Original Research Article

Relationship between platelet-lymphocyte ratio and tumour infiltrating lymphocyte on metastatic in breast cancer patient

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ABSTRACT

Background: Platelet-lymphocyte ratio (PLR) is known associated with the prognosis of distant metastatic breast cancer. Tumor-infiltrating lymphocyte (TIL) in breast cancer also associated with the prognosis of distant metastatic breast cancer. In this study, we will examine the relationship between PLR and TIL, in association with the metastatic incidence in breast cancer.

Methods: This research is a retrospective, analytic, cross-sectional study. Data was taken from medical records of breast cancer patients at Sanglah general hospital. Samples were taken by nested sampling by selecting all breast cancer patients from the period of January 1st, 2017, to December 31st, 2018, which had complete medical record data, with total sample 211. The PLR and TIL were calculated and analyzed in relation to metastasis incidence of breast cancer.

Results: The sample characteristics were sorted by age, education, occupation, the area of origin, menstrual status, breast cancer staging, breast cancer subtype, TIL levels, lymphovascular invasion (LVI) status, metastatic status, and breast cancer grading. The data were analyzed to know the association of PLR, TIL, confounding factors in relation to metastatic incidences. In the sample group with PLR $\geq$ 156 10μ /μL, there were 22.9% cases of metastases (p = 0.002). The sample group at low TIL had metastatic event 12.5% with (p=0.442).

Conclusions: PLR was associated with higher metastasis in breast cancer patients and low TIL had no association with breast cancer metastasis.

Keywords: Breast cancer, Metastasis, PLR, TIL

INTRODUCTION

Breast cancer is in the 5th place that caused death in the world (627.000 deaths) after lung cancer (1,76 million deaths), colorectal cancer (862.000 deaths), gastric cancer (783.000 deaths), liver cancer (782.000 deaths).[1,2] Development of breast cancer depends on the cancer's microenvironment, and the microenvironment of cancer very supported the development and transmission of breast cancer.[3] The microenvironment in breast cancer induced patient's inflammation responses, It has important role in carcinogenic process and the progressivity of cancer, which cancer progressivity and prognosis is influenced by body's inflammation responses in tumor microenvironment.[4] Breast cancer metastasis can happen in all organs, the most common is

the transmission to the bone and viscera and the other place with the small amount of tissue (example: brain, lungs).[5]

The cancer that had been metastasized to the other place define the outcome of the patient whereas the patient with cancer that had been metastasized have a poor prognosis.[6] Because it is said that if it had been metastasized far away, cancer cannot be able to heal and the average survival age of the patient is about 2-3 years.[7] The systemic inflammatory response components will increase such as a lymphocyte, neutrophil, and platelet, it is known to have an important role in carcinogenesis and tumor progressivity.[8-11] Nowadays, platelet is known to have an important role in the tumor transmission which the platelet in the circulation will increase the survival ability of the tumor by enfolding the tumor and make it be unknown by the immune system.[12,13] Platelet lymphocyte ratio (PLR) is the ratio of the platelet and lymphocyte has known as one of the inflammatory response and had been studied much and closely related with the prognostic of the breast cancer.[9-14] The higher PLR related to poor prognostic and the outcome of cancer will getting worse.[15,16]

Different with the platelet, lymphocyte infiltration is known to made a better prognosis in various cancer patients, in this case, tumor infiltrating lymphocyte (TIL) in breast cancer is one of the body's response to cancer whereas the TIL will increase when cancer happen which is the mark of the body that against the cancer cell.[17,18] If the lymphocyte infiltration (CD8+cell) is increasing, the breast cancer patient tends to have a better prognosis. In some of the study, the increasing of PLR and the decreasing of TIL is used as the prognostic indicator of breast cancer patient.[9,19] In this study, authors want to see about the relationship between the increasing of PLR and the decreasing of TIL in breast cancer metastasis, with the assumption if there is a metastasis, breast cancer patients will have poor prognosis.[20]

METHODS

This study is an analytic, retrospective, cross-sectional study to know about the relationship of platelet lymphocyte ratio (PLR) and tumor infiltrating lymphocyte (TIL), to the metastasis risk in the breast cancer patient. This study is done in Sanglah hospital Denpasar in January 2019. This study is in the general surgery area, especially surgical oncology division, faculty of medicine Udayana University in Sanglah general hospital.

The samples are taken by nested sampling, which is by choosing all the breast cancer patients in the period of 1st January 2017 until 31st December 2018 which had complete medical record data. The sample that has been taken must fulfill the inclusion and exclusion criteria in the group of metastasis patients and nonmetastatic patients. The data is taken from the breast cancer medical record which fulfills the inclusion and exclusion criteria in Sanglah general hospital Denpasar. The number of the sample that had been found is 211 patients. The register of PLR is taken from the examination before the biopsy, and TIL is taken from the histopathology. The filling of the data in the questionnaire was done by searching the medical record data of the patient.

The data that had been collected is processed by computer using SPSS v.17. The analysis include descriptive analysis, defining the PLR cutoff point number with ROC curve, bivariate analysis to know about the relationship of PLR number to the metastasis and TIL number to the metastasis, and multivariate analysis using the logistic regression to control the confounding variable to the relationship between PLR and TIL to the metastasis.

