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CD8+ as a Predictive Factor for Response to Anthracycline-Based Neoadjuvant Chemotherapy in Patients with Locally Advanced Breast Cancer at Dr. Soetomo General Hospital, Surabaya, January 2024-August 2025

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ABSTRACT

Breast cancer remains a major global health issue. In Indonesia, approximately 70% of breast cancer cases are diagnosed at the locally advanced stage (LABC). Neoajuvant chemotherapy (NAC) is the treatment of choice for LABC, significantly reducing tumor size prior to surgical resection. CD8+ cytotoxic T-cells are a major component of tumor-infiltrating lymphocytes (TILs) and play a key role in inhibiting tumor proliferation. High CD8+ T cell infiltration has been associated with improved NAC response. This study aims to evaluate the potential of CD8+ T cell density as a significant predictor of clinical response to NAC. This retrospective cohort study involved 54 LABC patients who underwent NAC prior to surgical breast resection at Dr. Soetomo General Hospital from January 2024 to August 2025. The association between CD8+ T cell density and clinical response to NAC was analyzed using the Chi-square test or Fisher's exact test, as appropriate. Logistic regression analysis was conducted to determine whether high CD8+ density served as a predictive factor for a positive chemotherapeutic response. Statistical significance was defined as $p<0.05$. A statistically significant association was found between high CD8+ T cell density and a positive clinical response to NAC ($p<0.001$; OR 5.19; 95% CI: 1.87–14.42). High CD8+ density was shown to be a significant predictor of a favorable clinical response to neoajuvant chemotherapy ($p<0.001$). High CD8+ T cell density may serve as an independent and significant predictor of a positive clinical response in LABC patients receiving neoajuvant chemotherapy.

KEYWORDS: LABC, CD8+ T Cells, Predictor, Chemotherapy Response.

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INTRODUCTION

Breast cancer is one of the most common malignancies among women worldwide, with increasing incidence across both developed and developing regions. Global data consistently demonstrate breast cancer as a leading cause of cancer morbidity and mortality [1]. In Indonesia, breast cancer frequently presents at an advanced stage, with the majority of patients arriving with Locally Advanced Breast Cancer (LABC), contributing to poorer prognosis and higher treatment complexity [2].

LABC represents a heterogeneous group of tumors characterized by large primary lesions, skin or chest wall involvement, and/or advanced regional nodal disease. Its management requires a multimodal approach, with neoadjuvant chemotherapy (NAC) established as a standard strategy to downstage tumors, increase operability, and provide early assessment of chemosensitivity [3]. Despite standardized regimens such as anthracycline-based therapy, clinical responses vary widely among patients, suggesting underlying biological differences that influence treatment outcomes.

The tumor microenvironment (TME) has become an essential focus of contemporary oncologic research. Tumor-infiltrating lymphocytes (TILs), particularly CD8+ cytotoxic T cells, serve as critical mediators of antitumor immunity by inducing apoptosis of malignant cells and shaping therapeutic responses [4]. Several studies have reported that higher CD8+ T cell density within the tumor stroma correlates with better outcomes in breast cancer, including enhanced sensitivity to NAC [5,6].

Given their biological importance, CD8+ T cells may represent a practical predictive biomarker for NAC response in LABC. However, data regarding CD8+ density and its predictive value in Indonesian breast cancer populations remain limited [7]. This study evaluates CD8+ T cell density as a predictor of clinical response to anthracycline-based NAC in patients with LABC treated at Dr. Soetomo General Hospital.

METHOD

This study utilized a retrospective cohort design involving female patients diagnosed with Locally Advanced Breast Cancer who underwent anthracycline-based neoadjuvant chemotherapy at Dr. Soetomo General Hospital between January 2024 and August 2025. Patient data, including clinical characteristics, histopathological results, treatment regimens, and response evaluations, were retrieved from medical records. Paraffin-embedded tumor tissues obtained before NAC initiation were collected for immunohistochemical (IHC) analysis.

