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Low tumor-infiltrating lymphocytes (TIL) and high platelet lymphocyte ratio (PLR), neutrophil-lymphocyte ratio (NLR), and mean platelet volume (MPV) as a risk factor of metastasis in triple-negative breast cancer (TNBC)

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ABSTRACT

Introduction: Triple Negative Breast Cancer (TNBC) is a subtype of breast cancer that is more aggressive and has a poor prognosis associated with an increased risk of metastasis. Cancer cells are known to trigger an inflammatory process which in turn causes tissue and vascular invasion, leading to changes in the levels of several hematological components. Unfortunately, research regarding various biomarkers in TNBC metastasis is still limited and argumentative. This study aims to determine the role of immune system-derived indices as biomarkers of metastasis in TNBC.

Method: This is a case-control study to examine the relationship between tumor-infiltrating lymphocyte (TIL), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and mean platelet volume (MPV) on metastasis in patients with TNBC subtype breast cancer. This research took place at the Medical Records Installation at RSUP Prof. DR. I.G.N.G Ngoerah Denpasar from August 2021 – April 2022. Research subjects were breast cancer patients with TNBC subtypes with metastases and without metastases who were registered in the Surgical Oncology Division in 2016-2021. All data analysis used SPSS for Windows version 23.0 with the p-value considered significant if $p<0.05$.

Results: A total of 100 research subjects were divided into metastatic (44 patients) and non-metastatic (56 patients) groups with a mean age of 52.97 ± 12.67 and 51.98 ± 10.86 years, respectively. Bivariate analysis showed a significant relationship between TIL (OR 0.163; IK95% 0.067 – 0.399; p=0.000), NLR (OR 3.644; IK95% 1.585 – 8.375; p=0.002), PLR (OR 9.755; IK95% 3.872 – 24.580; p=0.000), MPV (OR 9.500; 95% CI 6.512 – 58.393; p=0.000) on the incidence of metastasis. Multivariate analysis showed a significant relationship between the NLR and MPV variables ($p<0.05$).

Conclusion: Patients with metastases had significantly higher NLR and MPV values and significantly increased risk of metastasis in TNBC.

Keywords: *Mean platelet volume, neutrophil to lymphocyte ratio, platelet to lymphocyte ratio,* triple-negative breast cancer, *tumor-infiltrating lymphocyte*.
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INTRODUCTION

Triple-negative breast Cancer (TNBC) is a subtype that does not express all three types of receptors and is a group of breast cancer that is clinically more aggressive than other groups and therefore has a worse prognosis.[1] TBNC is breast cancer with the worst prognosis. This can be seen from the low 5-year survival of TNBC which is only 62.1% compared to 80.8% compared to non-TNBC subtypes.[1]

Tumor-infiltrating lymphocytes (TIL) are one of the body's responses to cancer and cause a better prognosis in various breast cancer patients. TILs will increase when cancer occurs as a sign that the body is trying to fight cancer cells.[2] If lymphocyte infiltration (CD8+ cells) is increased, breast cancer patients tend to have a better prognosis. In several studies, increased PLR and decreased TIL were used as prognostic indicators for breast cancer patients.[3]

Cancer cells are known to trigger an inflammatory process which in turn causes tissue and vascular invasion. This process causes an increase in several components such as lymphocytes, neutrophils, platelets, and mean platelet volume (MPV) which are known to have an important role in carcinogenesis and tumor progression. The presence of platelets in circulation has an important contribution to tumor transmission and increases tumor survival by enveloping tumors and making them undetectable by the immune system.[4] High MPV levels are significantly associated with lower disease-free survival (DFS) rates.[5]

Platelet-to-lymphocyte ratio (PLR) is the ratio of platelets and lymphocytes which is known as an inflammatory

response and has been widely studied and is closely related to breast cancer prognostics. The higher the PLR is associated with worse prognostic and cancer outcomes.[3] Regarding the relationship between platelets and CTCs, the epithelial-mesenchymal transition phenomenon has been discovered, where platelets are thought to facilitate the extravasation of tumor cells in the circulation to the extravascular side in certain organs.[3]

