DOI:10.31557/APJCP.2024.25.5.1607
Biomarkers for Chemotherapy Response

RESEARCH ARTICLE

Editorial Process: Submission:11/22/2023 Acceptance:05/04/2023

Response to Neoadjuvant Chemotherapy in Invasive Breast Cancer Predicted by CD4+, CD8+, and FOXP3+ Tumor-Infiltrating Lymphocytes

Primariadewi Rustamadji*, Elvan Wiyarta, Meike Pramono, Sinta Chaira Maulanisa

Abstract

Background: Response to neoadjuvant chemotherapy (NC) in individuals with invasive breast cancer (IBC) must be monitored, and biomarkers are needed. NC can activate an anti-tumour immune response in its microenvironment, known as Tumor-infiltrating Lymphocytes (TIL). TIL components believed to have great potential as predictors are CD4+, CD8+, and FOXP3+ TIL. This study aims to explore TIL components that can potentially be predictive biomarkers of NC pathological responses. **Methods:** A sample size of 40 were analyzed based on the relationship between CD4+, CD8+, and FOXP3+ TIL expression with the Miller-Payne (MP) grading system. Age, tumour grade, PR, ER, Ki-67, and HER2 were also evaluated. CD4+, CD8+, and FOXP3+ TIL expressions were analayzed by IHC staining, while other data were collected from archives. Data was analyzed using univariate and multivariate analysis. **Results:** Univariate analysis showed a significant relationship between CD4+ TIL and MP ($p<0.001$), CD8+ and MP ($p=0.004$), and FOXP3 with MP ($p<0.001$). The simultaneous integration of the three biomarkers in one model was not good enough to be a predictive model. Therefore, an exploratory analysis was conducted by testing several alternative models that combined two of the three existing biomarkers. It turned out that CD4+ TIL in model 2 (CD4+CD8+) and FOXP3+ TIL in model 4 (CD8+FOXP3+) showed significant coefficient values. Moreover, all of the threshold coefficients in model 4 are significant. **Conclusion:** This study shows that CD4+, CD8+, and FOXP3+ TIL have promising potential as predictive biomarkers. In particular, FOXP3+ is dominant in predictive models of pathological response in patients with IBC.

Keywords: CD4- CD8- FOXP3- prognosis- chemotherapy response- predictor

Asian Pac J Cancer Prev, **25 (5)**, 1607-1613

Introduction

Breast cancer is a heterogeneous and complex malignant disease in terms of diagnosis and treatment and is still a global public health problem [1]. Breast cancer accounts for 23% of all female malignant neoplasms [1]. The incidence of breast cancer in the world in 2018 even reached 2.09 million new cases [2]. Depending on their proximity to the basement membrane, benign and invasive breast cancers are typically distinguished during the histopathological categorization process [3]. Seventy percent of breast cancer cases involve invasive breast cancer (IBC), which must be treated aggressively [4].

Current IBC treatment relies on chemotherapy, particularly neoadjuvant chemotherapy (NC), which is administered to patients before they receive surgery [5]. The standard of care for locally advanced breast cancer has shifted to NC, and it is increasingly being offered to patients with early-stage, treatable illness [6]. Although the selection of type of treatment in breast cancer patients is multifactorial and decision making should be done by multidisciplinary teams [7], NC uses anthracycline-based chemotherapy as an essential component and aims to reduce tumour size, reduce surgical field, increase the chance of breast-conserving surgery, control micrometastasis, and provide prognostic information based on pathological response rate [6, 8]. NC in IBC can cause high clinical response rates of up to 70% to 90% [9]. In addition to the clinical response, the success of NC can also be seen from the pathological response [10]. Evaluation of the pathological response of NC can be done using the Miller-Payne (MP) grading system, which divides the response scores into grades 1 to 5 [10]. The expected pathological response is a complete pathological response (MP 5) with an excellent survival prognosis [11, 12]. After NC administration, the complete pathological response is the state of clearance of invasive tumor cells in tumor bed and lymph nodes [13].

NC can activate an anti-tumour immune response in its microenvironment, known as Tumor-infiltrating

*Department of Anatomic Pathology, Faculty of Medicine Universitas Indonesia-Dr. Cipto Mangunkusumo National Hospital, Jakarta, Indonesia. *For Correspondence: primariadewi.rustamadji@ui.ac.id*

Lymphocytes (TIL) [14]. TIL is a stimulated immune system that will attack cancer cells, which consist of Cytotoxic Cluster of Differentiation 4 (T CD4+) T cells, CD8+ T cells, T regulatory forkhead box P3 (Treg FOXP3+) cells, and other immune cells that are in the area. peritumoral and intratumoral [15, 16]. This interaction of TIL with cancer will determine the success of immunotherapy in cancer and other cancer therapies that can induce the immune system [9, 14]. Therefore, the amount of TIL before NC administration is thought to be related to the pathological response status of NC, so it can be a biomarker that predicts the level of pathological response of NC [12]. However, the evaluation of TIL as a predictive biomarker of the pathological response of NC is controversial. Several studies have stated that TIL consists of various lymphocyte cells with two different activities, namely pro-tumour and anti-tumour, so not all of them represent the process of tumour eradication [17-19]. Therefore, several studies have focused on finding TIL components that can provide good predictive value for the pathological response of NC [20].