CD8+ T-cell density was assessed using immunohistochemistry performed on 4 µm paraffin sections. Staining evaluation focused on stromal tumor-infiltrating lymphocytes. For each sample, five representative high-power fields (400×) were captured and analyzed using ImageJ to quantify mean CD8+ cell density, following previously validated approaches [4]. Cutoff determination for high versus low CD8+ density used ROC curve analysis.

Clinical response to NAC was evaluated using RECIST 1.1 criteria, categorizing patients into complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD) [8]. CR and PR were classified as positive responses; SD and PD as negative responses. Associations were tested using Chi-square or Fisher's exact tests, and logistic regression assessed independent predictive value.

An additional strength of this study is the establishment of a quantitative cut-off value for $CD8^+$ T-cell density in LABC tissue samples. The cut-off value of 15 positive cells per five high-power fields was determined based on the mean lymphocytic density obtained from immunohistochemical evaluation. This data-driven threshold allows more objective stratification of patients and enhances the applicability of $CD8^+$ infiltration as a predictive biomarker in clinical decision-making.

RESULT

At the initial planning stage, the targeted sample size was 80 patients diagnosed with locally advanced breast cancer (LABC) who received anthracycline-based neoadjuvant chemotherapy at Dr. Soetomo General Hospital between January 2024 and August 2025. However, after reviewing medical records and verifying tissue availability, only 54 patients met all inclusion criteria and had adequate paraffin block specimens for analysis. The reduced final sample size was due to several factors, including: some patients being classified as early breast cancer, incomplete clinical or histopathological data, absence or poor condition of paraffin block specimens unsuitable for immunohistochemistry, and incomplete chemotherapy cycles or lack of post-treatment response assessment data.

Most subjects were older than 40 years (44 patients; 81.5%). Based on clinical T staging, the majority were classified as T4 (41 patients; 75.9%). The most common nodal involvement was N1 (32 patients; 59.3%). According to AJCC TNM staging, stage IIIB was the predominant stage (34 patients; 63%). The most frequent immunohistochemical molecular subtype was luminal B (40 patients; 74.1%). Positive estrogen receptor (ER) and progesterone receptor (PR) expression was found in 49 patients (90.7%). High Ki67 expression was observed in 42 patients (77.8%). The most commonly used anthracycline-based neoadjuvant chemotherapy regimen was cyclophosphamide, doxorubicin, and fluorouracil (CAF), accounting for 77.8% of cases.

$CD8^+$ lymphocyte expression was assessed using immunohistochemistry on biopsy specimens. The number of positively stained $CD8^+$ cells was determined by calculating the mean cell density across five representative high-power fields (400×). Normal control tissue showed minimal $CD8^+$ expression, whereas tumor samples demonstrated increased $CD8^+$ expression, characterized by numerous dark yellow-brown lymphocytes among blue-stained tumor nuclei.

The initial cut-off value for defining strong versus weak $CD8^+$ density was determined using the receiver operating characteristic (ROC) curve, with an area under the curve (AUC) of 99.5%. The optimal cut-off point was identified using a linear curve intersection between sensitivity and specificity (Figure 4). Using this cut-off, $CD8^+$ density was categorized into strong or weak groups. Most subjects exhibited strong $CD8^+$ lymphocyte density (38 patients; 70.4%).

A notable strength of the current findings is the identification of a $CD8^+$ T-cell density cut-off value (15 positive cells per five high-power fields), which provides a clinically meaningful threshold for distinguishing patients with strong versus weak immune infiltration. This cut-off enhances the interpretability of the response analysis and supports the potential use of $CD8^+$ infiltration as a practical predictor of neoadjuvant chemotherapy response.