Inflammation will have systemic and local impacts (microenvironment) that support the development of malignancy. Recent research also found that indices derived from peripheral blood cellular components such as neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), mean platelet volume (MPV) also show an increased pattern in inflammatory processes and various types of malignancy.[3] Of the four hematological biomarkers, NLR is a potential biomarker in TNBC because it is effective and economical.[6] A higher pretreatment NLR examination significantly and independently indicates a poor prognosis for breast cancer, especially TNBC.[7]

Metastasis is defined as the process of spread to nearby secondary sites or distant, non-contiguous locations, and the formation of macroscopic secondary foci.[8] One of the latest findings is the close connection of the immune system in the metastasis process, where TILs, neutrophil cells, lymphocytes, and platelets play an important role both locally and in circulation. However, unfortunately, research regarding these various biomarkers in TNBC metastasis is still limited and argumentative. Therefore, this study aims to examine the influence of several immune system-derived indices on the incidence of metastasis in TNBC patients.

METHOD

Study design

This research is an analytical study with a case-control method that aims to evaluate the relationship between TIL (tumor-infiltrating lymphocytes), PLR (platelet lymphocyte ratio), NLR (neutrophil-lymphocyte ratio), and MPV (mean platelet volume) on metastasis in breast cancer subtype TNBC. The research took place at the Medical Records Installation at RSUP Prof. DR. I.G.N.G Ngoerah Denpasar which took place from August 2021 – April 2022.

Participant

The samples in this study were breast cancer patients with TNBC subtypes with metastases and without metastases who were registered in the Surgical Oncology Division of Prof. DR Hospital. I.G.N.G Ngoerah Denpasar in 2016-2021. Based on the minimum sample size calculation, 100 patients were obtained. The inclusion criteria used in this study were TNBC patients who had undergone neoadjuvant chemotherapy, metastatic status that had been diagnosed, and complete clinicopathological data in the medical record. Meanwhile, patients with a history of other malignancies unless deemed disease-free for 5 years or more as recorded in the medical record, patients who suffered from breast cancer during pregnancy or became pregnant during therapy as recorded in the medical record, and patients with recurrent breast cancer and incomplete data were excluded from this study.

Variables and measurement

This study evaluated TIL (tumor-infiltrating lymphocytes), PLR (platelet lymphocyte ratio), NLR (neutrophil-lymphocyte ratio), and MPV (mean platelet volume) taken from patient blood samples before chemotherapy was given in the medical record. This study also evaluated age, histological grading, menopausal status, histological subtype, tumor size, and cancer stage obtained from medical records.

Data analysis

Data analysis was carried out using SPSS for Windows version 23.0 software. The p-value was considered significant if $P<0.05$.

RESULTS

Baseline Characteristics of Research Subjects

This study involved 100 research subjects divided into 44 subjects in the metastatic group and 56 subjects in the non-metastatic group (Table 1). The mean age of subjects in the metastatic group was 52.97 ± 12.67 years while in the non-metastatic group, it was 51.98 ± 10.86 years. The mean MPV in the metastatic group was 5.74 ± 0.58 fL which seemed lower when compared to the non-metastatic group (6.92 ± 1.10 fL). However, the metastatic group had higher neutrophil and platelet counts when compared with the metastatic group (5.80 ± 2.28 x 10^3/mL and 290.73 ± 68.86 x 10^3/mL vs. 4.70 ± 2.01 x 10^3/mL and 260.81 ± 66.61 x 10^3/mL). Judging from the number of lymphocyte counts, the metastatic group appeared to have a lower lymphocyte count.

The non-metastatic group is dominated by stage 3 cancer with a frequency of 89.3%. Judging from TIL, 77.3% of the metastatic group had low TIL while 64.9% of the non-metastatic group had high TIL. Judging from the histological grade, both groups were dominated by moderate-low grade (metastasis vs. non-metastasis: 61.4% vs. 73.2%).

