumors characterized by high CD8 scores demonstrate substantial infiltration by anti-cancer immune cells such as CD4 memory T cells, M1 macrophages, and B cells [28].

Chemotherapeutic agents exert their tumor-killing effects by inducing cell cycle arrest and apoptosis. Research indicates that these agents possess the capacity to bolster antitumor immunity [36, 37]. Following chemotherapy, the release of pro-inflammatory cytokines like IL-8 and CCL-2 can prompt apoptosis and stimulate an immune response [36]. Chemotherapy may further potentiate the cytotoxic lymphocyte response by liberating tumor antigens for processing by antigen-presenting cells (APCs), thereby fostering enduring antitumor immunity. Consequently, the immune profile preceding therapy not only supports chemotherapy but may also serve as a predictor for the response to neoadjuvant chemotherapy (NAC) [38]. Enhanced immune evasion by cancer cells can also dampen the antitumor immune response to

Table 3. Bivariate and Multivariate Analysis of Immunoscore C3/CD8 and CD3/CD45RO, FOXP3 Expression, and Confounding Variables (Age and LVI) to NAC Response

Variable	Groups		Bivariate			Multivariate		
	Case (n=35)	Control (n=35)	OR	95% CI	P value	aOR	95% CI	P value
Age								
≥ 40 years	28 (80)	32 (91.4)	0.260416667	0.088-1.590	0.1194	3,343	0.471-23.754	0.1583
< 40 years	7 (20)	3 (8.6)						
LVI (Positive)								
Positive	16 (45.7)	7 (20.0)						0.073
Negative	17 (48.6)	20 (57.1)	2,689	0.896-8.067	0.074	4,348	0.871-21.699	
Immunoscore CD3/CD8								
Low	22 (62.9)	6 (17.2)	8,179	2.683-24.939	0.000	10,930	1.336-89.420	0.026
High	13 (37.1)	29 (82.8)						
Immunoscore CD3/CD45RO								
Low	19 (54.3)	9 (25.7)	3,431	1.251-9.404	0.015	1,443	0.207-10.049	0.49375
High	16 (45.7)	26 (74.3)						
FOXP3 (High)								
High	25 (71.4)	13 (37.1)	4,231	1.550-11.546	0.004	11,775	2.537-54.656	0.002
Low	10 (28.6)	22 (62.9)						

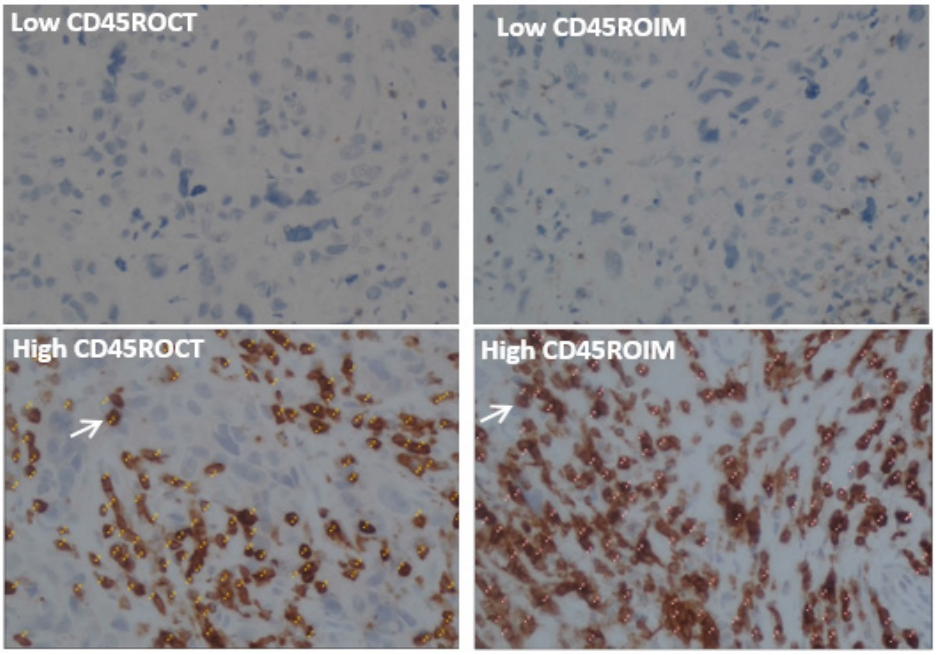

Figure 4. The Immunohistochemical Staining Results of *CD45RO*. White arrows indicate positive results

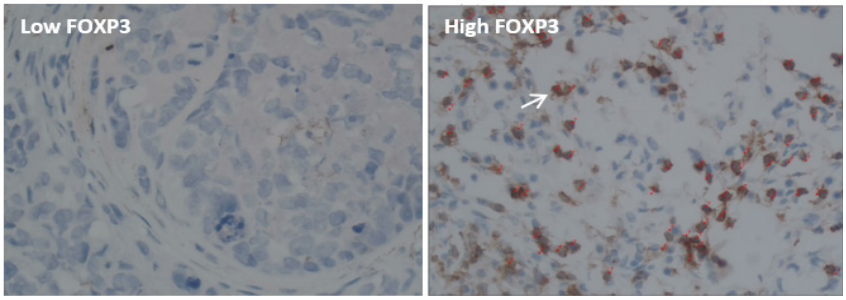

Figure 5. The Immunohistochemical Staining Results of *FOXP3*. White arrow indicates positive result

chemotherapy. Furthermore, studies have reported that 5-fluorouracil and paclitaxel can enhance immune evasion by restoring CD8 T cell sensitivity and suppressing Treg cells [39].