Clinical response was evaluated using RECIST criteria based on objective tumor size reduction measured with calipers. The distribution of responses was as follows: Complete response: 5 patients (9.3%), Partial response: 35 patients (64.8%), Stable disease: 10 patients (18.5%), Progressive disease: 4 patients (7.4%). For analysis, the responses were grouped into two categories: Positive response (complete + partial): 40 patients (74.1%), Negative response (stable + progressive): 14 patients (25.9%).

Fisher's exact test revealed a statistically significant association between $CD8^+$ density and clinical response, showing that patients with weak $CD8^+$ density were more likely to exhibit negative clinical responses to chemotherapy.

Multivariate logistic regression analysis further confirmed these findings. Strong $CD8^+$ density was identified as an independent protective factor against negative clinical response ($p < 0.001$; OR = 160.3; 95% CI: 15.29–1680.7). The predictive model demonstrated an accuracy of 92.6% in identifying patients with a positive response to neoadjuvant chemotherapy.

DISCUSSION

This study investigated the association between $CD8^+$ T-cell infiltration and clinical response to anthracycline-based neoadjuvant

chemotherapy (NAC) in patients with locally advanced breast cancer (LABC). Breast cancer remains a major global health burden, with increasing incidence and mortality in many regions, including Indonesia [1,2]. According to the AJCC 8th edition staging system, LABC includes tumors >5 cm with regional nodal involvement, or tumors of any size with chest-wall or skin invasion [9]. The predominance of T4 and N1 lesions in this study is consistent with this definition and reflects the advanced stage typically encountered in referral centers. Table 1 summarizes demographic, clinical, and biological characteristics of the 54 included patients. Most patients were older than 40 years (81.5%), with the majority presenting as T4 (75.9%) and N1 (59.3%), consistent with typical LABC patterns in referral centers. Stage IIIB was the predominant TNM stage (63%). Luminal B was the most common molecular subtype (74.1%), with high ER/PR positivity (90.7%) and elevated Ki-67 expression (77.8%). The CAF regimen was the most frequently used therapy (77.8%). Overall, this table highlights that the cohort predominantly consisted of advanced-stage tumors with high proliferative activity, providing a suitable population for evaluating $CD8^+$ as a predictive marker for neoadjuvant chemotherapy response.

Table 1: Characteristics of LABC Subjects Receiving Neoadjuvant Chemotherapy at Dr. Soetomo Regional General Hospital from January 2024 - August 2025

Variable	Category	N (Count)	% (Percentage)
Age	<40 years	10	18.5
	>40 years	44	81.5
Gender	Female	54	100
Age at Initial Cancer Diagnosis	<60 years	22	40.7
	>60 years	32	59.3
Occupation	Entrepreneur	6	11.1
	Homemaker	33	61.1
	Private Employee	10	18.5
	Civil Servant	3	5.5
	Health Worker	2	3.7
History of Shift Work	Yes	12	22.2
	No	42	77.8
History of Contraception	Hormonal	42	77.8
	Non-hormonal	12	22.2
Duration of Contraception Use	<4 years	11	20.3
	>4 years	43	79.7
Clinical T Stage	T1	3	5.6
	T2	2	3.7
	T3	8	14.8
	T4	41	75.9
Regional Node Status	N0	4	7.4
	N1	32	59.3
	N2	15	27.8
	N3	3	5.6
TNM Stage	IIIA	16	29.6
	IIIB	34	63.0
	IIIC	4	7.4
Immunohistochemistry Molecular Subtype	Luminal A	9	16.7
	Luminal B	40	74.1
	HER-2	2	3.7
	TNBC (Triple Negative Breast Cancer)	3	5.6
Estrogen Receptor (ER) Status	Positive	49	90.7
	Negative	5	9.3
Progesterone Receptor (PR) Status	Positive	49	90.7
	Negative	5	9.3
Ki67 Status	High	42	77.8
	Low	12	22.2
Chemotherapy Regimen	CAF	42	77.8
	CEF	2	3.7
	Paclitaxel-Cisplatin	6	11.1
	Paclitaxel-Herceptin	2	3.7
	Docetaxel-Carboplatin	2	3.7