Based on Table 2, it can be seen that the mean age of the research sample at stage II was found to be 56.143 ± 6.466 years, which is higher compared to the mean age at higher stages. Apart from that, if we look at the mean MPV value, it was found that

Table 1. Baseline Characteristics of Research Subjects Based on Metastasis Status

Variable	Metastatic Status	
	Metastasis (n=44)	Non-Metastasis (n=56)
Age (years)	52.97 ± 12.67	51.98 ± 10.86
MPV (fL)	5.74 ± 0.58	6.92 ± 1.10
Neutrophil (10^3/mL)	5.80 ± 2.28	4.70 ± 2.01
Platelet (10^3/mL)	290.73 ± 68.86	260.81 ± 66.61
Lymphocyte (10^3/mL)	1.82 ± 0.59	3.40 ± 6.66
TIL		
High	10 (22.7%)	36 (64.3%)
Low	34 (77.3%)	20 (35.7%)

Table 2. Baseline Characteristics of Research Subjects Based on Stage

Variable	Stage		
	Stage II (n=7)	Stage III (n=51)	Stage IV (n=42)
Age (years)	56.143 ± 6.466	51.745 ± 11.203	52.619 ± 12.787
MPV (fL)	6.288 ± 0.692	6.957 ± 1.129	5.752 ± 0.588
Neutrophil (10^3/mL)	6.916 ± 2.969	4.561 ± 1.886	4.609 ± 2.109
Platelet (10^3/mL)	221.257 ± 34.662	264.433 ± 67.863	262.940 ± 69.621
Lymphocyte (10^3/mL)	1.370 ± 0.472	3.595 ± 6.690	1.855 ± 0.580
TIL			
High	6 (85.7%)	31 (60.8%)	9 (21.4%)
Low	1 (14.3%)	20 (39.2%)	33 (78.6%)

Table 3. Baseline Characteristics of Research Subjects Based on Grade

Variable	Grade	
	High (n=32)	Low-Moderate (n=68)
Age (years)	53.412 ± 11.634	50.313 ± 11.465
MPV (fL)	6.478 ± 1.195	6.248 ± 0.777
Neutrophil (10^3/mL)	4.665 ± 2.080	4.916 ± 2.251
Platelet (10^3/mL)	262.602 ± 67.555	256.918 ± 67.574
Lymphocyte (10^3/mL)	2.918 ± 6.102	2.262 ± 0.713
TIL		
High	32 (47.1%)	14 (43.8%)
Low	36 (52.9%)	18 (56.3%)

Table 4. Association of TILs and risk of metastasis in TNBC

Variable	Group		OR	95%CI	p Value
	Metastasis	Non-metastasis			
TIL					
High	10	36	0.163	0.067 – 0.399	<0.001*
Low	34	30			

*Analysis was carried out using the Chi-Square Test. Results were considered significant if p≤0.05.

Table 5. Analysis of mean differences between PLR and metastasis in TNBC

Variable	Group		Mean differences	p Value
	Metastasis	Non-metastasis		
PLR	191.84 ± 74.07	133.38 ± 63.89	58.45	<0.001*

*Analysis was carried out using the Mann-Whitney U Test. Results were considered significant if p≤0.05.

Table 6. Association between PLR and risk of metastasis in TNBC

Variable	Group		OR	95%CI	p Value
	Metastasis	Non-metastasis			
PLR					
High	27	17	3.644	1.585 – 8.375	0.002*
Low	17	39			

*Analysis was carried out using the Chi-Square Test. Results were considered significant if p≤0.05.

the highest mean was in stage III at 6.957 ± 1.129 fL. Similar results were also found in platelet and lymphocyte parameters which were found to be highest in stage III. On the other hand, the highest neutrophil count value was found in stage II at 6,916 ± 2,969 with the highest TIL count in stage III and the lowest in stage IV.

Based on the results of the analysis in Table 3, it was found that the average patient age was older in the high-grade group compared to the moderate-low grade, 53,412 ± 11,634. These results were also followed by MPV, platelet, and lymphocyte parameters which were found to have a higher mean at high grade. On the other hand, if we look at the neutrophil parameters, it was found that lower grades had a higher mean neutrophils compared to higher tumor grades. When looking at the results of the TIL examination, there were no consistent results or a tendency that in patients with a high grade there would be a preponderance of high or low TILs.