This study aims to explore TIL components that can potentially be predictive biomarkers of NC pathological responses. TIL components believed to have great potential as predictors are CD4+, CD8+, and FOXP3+ TIL. Several studies still show inconclusive things about the role of these three biomarkers [20-23]. Therefore, apart from analyzing individually, this study also assessed the integration of these three biomarkers as predictors of NC pathological response in IBC to be assessed by MP.

Materials and Methods

Study Design

The University of Indonesia's Anatomical Pathology Laboratory supported this retrospective cohort study. In February 2022, the Universitas Indonesia Ethics Committee authorized the experimental procedures with protocol number KET-131/UN2.F1/ETIK/PPM.00.02/2022. Each participant gave a written agreement and understood the goal of the research. The research adheres to the Declaration of Helsinki [24]. The data collection process was carried out in May 2022. Data collection was carried out to retrieve departmental archives data dated January 2014 to June 2016. The population of this study were all preparations of hematoxylin-eosin (HE) and paraffin block breast carcinoma in female patients who had been histopathologically diagnosed as IBC and had been treated with anthracycline NC from January 2014 to June 2016. Individuals with non-IBC diseases and systemic illnesses, such as poorly controlled diabetes (defined as HbA1c >8% in the previous three months) and hypertension, were judiciously excluded to reduce potential confounding influences on inflammatory markers central to our study, while ensuring all paraffin blocks, regardless of initial inclusion, were retained and stored in accordance with institutional and ethical guidelines. Sample selection was carried out by consecutive sampling, namely collecting as many samples as possible from archives from January 2014 to June 2016. The study's power is expected to be 80% (resulting in a Type II error rate, or beta, of 20%) and its confidence level is 95% (implying a Type I error rate, or alpha, of 5%). We collected data on the age of the patients, the grade of their tumors, whether they were positive for the estrogen receptor (ER), the progesterone receptor (PR), HER2, and the proliferation marker Ki67(20% as the cut-off value). Quantitative information on CD4+, CD8+, and FOXP3 TIL expression was also collected. All TIL data calculated in this study were taken from patients before being given NC (pretreatment). After mastectomy, a Miller-Payne grading system was performed on the preparations to determine the pathological response. Patients included were patients who tolerated mastectomy after NC.

Slides Preparation

In this study, breast cancer biopsy samples were processed to examine the expression of CD4+, CD8+, and FOXP3+ TIL. The process started with the preparation of slides from tissue paraffin blocks, which were then marked with the relevant sample number and antibody type for staining.

These slides were first dried and then heated to facilitate subsequent staining procedures. The slides were carefully deparaffinized, rehydrated, and washed. An essential part of the process involved blocking endogenous peroxidase to prevent false positives.

Next, antigen retrieval was performed to improve the antibodies' access to their target proteins in the tissue. This was followed by further blocking and washing. Then, the slides were incubated with monoclonal mouse primary antibody against: CD4 (Biocare [USA]; clone: 4B12, dilution 1:50), CD8 (Biocare [USA]; clone: C8/144, dilution 1:300), and FOXP3 (Biocare [USA]; clone: 86D, dilution 1:100), washed again, and exposed to a secondary antibody.

After more washing, the slides were treated with a mix of DAB chromogen and substrate buffer until they took on a brown hue, indicative of the presence of the TIL. The slides were then counterstained to aid visualization and stained for better contrast, with washes between each step.

Finally, the slides underwent a process of dehydration and clearing to remove tissue fluid and dehydrants respectively. Alongside these samples, negative and positive controls were run for validation, with tonsil tissue serving as the positive control. This detailed procedure enabled a thorough examination of TIL expression in breast cancer biopsy samples.

Slide Reading

In our study, the expression of CD4+, CD8+, and FOXP3+ Tumor Infiltrating Lymphocytes (TIL) and the pathological response of the malignant pleural (MP) were examined using immunohistochemical staining. Two researchers independently assessed five distinct visual fields at 400x magnification in representative areas for each sample. To ensure the consistency and validity of their assessments, an Intraclass Coefficient Correlation was calculated, revealing an interobserver difference of less than 10%. This evaluation was conducted in a blinded manner, with evaluators unaware of sample groupings, utilizing the ImageJ software to manually determine both

intratumoral and peritumoral expression levels through careful counting across all samples.