Tumor-infiltrating lymphocytes (TILs), especially $CD8^+$ cytotoxic T cells, play a central role in antitumor immunity. The immune contexture including density, functional state, and spatial distribution of immune cells has a strong impact on clinical outcomes across human cancers [4]. $CD8^+$ T cells mediate tumor cell elimination through perforin–granzyme pathways, FAS/FAS-L

interactions, and pro-inflammatory cytokines such as IFN-γ [10–12]. These mechanisms are part of broader immune surveillance and immunoediting processes that shape tumor progression [13,14]. Figure 1 shows normal breast tissue used as a control for $CD8^+$ immunohistochemical staining. Only minimal $CD8^+$ staining is visible, reflecting the physiologically low presence of cytotoxic T cells in non-tumoral tissue. This baseline appearance is essential as a reference point to highlight the increased $CD8^+$ infiltration observed in malignant samples. The figure demonstrates the contrast between healthy tissue and tumor-associated immune activation.

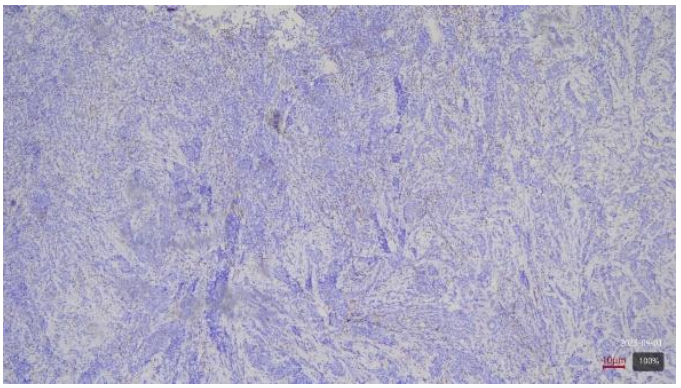

Figure 1: normal tissue (control). Immunohistochemistry of normal breast tissue showing minimal CD8+ lymphocyte presence. This represents the physiological baseline and serves as the reference for comparing CD8+ infiltration in tumor samples.

Figure 2 displays tumor tissue infiltrated by $CD8^+$ lymphocytes, identified as brownish-yellow stained cells among malignant nuclei. The prominent accumulation of $CD8^+$ cells indicates an active host immune response directed toward the tumor. This visual difference from the control specimen confirms the biological heterogeneity of $CD8^+$ infiltration among patients, forming the basis for categorizing $CD8^+$ density as "high" or "low" and assessing its predictive relevance to chemotherapy response.

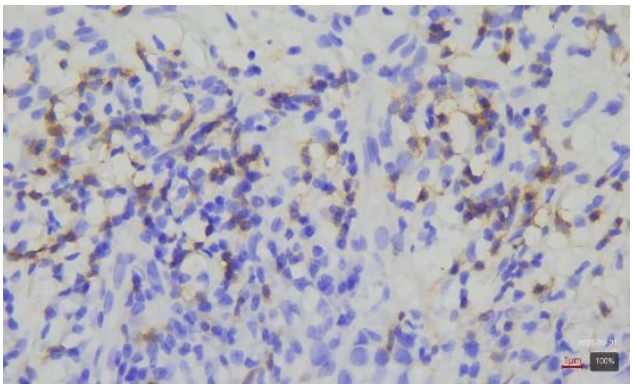

Figure 2: CD8+ expression in tumor (magnified 400x). dense clusters of CD8+ T cells. The markedly increased infiltration compared to normal tissue indicates an active cytotoxic immune response associated with better neoadjuvant chemotherapy sensitivity.

Figure 3 presents the ROC (Receiver Operating Characteristic) curve illustrating the ability of $CD8^+$ T-cell density to discriminate between patients with positive and negative responses to neoadjuvant chemotherapy. The Area Under the Curve (AUC) of 99.5% demonstrates exceptionally high predictive accuracy. The curve's proximity to the upper-left corner reflects excellent sensitivity and specificity, confirming $CD8^+$ density as a near-perfect biomarker for predicting treatment response in LABC.