The relationship between tumor-infiltrating lymphocytes (TIL) and the risk of metastasis in triple-negative breast cancer (TNBC) subtype breast cancer

The relationship between TIL and metastasis risk status in this study was analyzed using chi-square analysis to calculate significance and odds ratio (OR). The results of the analysis showed that TIL was significantly associated with metastasis in TNBC-type breast cancer. From Table 4 it can also be seen that TIL is a protective factor with an OR of 0.163 (95% CI = 0.067 – 0.399; p<0.001) which shows that patients with high TIL have a 6 times lower risk of metastasis compared to TNBC patients with low TIL (Table 4).

The relationship between platelet-to-lymphocyte ratio (PLR) and the risk of metastasis in triple-negative breast cancer (TNBC) subtype breast cancer

In contrast to the analysis of the relationship between TIL and the risk of metastasis, the relationship between PLR and the risk of metastasis was analyzed using the Mann-Whitney test to see the difference in mean PLR between the metastatic and non-metastatic groups. The results of the analysis showed that PLR was significantly associated with metastasis in TNBC-type breast cancer. From Table 5 it can also be seen that the metastatic group had a significantly higher mean PLR compared to the non-metastatic group (191.84 ±

Table 7. Analysis of mean differences between NLR and metastasis in TNBC

Variable	Group		Mean differences	p Value
	Metastasis	Non-metastasis		
NLR	3.78 ± 2.11	2.30 ± 1.45	1.48	<0.001*

*Analysis was carried out using the Mann-Whitney U Test. Results were considered significant if p≤0.05.

Table 8. Association between NLR and risk of metastasis in TNBC

Variable	Group		OR	95%CI	p Value
	Metastasis	Non-Metastasis			
NLR					
High	31	11	9.755	3.872 – 24.580	<0.001*
Low	13	45			

*Analysis was carried out using the Chi-Square Test. Results were considered significant if p≤0.05.

Table 9. Analysis of mean differences between MPV and metastasis in TNBC

Variable	Group		Mean differences	p Value
	Metastasis	Non-metastasis		
MPV	5.74 ± 0.58 fL	6.92 ± 1.10 fL	1.17	<0.001*

*Analysis was carried out using the Mann-Whitney U Test. Results were considered significant if p≤0.05.

Table 10. Association between MPV and metastasis in TNBC

Variable	Group		OR	95%CI	p Value
	Metastasis	Non-Metastasis			
MPV					
Low	39	16	9.500	6.512 – 58.393	<0.001*
High	5	40			

*Analysis was carried out using the Chi-Square Test. Results were considered significant if p≤0.05.

Table 11. Results of multivariate logistic regression analysis

Variable	Adjusted OR	95%CI	p Value
NLR	15.081	3.980-57.191	<0.001*
MPV	9.710	7.013-117.527	<0.001*

*Analysis was carried out using a logistic regression test. Results were considered significant if p≤0.05

74.07 vs. 133.38 ± 63.89; p<0.001) (Table 5).

To divide PLR into two groups, ROC analysis was carried out to calculate the optimal cut point. The results of the ROC analysis show that the optimal cut point for PLR is 151.44 so the PLR variable is then divided into two groups based on this division (high and low). The analysis was continued with the chi-square test to see the relationship between classified PLR and the risk of metastasis. The results of the analysis show that a high PLR is significantly associated with the risk of metastasis with an OR value of 3.644, which indicates that a high PLR value can increase the risk of metastasis in TNBC by 3.644 times compared to patients with a low PLR value (95% CI = 1.585 – 8.375; p =0.002) (Table 6).

The relationship between neutrophil-to-lymphocyte ratio (NLR) and the risk of metastasis in triple-negative breast cancer (TNBC) subtype breast cancer

Analysis of the relationship between NLR and risk of metastasis was analyzed using the Mann-Whitney test to see the difference in mean NLR between the metastatic and non-metastatic groups. The results of the analysis showed that NLR was significantly associated with metastasis in TNBC-type breast cancer. From Table 7 it can also be seen that the metastatic group had a significantly higher mean NLR compared to the non-metastatic group (3.78 ± 2.11 vs. 2.30 ± 1.45; p<0.001) (Table 7).