RESULTS

The sample of the study had been data analysis tested by the age variable, age characteristic in the normal distribution study sample that is $p > 0.05$ in the Kolmogorov-Smirnov test, so mean is used as the concentration measure and standard deviation (SD) as the spreading measure. In (Table 1) authors showed the characteristic in present study, average numbers of each data is found: age 48.6±9.8 years old, the most breast cancer incident is in level of education of senior high school about 68 patients (32.2%), most occupation which has breast cancer is housewife about 91 patients (43.1%), most patients are from Bali regency about 184 patients (87.2%), most patients are in premenopausal state about 113 patient (53.6%), most cases are in stage III about 91 patients (44%), most subtype is B luminal about 89 patients (42.2%), patients with mild TIL is the most number about 37 patients (72.6%), numbers of breast cancer with negative LVI is the most cases about 137 patients (64.9%) (Table 1).

In the sample with metastasis is about 29 patients (13.7%) and nonmetastatic is 182 patients (86.3%), with the metastasis spreading as the table below. And for the most incident is in the high grade about 198 patients (93.8%).

Before doing the bivariate analysis, the PLR variable is grouped first. The basic of the PLR group is one point that is called cut off point which is defined by using the ROC curve. The best cut off point is taken ≥156 (Figure 1).

In the sample group with PLR ≥156 10μ/μL, metastasis is found about 22.9% and nonmetastasis is about 77.1%, meanwhile, in sample group with the PLR <156 10μ/μL, nonmetastasis is found about 92.2% and metastasis is about 7.8%. Patient with PLR ≥156 10μ/μL has 3.5 times chances to be metastasis compared to the patient with PLR <156 10μ/μL and present study showed only PLR was significant relationship with metastasis ($p<0.002$). If

authors review it from the sample data validity in the population, where they believe interval is 1.573-7. 985 shown that this situation has happened in the population (Table 2). Sample group in low TIL have 0.72 times chance to metastasis compared to the high TIL patient group and that result is not significant statistically (p>0,05). Low TIL group include the negative TIL and mild positive TIL while the high TIL group covers the moderate positive TIL and high positive TIL. In low TIL group, only 12.5 % were had metastasis. Confounding factor (age, subtype, grade, LVI) also has been analized by bivariate analysis our data shown confounding factor are not significant statistically (Table 2).

After doing the bivariate analysis in the independent and dependent variable, it will continue to do the multivariate analysis to compare the relationship of independent variable and confounding variable to the dependent variable. It is done to control the confounding variable by analysis so the result of the conclusion is absolute without the impact of the confounding variable, in the multivariate analysis, the writer only includes the variable with p < 0.25 which is PRL and LVI variable, it is done so the analysis result will be more directed. (Table 2).

Table 1: The subject characteristic in the study.

Variable		n =211
Aged average±SD		48.64±(9,8)
Education	Primary school	64 (30.3%)
	Junior high school	19 (9%)
	Senior high school	68 (32.2%)
	Bachelor's degree	57(27.1%)
	Master's degree	3 (1.4%)
Occupation	Midwife	1 (0.5%)
	Teacher	7 (3.3%)
	Housewife	91 (43.1%)
	Merchant	18 (8.5%)
	Civil servant retired	2 (0.9%)
	Farmer	18 (8.5%)
	Civil servant	15 (7.1%)
	Private employee	23 (10.9%)
	Waitress	1 (0.5%)
	Doctor	3 (1.4%)
	Laborer	1 (0.5%)
	Lecturer	1 (0.5%)
	Entrepreneur	30 (14.2%)
Origin	Bali	184 (87.2%)
	Outside Bali	27 (12.8%)
Menstruation state	Premenopausal	113 (53.6%)
	Post menopause	98 (46.4%)
Stadium	I	4 (1.9%)
	II	86 (40.8%)
	III	91 (44%)
	IV	28 (13.3%)
Subtype	Luminal A	55 (26.1%)
	Luminal B	89 (42.2%)
	HER2 type	36 (17.1%)
	TNBC	31 (14.7%)
TIL	High	67 (31.8%)
	Low	144(68.2%)
LVI	Negative	137(64.9%)
	Positive	74 (35.1%)
Metastasis status	Metastasis	29 (13.7%)
	Non-metastasis	182(86.3%)
Grading	High grade	198(93.8%)
	Low grade	13 (6.2%)

Table 2: Relationship between PLR, TIL, age, subtype, grading, and LVI to the metastasis.