The assessment of CD4+, CD8+, and FOXP3+ TILs was conducted quantitatively with an Olympus Bx51 microscope. Lymphocyte counts were accurately determined by averaging their presence across five distinct fields of view at 400x magnification. This process encompassed both peritumoral (surrounding the tumor) and intratumoral (within the tumor) regions to provide a comprehensive analysis. The combined counts from these two regions formed the total expression of each TIL type, ensuring a detailed and precise representation of their distribution and abundance within the tumor microenvironment.

Statistical Analysis

Before analysis, Microsoft Excel was used to input data gathering into a main table (Microsoft Corp, Redmond, WA, USA). Statistical Package for the Social Sciences / SPSS version 20 was used to analyze and display the tabulated data (IBM Corp, Armonk, NY, USA). All categorical variables are expressed as frequency and analyzed with Fisher's exact test. Then, ordinal logistic regression was utilized to conduct a multivariate study of CD4+, CD8+, and FOXP3+ TIL incorporation.

Results

The expressions of CD4+, CD8+, and FOXP3+ TIL was evaluated in each of the forty samples using immunohistochemistry. Intratumoral and peritumoral TIL images on each stain, both CD4+, CD8+, and FOXP3, can be seen in Figure 1. In addition to the expression of CD4+, CD8+, and FOXP3+ TIL, clinicopathological characteristics data were also analyzed against MP, as shown in Table 1. Univariate analysis showed a significant relationship between CD4+ TIL and MP ($p<0.001$), CD8+ and MP ($p=0.004$), and FOXP3 with MP ($p<0.001$). There are no other variables that have a significant relationship. The results of this univariate analysis were followed by an inter-biomarker integration analysis using ordinal logistic regression, as shown in Table 2. In this multivariate analysis, there were four models tested, namely CD4+ CD8+ FOXP3+ (model 1), CD4+ CD8+ (model 2), CD4+ FOXP3+ (model 3), and CD8+ FOXP3+ (model 4).

The impact of CD4+, CD8+, and FOXP3+ TIL on the MP was calculated using an ordinal logistic regression with proportionate odds for odds ratio (OR), as shown in Table 2 (no missing value was observed). In model 1, there were some positive associations between MP and CD4+TIL (OR: 1.013; 95% CI, 0.722-1.421), CD8+ TIL (OR: 1.050; 95% CI, 0.996 to 1.107), along with FOXP3 (OR: 1.979; 95% CI, 0.815 to 4.807). In model 2, its OR were 1.339 (95% CI, 1.134 to 1.581) for CD4+ and 1.053 (95% CI, 0.993 to 1.116) for CD8+. In model 3, its OR were 1.049 (95% CI, 0.771 to 1.426) for CD4+ and 2.039 (95% CI, 1.327 to 3.132) for FOXP3. In model 4, its OR were 1.050 (95% CI, 0.996 to 1.106) for CD8+ and 2.039 (95% CI, 1.327 to 3.132) for FOXP3.

Discussion

This study shows that CD4+, CD8+, and FOXP3+ TIL have promising potential as predictive biomarkers. This statement is illustrated by the univariate analysis results, which show a significant association with the MP grading system without significant confounding. This univariate analysis was followed by multivariate analysis with ordinal logistic regression, which yielded surprising results. The simultaneous integration of the three biomarkers in one model (model 1) was not good enough to be a predictive model. Therefore, an