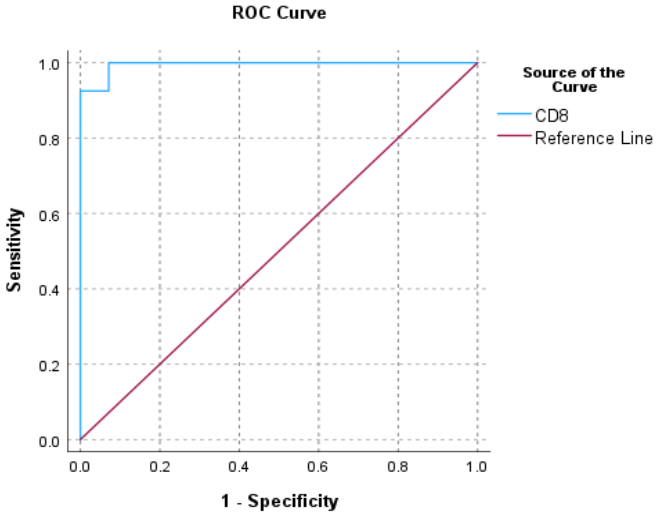

Figure 3: ROC curve graph of CD8+ lymphocyte levels and neoadjuvant chemotherapy response. This curve demonstrating excellent predictive performance of CD8+ density for neoadjuvant chemotherapy response, with a high AUC indicating strong discrimination between responders and non-responders.

Figure 4 illustrates the optimal cut-off point determined for categorizing $CD8^+$ density. The intersection of the sensitivity and specificity curves identifies a cut-off value of 15 $CD8^+$-positive cells per five high-power fields (HPF). This threshold maximizes diagnostic accuracy and allows objective classification into high and low $CD8^+$ density groups. Establishing this numerical cut-off strengthens the reproducibility and clinical applicability of the biomarker.

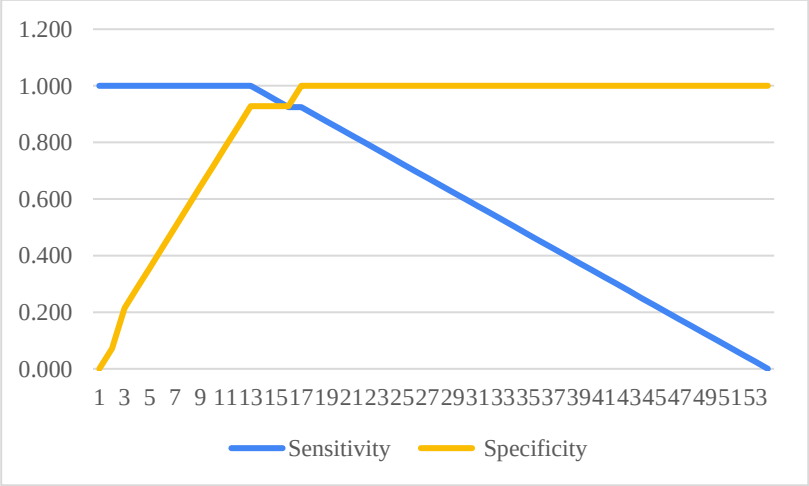

Figure 4: Cut-off value of CD8+ sensitivity and specificity. The intersection point identifies the threshold that best differentiates high versus low CD8+ expression for predicting chemotherapy outcomes

In breast cancer, the leukocyte composition of the tumor microenvironment (TME) influences treatment response and prognosis [15]. High $CD8^+$ infiltration is associated with more immunogenic tumor phenotypes [16] and has consistently been linked to improved outcomes across several studies. A systematic review and meta-analysis by Sun et al. demonstrated that strong $CD8^+$ T-cell infiltration correlates with better overall survival and disease-free survival in breast cancer [17]. Findings from Asano et al. further showed that TIL levels vary across molecular subtypes and predict NAC responsiveness [18].