To divide the NLR into two groups, ROC analysis was carried out to calculate the optimal cut point. The results of the ROC analysis show that the optimal cut point for NLR is 3.026 so the NLR variable is then divided into two groups based on this division (high and low). The analysis was continued with the chi-square test to see the relationship between classified NLR and the risk of metastasis. The results of the analysis showed that a high NLR was significantly associated with the risk of metastasis with an OR value of 9.755 indicating that a high NLR value could increase the risk of metastasis in TNBC by 9.755 times compared to patients with a low NLR value (95% CI = 3.872 – 24.580; p< 0.001) (Table 8).

The relationship between mean platelet volume (MPV) and the risk of metastasis in triple-negative breast cancer (TNBC) subtype breast cancer

Analysis of the relationship between MPV and the risk of metastasis was analyzed using the Mann-Whitney test to see the difference in mean MPV between the metastatic and non-metastatic groups. The results of the analysis showed that MPV was significantly associated with metastasis in TNBC-type breast cancer. From Table 9 it can also be seen that the metastatic group had a significantly higher mean MPV compared to the non-metastatic group (5.74 ± 0.58 fL vs. 6.92 ± 1.10 fL; p<0.001) (Table 9).

To divide MPV into two groups, ROC analysis was carried out to calculate the optimal cut point. The results of the ROC analysis show that the optimal cut point for MPV is 6.375 so the MPV variable is then divided into two groups based on this division (high and low). The analysis was continued with the chi-square test to see the relationship between classified MPV and the risk of metastasis. The results of the analysis showed that high MPV was significantly associated with the risk of metastasis with an OR value of 6.375 indicating that a low MPV value could increase the risk of metastasis in TNBC by 6.375 times compared to patients with high MPV values (95% CI = 6.512 – 58.393; p< 0.001) (Table 10).

Multivariate analysis between TIL, PLR, NLR, and MPV with the risk of metastasis in triple-negative breast cancer (TNBC) subtype breast cancer

Following up on the findings from the bivariate analysis, a multivariate analysis

was carried out in the form of logistic regression because the target variable (metastasis) was binary nominal data. In this analysis, TIL, PLR, NLR, and MPV as well as confounding variables such as age and histological grade are included in the regression calculation so that an independent relationship can be obtained between the independent variable and the dependent variable (by controlling confounding variables and interactions between independent variables).

From the results of logistic regression, it can be seen that there is a significant relationship between NLR and MPV and the risk of metastasis. The adjusted OR obtained was 15.081 (IK95% = 3.980-57.191) for NLR and 9.710 (IK95% = 7.013-117.527) for MPV (Table 11). This shows that only NLR and MPV significantly influence and increase the risk of metastasis in TNBC subtype breast cancer by 15,081 times for NLR and 9,710 times for MPV, respectively.

DISCUSSION

TBNC is breast cancer with the worst prognosis and TNBC has high genomic and phenotypic variability so it is classified into Basal-like 1 (BL-1), Basal-like 2 (BL-2), immunomodulatory (IM), Messenchymal (M) or Messenchymal-like (MSL) and luminal androgen receptor (LAR).[9] Although in general TNBC is chemosensitive, it is estimated that 20% of the patient population with TNBC is chemoresistant which cannot be predicted using current clinical parameters.[1]

In the last decade, inflammatory responses, both local to the tumor area and systemic, have played an important role in the pathophysiology of breast cancer progression. Lymphocyte infiltration in cancer tissue is an example of an immune response that can be assessed clinically and has prognostic significance. The dualism of the role of the immune system can be seen in several contradictory reports regarding TIL where some studies support that TIL is a better prognostic factor for survival while several other studies show no relationship.[10]

This study proves that TIL, PLR, NLR, and MPV have a significant relationship with metastasis. However, only NLR and MPV appeared as independent factors in the results of multivariate analysis, indicating that TIL and PLR were influenced by NLR and MPV or confounding variables.