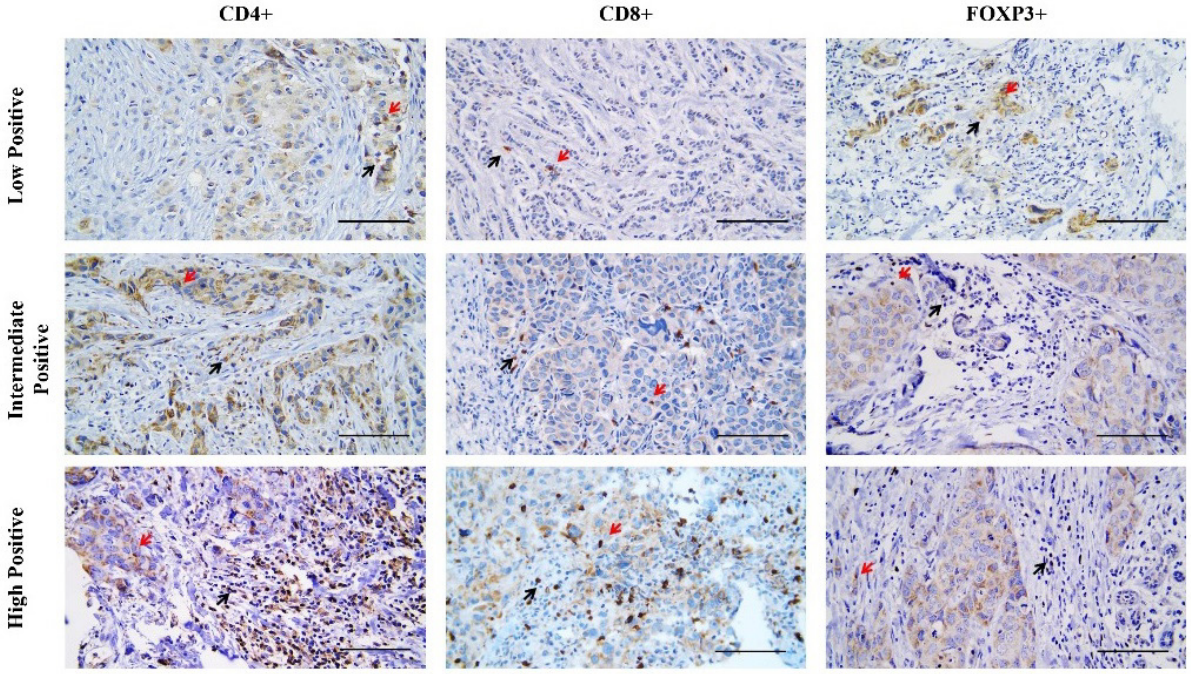

Figure 1. Intratumoral and Peritumoral TIL Images on (A) CD4+, (B) CD8+, and (C) FOXP3. The black arrow indicates peritumoral TIL. The red arrow indicates intratumoral TIL. Scale bar represents 50 μm for all images.

Table 1. Clinicopathological Characteristics of Invasive Breast Cancer Patients Treated with Neoadjuvant Chemotherapy at Dr. Cipto Mangunkusumo National Hospital, Jakarta, Indonesia

Variables	Miller-Payne					P-value
	1	2	3	4	5	
Age						
<50 y.o.	0 (0.0%)	6 (66.7%)	3 (33.3%)	0 (0.0%)	0 (0.0%)	0.368
≥50 y.o.	4 (12.9%)	9 (29.0%)	13 (41.9%)	3 (9.7%)	2 (6.5%)	
Tumour grade						
1	0 (0.00%)	2 (66.7%)	1 (33.3%)	0 (0.0%)	0 (0.0%)	0.762
2	4 (16.7%)	7 (29.2%)	9 (37.5%)	2 (8.3%)	2 (8.3%)	
3	0 (0.0%)	6 (46.2%)	6 (46.2%)	1 (7.7%)	0 (0.0%)	
ER status						
Negative	0 (0.00%)	0 (0.0%)	1 (50.0%)	1 (50.0%)	0 (0.0%)	0.245
Positive	4 (10.5%)	15 (39.5%)	15 (39.5%)	2 (5.3%)	2 (5.3%)	
PR status						
Negative	2 (11.10%)	5 (27.8%)	10 (55.6%)	1 (5.6%)	0 (0.0%)	0.354
Positive	2 (9.1%)	10 (45.5%)	6 (27.3%)	2 (9.1%)	2 (9.1%)	
HER2 status						
Negative	3 (10.30%)	11 (37.9%)	11 (37.9%)	2 (6.9%)	2 (6.9%)	1
Positive	1 (9.1%)	4 (36.4%)	5 (45.5%)	1 (9.1%)	0 (0.0%)	
Ki67 status						
Low	1 (7.10%)	6 (42.9%)	6 (42.9%)	1 (7.1%)	0 (0.0%)	0.953
High	3 (11.5%)	9 (34.6%)	10 (38.5%)	2 (7.7%)	2 (7.7%)	
CD4+	2.35 (2.08)	13.32 (4.72)	36.23 (14.09)	55.33 (20.02)	105.10 (21.07)	<0.001*
CD8+	14.80 (6.27)	31.07 (12.34)	43.80 (20.99)	52.07 (47.92)	55.90 (32.35)	0.004*
FOXP3+	2.20 (0.40)	7.91 (3.19)	17.34 (4.27)	24.53 (8.75)	55.40 (27.72)	<0.001*

CD, cluster of differentiation; ER, estrogen receptor; FOXP3, forkhead box P3; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor; All categorical variables are expressed as frequencies and analyzed using Fisher's exact test; *P-value less than 0.05 is considered statistically significant.