The present study found that a strong pre-treatment $CD8^+$ density was significantly associated with positive clinical response to NAC. This aligns with evidence that NAC not only reduces tumor burden but also modulates the TME, potentially enhancing $CD8^+$-driven antitumor immunity. $CD8^+$ T-cell–mediated cytotoxicity is inherently immunogenic, promoting the release of tumor antigens and sustaining immune activation [12].

Anthracyclines, a key component of NAC regimens, can trigger immunogenic cell death, further stimulating antigen presentation and improving the efficacy of cytotoxic T-cell responses. This synergy may explain why tumors with high baseline $CD8^+$ infiltration exhibit better clinical responses, as supported by studies from Miskad et al. and Deswangga et al., both of which identified $CD8^+$ expression as a significant predictor of NAC response in Indonesian breast cancer populations [5,6].

Yu et al. also described multiple biological predictors of NAC effectiveness, including Ki-67, molecular subtype, and host immune parameters, reinforcing that chemotherapy response involves both tumor-intrinsic and immune-mediated mechanisms [19].

The present study utilized the RECIST criteria for evaluating clinical response. RECIST provides reproducible unidimensional measurements and remains the standard for assessing therapeutic outcomes in solid tumors [8]. Complementary reviews, such as that by Tirkes et al., emphasize the importance of standardized imaging criteria to ensure consistency in response assessment in oncology [20]. The favorable response profile observed in this cohort is consistent with prior local studies demonstrating high responsiveness to anthracycline-based NAC in LABC [5,6,18].

Although $CD8^+$ T cells are crucial for antitumor activity, their effectiveness may be inhibited by immunosuppressive components of the TME, including $FOXP3^+$ regulatory T cells (Tregs). Mao demonstrated that FOXP3 expression in breast cancer cells can influence apoptosis pathways, potentially altering treatment sensitivity [17]. Studies in melanoma also highlight the prognostic significance of TIL composition, including the interplay between effector and regulatory subsets [21]. These findings support the concept that the balance between $CD8^+$ and regulatory immune elements may refine the predictive value of $CD8^+$ density alone.

An important strength of this study is the establishment of a $CD8^+$ cut-off value derived from quantitative immunohistochemistry, which may be clinically applicable for patient stratification. The findings suggest potential utility in tailoring NAC approaches based on immune infiltration profiles.

However, limitations include the retrospective design, a relatively small sample size, and the restriction to a single institution, potentially limiting generalizability. Differences in population-based risk profiles, such as occupational exposures reported by Li et al. [22], may also influence disease characteristics across regions. Future research using prospective, multicenter designs and integration of additional immune markers may improve predictive accuracy and validate the clinical relevance of $CD8^+$ density in NAC response.

CONCLUSION

High CD8+ T-cell density is strongly associated with favorable clinical response to anthracycline-based neoadjuvant chemotherapy in patients with LABC. CD8+ density may serve as a valuable predictive biomarker to guide individualized therapeutic strategies. Further prospective, multi-center studies are needed to validate these findings.