The results of this study indicate that there is a significant relationship between PLR and the risk of metastasis in TNBC-type breast cancer. Until now, there has been no research that specifically reports the relationship between TIL and TNBC breast cancer metastasis. Previous studies used breast cancer samples and did not specifically address TNBC. The study by Sejati et al (2019) examined the relationship between PLR and TIL about the incidence of breast cancer metastasis. The study reported that in the sample group with PLR ≥ 156 10µ /µL, there were 22.9% cases of metastasis (p = 0.002). Meanwhile, the sample group with low TIL experienced a metastasis incidence of 12.5% with a p-value of 0.442. The study confirmed that PLR was associated with higher metastasis in breast cancer patients.[11] PLR was found to be the only variable that had a statistically significant association with metastasis. Nonetheless, further multicenter studies are needed with sample homogenization and examining each variable to prove that other variables do not correlate with metastasis, for which data for each group of TIL, age, grade, and LVI are uneven. This might affect data validation.[11]

The relationship between platelets and cancer has long been documented and platelets are an important factor in the metastatic process. Tumor cells can cause platelet activation to form clotting aggregates which act as a barrier to the immune system and physical stress during the process of hematogenous metastasis.[12] This has been reported in several studies. The ability of tumor cells to induce platelet aggregation was first reported in 1968 and is known as tumor cell-induced platelet aggregation (TCIPA). It appears that tumor cells that enter and participate in the bloodstream will bind and activate platelets (cohesion) and also leukocytes. The host's immune system will attract tumor cells to the blood vessel walls through platelets (adhesion) and survive in the blood vessel walls (immune evasion). These bonds can also come out of the circulation (extravasation). Furthermore, tumor cells will survive and proliferate in other cells/tissues as metastases.[12,13]

The PLR value is calculated as the number of platelets divided by the number of lymphocytes. According to several studies, PLR is associated with a poor prognosis in other cancers such as colorectal cancer, lung cancer, and cancer.[14,15] Research also shows that PLR can provide implications for the selection of therapeutic modalities and prediction of prognosis in breast cancer patients. However, the relationship between PLR and breast cancer prognosis is still a matter of debate because relevant studies present varying results. These differences may be caused by different study designs and small sample sizes. However, a meta-analysis study in 2017 stated that PLR was associated with poor prognosis and clinicopathology in breast cancer.[16]

Previous research found that platelets have an important role in the survival of cancer cells. PLR is a comparison of platelet and lymphocyte values which is known as an indicator of the inflammatory response. PLR values were significantly higher in malignant tumors than in benign tumors. A higher PLR value is associated with worse prognostic and outcome.[3,17]

Regarding the role of PLR in TNBC, a study conducted by Onagi et al aimed to determine the relationship between TIL and peripheral blood markers, PLR and NLR, in 502 TNBC patients. A positive correlation between PLR and TIL was found in TNBC (P=0.013). mfIHC results showed tumors in patients with high PLR and NLR contained more CD3+CD4+FOXP3+ T cells (P=0.049 and 0.019, respectively), while no trend was observed in CD8+ T cells. TNBC patients had different outcome patterns according to TIL and PLR, with the TIL-high/PLR-low group having the lowest rates of disease recurrence and mortality, and the longest overall and distant metastasis-free survival, whereas the TIL-low/PLR-high group had the shortest life. The study confirmed that the combination of PLR with TIL can be used to determine more accurate outcome predictions for patients with TNBC.[18]

The role of PLR in TNBC has also been investigated by the study of Loi et al. The study used 92 samples dividing

patients into a pathological complete response (pCR) group (n=37) and a non-pathological complete response (non-pCR) group (n=55) according to the efficacy of NAC. In addition, patients with high NLR, high PLR, and low HALP had a lower 3-year survival rate than patients with low NLR, low PLR, and high HALP (P<0.05). Multivariate regression analysis showed that TNM stage III (OR (95% CI): 1.742 (1.209-2.631), P=0.003), lymph node metastasis (OR (95% CI): 1.922 (1.492-2.983), P = 0.005), high NLR (OR (95% CI): 2.261 (1.625-2.754), P<0.001), high PLR (OR (95% CI): 2.062 (1.692-2.791), P<0.001) and low HALP (OR (95% CI): 0.518 (0.365-0.734), P<0.001) was a risk factor for poor efficacy of NAC for TNBC. Patient mortality in the non-pCR group was higher than that in the pCR group within 3 years (P<0.05). Survival analysis showed that the 3-year survival rate of the non-pCR group was lower than that of the pCR group (P<0.05).[19]