Table 2. Ordinal Logistic Regression Analysis Predicting Neoadjuvant Chemotherapy Response in Invasive Breast Cancer Based on CD4+, CD8+, and FOXP3+ TIL Expression at Dr. Cipto Mangunkusumo National Hospital, Jakarta, Indonesia

Variables	Model 1 (CD4+CD8+FOXP3+)	Model 2 (CD4+CD8+)	Model 3 (CD4+FOXP3+)	Model 4 (CD8+FOXP3+)
Miller-Payne Threshold				
Grade 1	3.043*	2.14	1.885	3.075*
Grade 2	10.070*	7.534*	8.350*	10.168*
Grade 3	19.653*	19.174*	16.896*	19.636*
Grade 4	27.632*	27.151*	25.132*	27.579*
CD4+	0.049	0.292*	0.048	
CD8+	0.683	0.051	0.601	0.049
FOXP3+	0.013			0.712*
Model Fitting (χ^2)	68.869*	65.856*	65.281*	68.864*
Goodness of fit				
Pearson (χ^2)	83.92	68.456	74.473	85.31
Deviance (χ^2)	35.822	38.836	39.41	35.828
Pseudo R^2				
Cox and Snell	0.821	0.807	0.804	0.821
Negelkerke	0.886	0.871	0.868	0.886
McFadden	0.658	0.629	0.624	0.658
Test of parallel lines (χ^2)	3.653	3.456	4.632	3.772

CD, cluster of differentiation; FOXP3, forkhead box P3; *P-value less than 0.05 is considered statistically significant.

exploratory analysis was conducted by testing several alternative models that combined two of the three existing biomarkers. It turned out that CD4+ TIL in model 2 and FOXP3+ TIL in model 4 showed significant coefficient values. Moreover, all of the threshold coefficients in model 4 are significant, which means that the model can discriminate against MP grading at each threshold well. These findings can explain and support various theories and research on using these predictive biomarkers.

The association between CD4+, CD8+, and FOXP3+ TIL and MP is predicated on several hypotheses about the anticancer function of this biomarker. The immune response to tumour progression is caused by innate and adaptive immune responses [25]. The main agents of adaptive immunity against tumour cells are T cells that require cross-presentation by host antigen-presenting cells, particularly dendritic cells [25]. In the early stages, tumour cells are digested and processed in APC. The peptide obtained then binds to MHC class I molecules which will later be recognized by T cell lymphocytes-tumours [25, 26]. Effector T cell lymphocytes-tumours are then ready and able to recognize and destroy target cells/tumour cells by delivering proteins (perforins and granzymes) that induce cell death [25, 26]. The presence of high levels of CD4+, CD8+, and FOXP3+ TIL causes tumour cells to be more sensitive to chemotherapy and have higher cytotoxic effects so that after chemotherapy, lower tumour cellularity correlates with high Miller Payne scores, good pathological response. NCs, including anthracyclines or taxane, have been shown to have immunomodulatory effects on tumour cells [27, 28]. It stimulates an immune response by inducing immunogenic cell death [27, 28]. Several mechanisms occur in this process, including the translocation of calreticulin from the lumen of the endoplasmic reticulum to the cell surface, which serves as a signal to kill cells and stimulates the elimination of tumour cells by phagocytes and dendritic cells [29]. Anthracyclines will also trigger ATP release in the post-mortem phase of tumour cell death and stimulate tumour cells to produce interferon type 1, which induces release [30]. Another mechanism is that anthracyclines can simulate the release of High mobility group box 1 (HMGB1) protein, which triggers cell maturation [31]. The endpoint of maturation of these interstitial dendritic cells is more expansion and differentiation of T cells against tumour cells [31]. In addition, the findings of this study are supported by several other studies that demonstrate that the value of CD4+ [32-34], CD8+ [35, 36, 22], and FOXP3+ [37, 23, 38] TIL before NC are predictive parameters, indicating that the systemic and tumour microenvironment immunological profiles play a crucial role in response to chemotherapy.

Interestingly, CD4+, CD8+, and FOXP3+ TIL did not show significant integration when analyzed in the same model (model 1). Further exploratory analysis even showed that FOXP3+ TIL had a dominant role as a predictive biomarker of pathological response, followed by CD4+ TIL and CD8+ TIL. In addition, the regression model that includes FOXP3+ and CD8+ (model 4) is the best model, with FOXP3+ as the dominant variable influencing the prediction model. This finding demonstrates the potential of FOXP3+ TIL as an independent predictive biomarker. This characteristic may be supported by the function of FOXP3+ primarily on T regulatory cells [39]. By repressing the production of SKP2 and HER2, two oncogenes associated with breast cancer, FOXP3 slows the spread of tumor cells [40, 41]. In addition, apoptosis and tumor suppression in animal models may result from activating FOXP3 expression in breast cancer cell lines [40-42]. Based on these biochemical observations, it appears that the activity of FOXP3 in cancer cells may be breast tumor subtype dependent, especially with respect to carcinogenic pathways.

In this study, several limitations can be addressed for further research. Although the sample size of this study is in line with the power in calculating the minimum sample size, more research samples are needed to obtain more accurate regression models as predictions. We are aware that this is a major limiting factor of this study. However, we have mitigated this by conducting an extensive and comprehensive statistical analysis. In addition, because it was conducted at a national referral centre, this study has a wide variation in breast cancer. This is indicated by the still at least IBC grade 1 included in this study. Nevertheless, this study can be a reference for further research on integrating predictive biomarkers in IBC.