REFERENCES

1. 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. doi:10.3322/caac.21492
2. Dinas Kesehatan Kota Surabaya. Profil Kesehatan Kota Surabaya Tahun 2021.; 2022.
3. Cardoso F, Paluch-Shimon S, Senkus E, et al. 5th ESO-ESMO international consensus guidelines for advanced breast cancer (ABC 5). Ann Oncol. 2020;31(12):1623-1649. doi:10.1016/j.annonc.2020.09.010
4. Fridman WH, Pagès F, Sautès-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer. 2012;12(4):298-306. doi:10.1038/nrc3245
5. Miskad UA, Rifai RA, Masadah R, 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. doi:10.3233/BD-219002
6. Deswangga MD, Alsoph Y. CD8+ Tumoral as Predictor of Neoadjuvant Chemotherapy Response in Patients with Local Advanced Stage Breast Cancer. Sriwij J Surg. 2018;1(2):1-11. doi:10.37275/sjs.v1i2.6
7. Rafi MA. Comparing Robot-Assisted, Minimally Invasive, Transcervical, and Transhiatal Esophagectomy for Esophageal Cancer: A Causal Deep Learning Meta-Analysis with Neural Architecture. Pharmacol Med REPORTS, Orthop Illn DETAILS. 2025;4(2):68-86. doi:10.55047/comorbid.v4i2.1683
8. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45(2):228-247. doi:10.1016/j.ejca.2008.10.026
9. Giuliano AE, Connolly JL, Edge SB, et al. Breast cancer—major changes in the American Joint Committee on Cancer eighth edition cancer staging manual. CA Cancer J Clin. 2017;67(4):290-303. doi:10.3322/caac.21393
10. Putri DNA, Badaruddin B, Kadir A. Implementasi Program "Menjelajah Desa Menjemput Pajak" Pada Kantor Badan Pendapatan Kabupaten Langkat". PERSPEKTIF. 2020;9(2). doi:10.31289/perspektif.v9i2.3373
11. Raskov H, Orhan A, Christensen JP, Gögenur I. Cytotoxic CD8+ T cells in cancer and cancer immunotherapy. Br J Cancer. 2021;124(2):359-367. doi:10.1038/s41416-020-01048-4
12. Jaime-Sanchez P, Uranga-Murillo I, Aguilo N, et al. Cell death induced by cytotoxic CD8+ T cells is immunogenic and primes caspase-3–dependent spread immunity against endogenous tumor antigens. J Immunother cancer. 2020;8(1):e000528. doi:10.1136/jitc-2020-000528
13. Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. Immunity. 2004;21(2):137-148.
14. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646-674.
15. Abdulloh A, Ni'mah AQ. BI-RADS Classification for Breast Ultrasound: A Review. Pharmacol Med Reports, Orthop Illn Details. 2023;2(2):67-84. doi:10.55047/comorbid.v2i2.840
16. Ruffell B, Au A, Rugo HS, Esserman LJ, Hwang ES, Coussens LM. Leukocyte composition of human breast cancer. Proc Natl Acad Sci. 2012;109(8):2796-2801. doi:10.1073/pnas.1104303108
17. Sun Y, Wang Y, Lu F, Zhao X, Nie Z, He B. The prognostic values of FOXP3+ tumor-infiltrating T cells in breast cancer: a systematic review and meta-analysis. Clin Transl Oncol. 2023;25(6):1830-1843. doi:10.1007/s12094-023-

03080-1
18. Asano Y, Kashiwagi S, Goto W, et al. Prediction of treatment response to neoadjuvant chemotherapy in breast cancer by subtype using tumor-infiltrating lymphocytes. Anticancer Res. 2018;38(4):2311-2321. doi:10.21873/anticanres.12476
19. Yu Y, Xiang H, He XM, Yang HJ, Zong XY. Predictive factors determining neoadjuvant chemotherapy outcomes in breast cancer-a single center experience. Asian Pacific J Cancer Prev. 2013;14(4):2401-2406. doi:10.7314/APJCP.2013.14.4.2401
20. Tirkes T, Hollar MA, Tann M, Kohli MD, Akisik F, Sandrasegaran K. Response criteria in oncologic imaging: review of traditional and new criteria. Radiographics. 2013;33(5):1323-1341. doi:10.1148/rg.335125214
21. Mao Y. Cancer cell-expressed FOXP3 and apoptosis in breast cancer cells. Cancer Lett. 2016;383(1):10–8.
22. Li A, Shen Z, Sun Z, et al. Occupational risk factors and breast cancer in Beijing, China: a hospital-based case–control study. BMJ Open. 2022;12(2):e054151. doi:10.1136/bmjopen-2021-054151