Neutrophil-to-lymphocyte or NLR is one of the significant variables shown by the results of the multivariate analysis of this study. NLR is a calculation method that compares the activation of innate (represented by neutrophils) and adaptive (lymphocytes) immune responses, where the innate immune response is often considered to support cancer progression. NLR examination has been associated with the prognosis of colorectal, lung, breast, pancreatic, hepatocellular carcinoma, and cervical cancer.[20]

One study stated that the NLR was slightly higher in the bone metastatic disease group. However, the NLR with bone metastases accompanied by other organ metastases was also significantly higher compared with normal patients. The neutrophil count was higher and the lymphocyte count was lower in patients with bone metastases and other organ metastases. On the other hand, lymphocytes are important in providing anti-tumor immunity. Increased lymphocytes have also been reported to be a good prognostic indicator in patients with colorectal cancer, breast cancer, and melanoma.[20]

Previous studies show that there is an increased risk of breast cancer with an increase in NLR, which is a calculation of the division of the number of neutrophils by lymphocytes (multivariate OR from the highest versus lowest category (IK95=1.93 (1.26-2.97), p- trend <0.001). Furthermore, the study also analyzed NLR with several subtypes of breast cancer, such as luminal A and Her-2 negative which showed a relationship with the multivariable OR value for the highest category (IK95=2.00 (1.17- 3.45), p-trend <0.001) and (IK95=1.87 (1.16-3.02), p-trend <0.001).[21]

A report evaluating TNBC specifically was also presented by Jia in 2015 where it was reported that high NLR was associated with increased mortality in TNBC with an NLR cutoff value of 2.0.[7] Other research on breast cancer that supports the effect of NLR on patient survivability has previously been reported showing that an increase in patient mortality rates is obtained at high NLR values reaching 5-7. An NLR value with a cut-off equal to 3 is a strong predictor of recurrence in breast cancer patients.[22] In line with previous studies, the Moldoveanu (2020) study showed that an increase in NLR with a value of more than 2.84 at the time of diagnosis was associated with a decrease in overall survival (OS) in TNBC patients (HR= 1.8; IK95= 1.023-3.176) compared with the group with low NLR. Furthermore, the study showed that the maximum NLR recorded in patients during this study showed a significant association with reduced OS (HR 10.76; IK95= 4.193-26.58) and progression-free survival (PFS) (HR=7 .49; IK95=3.279-16.48). This study also analyzed the NLR of patients who died and patients who were examined during the 24 months before death or loss-to-follow-up. There were differences in NLR that emerged in the group of patients who died and patients who had been alive for 18 months before the examination.[23]

Analysis of the relationship between NLR and OS in TNBC patients was also carried out univariately and multivariately. Based on a study conducted by Moldoveanu in 2020, it was found that fluctuations in NLR that occurred temporally were related to recurrence and patient survival. Furthermore, studies show that NLR is an independent significant predictor of OS and PFS in TNBC patients.[23]

In addition, the association between NLR and distant metastasis has been reported in several studies evaluating cervical cancer, endometrial cancer, ovarian cancer, and colorectal cancer.[3] Caliskan et al reported that the number of neutrophils was higher and the number of lymphocytes was lower in patients with bone metastases and other organ metastases.[20] In this study, NLR was found to be significantly associated with and increase the risk of metastasis. The cut point identified in this study is in line with Orditura et al, namely 3.026, which appears to be the optimal cut point for identifying the risk of metastasis. Previous studies showed that in patients with TNBC who experienced metastases, it was found that the most common location for metastases was the lungs (n=63; 30.7%), then bones (n=47; 22.9%), liver (n=37; 18%), and brain (n=22;10.7%). Based on the results of the study analysis, it was found that NLR >2.5 was significantly associated with mortality in TNBC patients (HR= 2.12; IK95=1.32-3.39). Furthermore, previous studies showed a significant difference in OS between NLR <2.5 and NLR >2.5 (6% vs 28%, p<0.001) at 2 years. A subgroup analysis of female patients who received chemotherapy alone found poor OS in patients with NLR >2.5 (8% vs 36%, p=0.001).[24]