In conclusion, this study shows that CD4+, CD8+, and FOXP3+ TIL have promising potential as predictive biomarkers. In particular, FOXP3+ is dominant in predictive models of pathological response in patients with IBC.

Author Contribution Statement

Conceptualization, PR, MP, SCM; methodology, PR, EW, SCM; software, EW; validation, PR; formal analysis, PR, EW, SCM; investigation, PR, EW, MP, SCM; resources, PR, MP; data curation, EW, SCM; writing—original draft preparation, PR, EW; writing—review and editing, all authors; visualization, EW, SCM; supervision, PR; project administration, EW; funding acquisition, PR All authors have read and agreed to the published version of the manuscript.

Acknowledgements

We thank the Directorate of Research & Development Universitas Indonesia and the Ministry of Research/ National Agency for Research and Innovation for the funds provided through Publikasi Terindeks Internasional (PUTI) Pascasarjana Q3 grant number NKB-193/UN2. RST/HKP.05.00/2022.

Ethics approval and consent to participate

The Ethics Committee of the Faculty of Medicine, the University of Indonesia, approved the report protocols, with protocol number KET-131/UN2.F1/ETIK/ PPM.00.02/2022, in February 2022. Informed consent for participation was obtained from all subjects involved in the study.

Consent for publication

Informed consent for publication was obtained from all subjects involved in the study.

Availability of data and materials

All data generated or analyzed during the study are included in this published article.

Funding

This work was supported by grants from the Directorate of Research & Development Universitas Indonesia and the Ministry of Research/National Agency for Research and Innovation via Publikasi Terindeks Internasional (PUTI) Pascasarjana Q3 grant number NKB-193/UN2. RST/HKP.05.00/2022.