Additionally, we observed that the high *FOXP3* expression cohort exhibited more than an 11-fold heightened risk of poor response to neoadjuvant chemotherapy (NAC) in comparison to the low *FOXP3* expression group. T-regulatory (T-reg) cells, identified by the presence of CD25+ populations among CD4+ T lymphocytes, inherently dampen the activity of other T cell effectors. FOXP3, belonging to the FOX protein family, plays a pivotal role as a key regulator in the development and function of T-reg cells [19]. These findings are consistent with several prior investigations that have similarly reported an association between *FOXP3* expression, NAC response, and prognosis in breast cancer [27, 40–42]. Notably, NAC led to a significant reduction in *FOXP3* expression within tumor cells, and the absence of FOXP3 infiltration in post-NAC histological specimens was correlated with a favorable response to chemotherapy [27].

The prognostic significance of FOXP3 in triple-negative breast cancer (TNBC) is also contingent upon cytokine levels. Recent research by Goda et al. revealed a robust correlation between *FOXP3* expression and the levels of interleukin (IL)-33 and transforming growth factor beta 2 (TGFB2). IL-33 receptors are present on T-regulatory cells (Tregs) and are released into the extracellular space during tissue injury to initiate inflammatory signals. Meanwhile, the TGFB family serves to suppress cellular immune responses and induce epithelial-to-mesenchymal transition in mammary cells [43]. Furthermore, diminished *FOXP3* expression has been associated with heightened levels of pro-inflammatory interleukins such as IL-6 and IL-8 in human pancreatic carcinoma cell lines [18, 44]. Tumors expressing FOXP3 deploy mechanisms to evade immune surveillance. FOXP3 can facilitate communication between tumor cells and their microenvironment, including the activation of the signal transducer and activator of transcription 3 (STAT3) pathway. This study did not establish the CD3/CD45RO immunoscore as an independent risk factor for neoadjuvant chemotherapy (NAC) response. The role of *CD45RO* expression in cancer prognosis remains inconclusive. Some prior studies have indicated that elevated *CD45RO* expression is associated with improved clinical outcomes and prognosis in breast cancer [45, 46]. A meta-analysis revealed that

CD45RO T cell infiltration had a beneficial prognostic impact across various solid tumors and indicated that heightened CD45RO T cell density was inversely correlated with TNM stage [47]. However, divergent findings were reported by Hotta et al, in patients with renal cell carcinoma (RCC). Notably, the assessment of CD3/CD45RO immunoscores in cancer has not been previously undertaken [48].

Memory T cells that arise following antigen exposure can be divided into two main subsets: central memory T cells (Tcm) and effector memory T cells (Tem). The evaluation of *CD45RO* expression on tumor-infiltrating lymphocytes (TILs) enables the identification of both Tcm and Tem, contingent upon their location within the tissue [48, 49]. Tcm cells represent CD8 T cells that have encountered an antigen; while they lack immediate effector function, they possess the ability to swiftly respond to specific antigens and can transform into Tem cells upon re-stimulation. Tcm cells retain migratory capabilities, allowing them to circulate within lymphatic organs. Conversely, Tem cells, representing the terminal differentiation stage of CD8 T cells, lack lymph-node-homing receptors (CD62L and CCR7) and remain within tumor tissue to promptly execute effector functions without further differentiation [47, 50]. This implies that CD45RO memory T cells do not directly combat tumor cells independently but instead operate through CD8 T cells as effector cells. Consequently, our study found that low CD3/CD45RO immunoscores did not emerge as significant independent risk factors for poor response to neoadjuvant chemotherapy (NAC) after adjusting for potential confounding variables..

However, this study is subject to certain limitations. Firstly, not all histopathological examinations incorporated assessments of tumor-infiltrating lymphocytes (TIL) and lymphovascular invasion (LVI), leading to instances of missing data. Secondly, the quantification of *CD3, CD8, CD45RO*, and *FOXP3* expression densities was performed manually rather than utilizing digital pathology or application programs, which could introduce inherent biases. To mitigate this, two independent pathologists conducted the evaluations, with any disparities resolved through discussion. Thirdly, the retrospective nature of the study and reliance on secondary data sources may contribute to assessment bias owing to the potential lack of objectivity and reliability in the data collection tools.

In conclusion, based on our findings, we infer that both a diminished CD3/CD8 immunoscore and elevated *FOXP3* expression act as autonomous predictors for suboptimal response to neoadjuvant chemotherapy among individuals with triple-negative breast cancer (TNBC). Future investigations should explore the prognostic implications of additional tumor-infiltrating lymphocyte (TIL) subtypes in shaping chemotherapy outcomes, particularly within the TNBC context. Moreover, this methodology could be extended to evaluate other subtypes of breast cancer.

Author Contribution Statement

Concept, Planning, and Design: PATA, Research Procedure: PATA, NPS, Draft Manuscript: PATA, IWS, Revision of Draft: IWS, KS, Final Manuscript Approval: PATA, IWS

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Ethical Approval

This study was approved by the Ethical Research Committee of the Faculty of Medicine, University of Udayana (Approval No: 2663/UN14.2.2.VII.14/LT/2021).

Data Availability

Data availability is not applicable to this research as it involves only a descriptive analysis without a database.

Conflict of Interest

The authors declare that they have no conflicts of interest related to this study.

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