Mean platelet volume (MPV) is often used to measure volumetric platelet size, which is considered a potential indicator of platelet reactivity and activation. Changes in MPV values have been reported in breast, lung, stomach, colon, and ovarian cancers. In addition to increasing size, thrombocytosis (increased platelet count) is also correlated with a worse prognosis in many cancers, such as breast, gastric, pancreatic, ovarian, and endometrial cancers.[25] Although several studies have shown that a large MPV value is a poor prognostic factor, in breast cancer and cases of bone and liver metastases, a small MPV value appears to be a significant metastatic prognostic factor.[26] In line with this study, a previous study conducted by Li in 2019 showed that lower MPV values were found in patients with breast cancer who had liver metastases compared to breast cancer patients without metastases. Furthermore, MPV was independently associated with the presence of liver

metastases. This study demonstrated the existence of MPV levels using menopausal status and TNBC subtype to stratify breast cancer patients. The findings in this study showed that there was a significant difference in MPV in postmenopausal TNBC patients based on liver metastasis status (9.3+1.6 fL vs 7.8+1.0 fL, p=0.003). In addition, this study also analyzed the prevalence of liver metastases calculated using MPV quartiles. This study found that the prevalence rates of liver metastases in Q1, Q2, Q3, and Q4 were 82.3% (93/113), 50.5% (52/103), 27.0% (30/111), and 36.4% (36/99). Furthermore, the prevalence rate of liver metastases in Q1 was significantly higher than in Q2, Q3, and Q4. The results of this study indicated that the prevalence of liver metastases decreased with increasing quartiles of MPV (p<0.001).[25]

The previous study also showed results that were similar to the results of other studies, where high pre-treatment MPV levels were a potential predictive factor and an independent prognostic factor. This was demonstrated through analysis in this study where it was found that the pre-treatment MPV levels of invasive breast cancer patients were significantly higher compared to the control group (8.65+0.98 vs 8.34+0.78, p=0.002) and preoperative MPV levels were significantly higher than those in the postoperative group in invasive breast cancer patients (8.65 ± 0.98 vs 8.44 ± 0.91, P = 0.042). Then, based on the results of univariate and multivariate analysis, it was found that MPV was a significant prognostic factor (p=0.035; HR= 1.86; IK95=1.06-3.25).[27]

Platelets that are activated by thrombin, which is a production component of malignant cells, are a component that plays an important role in tumor metastasis. Apart from playing a role in binding malignant cells that are transmitted through the blood to vascular endothelial cells, activated platelets release heparinase to help the migration of malignant cells toward the blood vessel walls and form metastases.[27]

The small value of MPV in cases of tumor or metastasis can be explained by the thrombogenesis induction effect of the tumor. Increased platelet production causes a decrease in the volume of platelets produced. By removing the tumor mass, the levels of platelets produced a return to normal so that the MPV value returns to normal.[28]

However, this study shows limitations, in that this research is a case-control study, which is a study design that cannot control and analyze the effects of confounding variables. In addition, this research is quite difficult in selecting a control group and cases that have comparable characteristics because there are several accompanying factors in the subjects that are difficult to control. This study was also only conducted at one health center located in Bali, so extrapolation of the data needs to be taken into account due to the lack of representation of the results of this research study. However, this research data can be considered as initial reference data regarding low TIL, PLR, NLR, and high MPV as risk factors for metastasis in TNBC patients.

CONCLUSION

Patients with metastases tend to have proportionally low TIL and high PLR. However, TILs were not independently associated with the risk of metastasis in TNBC. However, patients with metastases had higher PLR values and lower MPV values significantly increased the risk of metastasis in TNBC.

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CONFLICT OF INTEREST

None.

ETHICAL STATEMENT

This research has obtained a certificate of ethical clearance from the Ethics Commission of the Faculty of Medicine, Universitas Udayana number 3071/UN14/2/2/VII/14/LT/2022.

AUTHOR CONTRIBUTION

All authors contributed equally to this study

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