Competing interests

The authors declare no conflict of interest

References

1. Tan PH, Ellis I, Allison K, Brogi E, Fox SB, Lakhani S, et al. The 2019 world health organization classification of tumours of the breast. Histopathology. 2020;77(2):181-5. https://doi.org/10.1111/his.14091.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424. https://doi.org/10.3322/caac.21492.
3. Akram M, Iqbal M, Daniyal M, Khan AU. Awareness and current knowledge of breast cancer. Biol Res. 2017;50(1):33. https://doi.org/10.1186/s40659-017-0140-9.
4. Makki J. Diversity of breast carcinoma: Histological subtypes and clinical relevance. Clin Med Insights Pathol. 2015;8:23-31. https://doi.org/10.4137/CPath.S31563.
5. Yao L, Pang Z, Wang M, Wang M, Sun X, Cui M, et al. The choice of a neoadjuvant chemotherapy cycle for breast cancer has significance in clinical practice: Results from a population-based, real world study. Cancer Biol Med. 2021;19(5):755-67. https://doi.org/10.20892/j.issn.2095-3941.2020.0800.
6. Lee A, Lim W, Moon BI, Paik NS, Koh SH, Song JY. Chemotherapy response assay test and prognosis for breast cancer patients who have undergone anthracycline- and taxane-based chemotherapy. J Breast Cancer. 2011;14(4):283-8. https://doi.org/10.4048/jbc.2011.14.4.283.
7. Bortot L, Targato G, Noto C, Giavarra M, Palmero L, Zara D, et al. Multidisciplinary team meeting proposal and final therapeutic choice in early breast cancer: Is there an agreement? Front Oncol. 2022;12:885992. https://doi.org/10.3389/fonc.2022.885992.
8. Jiang B, Liu Q, Gai J, Guan J, Li Q. Analysis of adjuvant chemotherapy on pathological remission of breast cancer. Comput Math Methods Med. 2021;2021:5440154. https://doi.org/10.1155/2021/5440154.
9. Denkert C, Loibl S, Noske A, Roller M, Müller BM, Komor M, et al. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol. 2010;28(1):105-13. https://doi.org/10.1200/jco.2009.23.7370.
10. Zhao Y, Dong X, Li R, Ma X, Song J, Li Y, et al. Evaluation of the pathological response and prognosis following neoadjuvant chemotherapy in molecular subtypes of breast cancer. Onco Targets Ther. 2015;8:1511-21. https://doi.org/10.2147/ott.S83243.
11. Pelekanou V, Carvajal-Hausdorf DE, Altan M, Wasserman B, Carvajal-Hausdorf C, Wimberly H, et al. Effect of neoadjuvant chemotherapy on tumor-infiltrating lymphocytes and pd-l1 expression in breast cancer and its clinical significance. Breast Cancer Res. 2017;19(1):91. https://doi.org/10.1186/s13058-017-0884-8.
12. Sun Y, Liao M, He L, Zhu C. Comparison of breast-conserving surgery with mastectomy in locally advanced breast cancer after good response to neoadjuvant chemotherapy: A prisma-compliant systematic review and meta-analysis. Medicine (Baltimore). 2017;96(43):e8367. https://doi.org/10.1097/md.0000000000008367.
13. Lee HJ, Park IA, Song IH, Kim S-B, Jung KH, Ahn J-H, et al. Comparison of pathologic response evaluation systems after anthracycline with/without taxane-based neoadjuvant chemotherapy among different subtypes of breast cancers. PLOS ONE. 2015;10(9):e0137885. https://doi.org/10.1371/journal.pone.0137885.
14. Park YH, Lal S, Lee JE, Choi YL, Wen J, Ram S, et al. Chemotherapy induces dynamic immune responses in breast cancers that impact treatment outcome. Nat Commun. 2020;11(1):6175. https://doi.org/10.1038/s41467-020-19933-0.
15. Denkert C, von Minckwitz G, Darb-Esfahani S, Lederer B, Heppner BI, Weber KE, et al. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: A pooled analysis of 3771 patients treated with neoadjuvant therapy. Lancet Oncol. 2018;19(1):40-50. https://doi.org/10.1016/s1470-2045(17)30904-x.
16. Hornychova H, Melichar B, Tomsova M, Mergancova J, Urminska H, Ryska A. Tumor-infiltrating lymphocytes predict response to neoadjuvant chemotherapy in patients with breast carcinoma. Cancer Invest. 2008;26(10):1024-31. https://doi.org/10.1080/07357900802098165.
17. Peña-Romero AC, Orenes-Piñero E. Dual effect of immune cells within tumour microenvironment: Pro- and anti-tumour effects and their triggers. Cancers (Basel). 2022;14(7). https://doi.org/10.3390/cancers14071681.
18. Yu P, Fu YX. Tumor-infiltrating t lymphocytes: Friends or foes? Lab Invest. 2006;86(3):231-45. https://doi.org/10.1038/labinvest.3700389.
19. Whiteside TL. The tumor microenvironment and its role in promoting tumor growth. Oncogene. 2008;27(45):5904-12. https://doi.org/10.1038/onc.2008.271.
20. Ruan M, Tian T, Rao J, Xu X, Yu B, Yang W, et al. Predictive value of tumor-infiltrating lymphocytes to pathological complete response in neoadjuvant treated triple-negative breast cancers. Diagn Pathol. 2018;13(1):66. https://doi.org/10.1186/s13000-018-0743-7.
21. Kuwahara T, Hazama S, Suzuki N, Yoshida S, Tomochika S, Nakagami Y, et al. Intratumoural-infiltrating cd4+and foxp3+t cells as strong positive predictive markers for the prognosis of resectable colorectal cancer. Br J Cancer. 2019;121(8):659-65. https://doi.org/10.1038/s41416-019-0559-6.
22. Goda N, Sasada S, Shigematsu H, Masumoto N, Arihiro K, Nishikawa H, et al. The ratio of cd8+lymphocytes to tumor-infiltrating suppressive foxp3+effector regulatory t cells is associated with treatment response in invasive breast cancer. Discov Oncol. 2022;13(1):27. https://doi.org/10.1007/s12672-022-00482-5.
23. Miyashita M, Sasano H, Tamaki K, Hirakawa H, Takahashi Y, Nakagawa S, et al. Prognostic significance of tumor-infiltrating cd8+ and foxp3+ lymphocytes in residual tumors and alterations in these parameters after neoadjuvant chemotherapy in triple-negative breast

cancer: A retrospective multicenter study. Breast Cancer Res. 2015;17(1):124. https://doi.org/10.1186/s13058-015-0632-x.
24. WMA. World medical association declaration of helsinki: Ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191-4. https://doi.org/10.1001/jama.2013.281053.
25. Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. Nat Immunol. 2013;14(10):1014-22. https://doi.org/10.1038/ni.2703.
26. Miskad UA, Rifai RA, Masadah R, Nelwan B, Ahmad D, Cangara H, et al. The value of tumor-infiltrating lymphocytes and cd8 expression as a predictor of response to anthracycline-based neoadjuvant chemotherapy in invasive breast carcinoma of no special type. Breast Dis. 2021;40(S1):S9-s14. https://doi.org/10.3233/bd-219002.
27. Alizadeh D, Trad M, Hanke NT, Larmonier CB, Janikashvili N, Bonnotte B, et al. Doxorubicin eliminates myeloid-derived suppressor cells and enhances the efficacy of adoptive t-cell transfer in breast cancer. Cancer Res. 2014;74(1):104-18. https://doi.org/10.1158/0008-5472.Can-13-1545.
28. Mattarollo SR, Loi S, Duret H, Ma Y, Zitvogel L, Smyth MJ. Pivotal role of innate and adaptive immunity in anthracycline chemotherapy of established tumors. Cancer Res. 2011;71(14):4809-20. https://doi.org/10.1158/0008-5472.Can-11-0753.
29. Abdullah TM, Whatmore J, Bremer E, Slibinskas R, Michalak M, Eggleton P. Endoplasmic reticulum stress-induced release and binding of calreticulin from human ovarian cancer cells. Cancer Immunol Immunother. 2022;71(7):1655-69. https://doi.org/10.1007/s00262-021-03072-6.
30. Vacchelli E, Sistigu A, Yamazaki T, Vitale I, Zitvogel L, Kroemer G. Autocrine signaling of type 1 interferons in successful anticancer chemotherapy. Oncoimmunology. 2015;4(8):e988042. https://doi.org/10.4161/2162402x.2014.988042.
31. Wahid A, Chen W, Wang X, Tang X. High-mobility group box 1 serves as an inflammation driver of cardiovascular disease. Biomed Pharmacother. 2021;139:111555. https://doi.org/https://doi.org/10.1016/j.biopha.2021.111555.
32. Zurlo IV, Schino M, Strippoli A, Calegari MA, Cocomazzi A, Cassano A, et al. Predictive value of nlr, tils (cd4+/cd8+) and pd-l1 expression for prognosis and response to preoperative chemotherapy in gastric cancer. Cancer Immunol Immunother. 2022;71(1):45-55. https://doi.org/10.1007/s00262-021-02960-1.
33. Poncette L, Bluhm J, Blankenstein T. The role of cd4 t cells in rejection of solid tumors. Curr Opin Immunol. 2022;74:18-24. https://doi.org/https://doi.org/10.1016/j.coi.2021.09.005.
34. Cachot A, Bilous M, Liu Y-C, Li X, Saillard M, Cenerenti M, et al. Tumor-specific cytolytic cd4 t cells mediate immunity against human cancer. Sci Adv.7(9):eabe3348. https://doi.org/10.1126/sciadv.abe3348.
35. Goode EL, Block MS, Kalli KR, Vierkant RA, Chen W, Fogarty ZC, et al. Dose-response association of cd8+ tumor-infiltrating lymphocytes and survival time in high-grade serous ovarian cancer. JAMA Oncol. 2017;3(12):e173290. https://doi.org/10.1001/jamaoncol.2017.3290.
36. Al-Saleh K, Abd El-Aziz N, Ali A, Abozeed W, Abd El-Warith A, Ibraheem A, et al. Predictive and prognostic significance of cd8+ tumor-infiltrating lymphocytes in patients with luminal b/her 2 negative breast cancer treated with neoadjuvant chemotherapy. Oncol Lett. 2017;14(1):337-44. https://doi.org/10.3892/ol.2017.6144.
37. Correale P, Rotundo MS, Del Vecchio MT, Remondo C, Migali C, Ginanneschi C, et al. Regulatory (foxp3+) t-cell tumor infiltration is a favorable prognostic factor in advanced colon cancer patients undergoing chemo or chemoimmunotherapy. J Immunother. 2010;33(4):435-41. https://doi.org/10.1097/CJI.0b013e3181d32f01.
38. Dieci MV, Tsvetkova V, Griguolo G, Miglietta F, Tasca G, Giorgi CA, et al. Integration of tumour infiltrating lymphocytes, programmed cell-death ligand-1, cd8 and foxp3 in prognostic models for triple-negative breast cancer: Analysis of 244 stage i–iii patients treated with standard therapy. Eur J Cancer. 2020;136:7-15. https://doi.org/https://doi.org/10.1016/j.ejca.2020.05.014.
39. Ladoire S, Arnould L, Mignot G, Coudert B, Rébé C, Chalmin F, et al. Presence of foxp3 expression in tumor cells predicts better survival in her2-overexpressing breast cancer patients treated with neoadjuvant chemotherapy. Breast Cancer Res Treat. 2011;125(1):65-72. https://doi.org/10.1007/s10549-010-0831-1.
40. Zuo T, Liu R, Zhang H, Chang X, Liu Y, Wang L, et al. Foxp3 is a novel transcriptional repressor for the breast cancer oncogene skp2. J Clin Invest. 2007;117(12):3765-73. https://doi.org/10.1172/jci32538.
41. Zuo T, Wang L, Morrison C, Chang X, Zhang H, Li W, et al. Foxp3 is an x-linked breast cancer suppressor gene and an important repressor of the her-2/erbb2 oncogene. Cell. 2007;129(7):1275-86. https://doi.org/10.1016/j.cell.2007.04.034.
42. Liu Y, Wang Y, Li W, Zheng P, Liu Y. Activating transcription factor 2 and c-jun–mediated induction of foxp3 for experimental therapy of mammary tumor in the mouse. Cancer Res. 2009;69(14):5954-60. https://doi.org/10.1158/0008-5472.CAN-09-0778.