Variable		Metastasis		Odds ratio (OR)	95% CI	p
		Yes	No			
PLR	≥156	19 (22.9%)	64 (77.1%)	3.5	1.537-7.985	0.002*
	<156	10 (7.8%)	118 (92.2%)			
TIL	Low	18 (12.5%)	126 (87.5%)	0.727	0.322-1.641	0.442
	High	11 (16.4%)	56 (83.6%)			
Age	≥45 yo	19 (14.2%)	115 (85.8%)	1.107	0.486-2,520	0.809
	<45 yo	10 (13%)	67 (87%)			
Subtype	TNBC	6 (19.4%)	25 (80.6%)	0.967	0.351- 2.665	
	HER2	5 (13.9%)	31 (86.1%)	1.106	0.322-3.789	0.815
	B luminal	11 (12.4%)	78 (86.6%)	1.646	0.499-5.425	
	A Luminal	7 (12.7%)	48 (87.3%)			
Grade	High	27 (13.6%)	171 (86.4%)	0.868	0.182-4.134	0.859
	Low	2 (15.4%)	11 (84.6%)			
LVI	Positive	13 (17.6%)	61 (82.4%)	1.612	0.729-3.565	0.236*
	Negative	16 (11.7%)	121 (88.3%)			

* p <0.25 is considered significant to do the multivariate analysis.
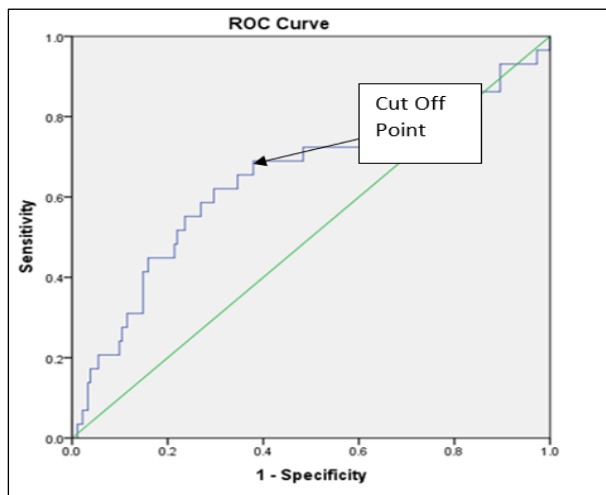
Figure 1: PLR cut off point based on ROC curve.

After doing data analysis with the independent and dependent variable and confounding variable, it is found that factor that correlates to the metastasis only PLR with the adjusted odds ratio 3.468 and significant statistically after considering the other variable such as age, subtype, histopathology grading, and LVI.

Table 3: Multivariate analysis result, Vorrelation PLR, TIL, with metastasis after controlling the age, subtype, histopathology grading and LVI variable.

Variable	Adjusted odds ratio	95%CI	P
PLR	3.468	1.518-7.924	0.003
LVI	1.566	0.694-3.534	0.280

Independent variable (TIL) and confounding variable (age, subtype, grading, and LVI) are not related to the metastasis (Table 3).

DISCUSSION

The relationship between confounding variable with metastasis

In this study result, age group more than 45 years old, metastasis is found about 14.2 %, meanwhile in age 45 years old or less group metastasis only about 13%. Most of the sample is in the age group of 45 years old or less. It may because of a very small difference between both age group to the metastasis. In a cohort which correlates between age when it diagnosed to the metastasis, the conclusion is that older the age so it is lower the metastasis risk, but if there is a metastasis so the mortality risk will be higher. The increasing of the age can be a protection factor to metastasis development. It can be related to the composition and remodeling of the extracellular matrix. Both factors are very important in tumorigenesis and metastasis progression. Aging process (increasing of age) can disturb the extracellular matrix by decreasing the matrix so the metastasis progression can be decreasing.[5]

Based on subtype breast cancer group, most metastases happen in the TNBC group (19.4%) compared to the other group, but it is meaningless statistically. It is fit the study before that there is no relationship between subtype to the metastasis nodule.[21]

In grading group, each grades the percentage mostly pointing to no metastasis about 86.4% so in this case there is no relationship between grade and metastasis.

Other study said that every cancer grading can cause metastasis, but especially is said that grading is correlated to the cancer recurrence speed, and spreading location.[22,23]

The percentage of metastasis in LVI positive group is about 17.6% and in the LVI negative group about 11.7%. So, in this case, LVI is not correlated to the metastasis. In a study explain the relationship between LVI and tumor development. Gene expression disturbance in the cytokine receptor pathway can cause the angiogenesis which in pathology is shown by LVI. Besides that, it also found that extracellular matrix components such as collagen are correlated to the LVI. Collagen is a matrix extracellular protein which has an important role in cell adhesion. In that study, the relationship between extracellular matrix receptors pathway and collagen alpha-1 (IV) chain (COL4A1 (and collagen alpha-1 (XI) chain (COL11A1). Some of the other extracellular matrix such as MMP-2 which involved in tumor progressivity and lymphoid nodule metastasis from gastric cancer and MMP-9 which involved in neovascularization and angiogenesis process. In conclusion, the imbalance between extracellular matrix and the tumor microenvironment can induce the LVI.[19]

Relationship between PLR with metastasis

Data from the study before showed that the increasing of PLR correlate to the prognostic indicator, so it makes the researcher want to study is there any relationship between the increase of PLR to the metastasis.[9,14]

Metastasis is a variable which is affected by PLR. In this study, metastasis is found in 22,9% patients with PLR level ≥ 156 and this result is significant statistically. If authors review it from the validity in the population, where they believe interval is 1.537-7.985, showed that this situation is really happening in the population. It is fit our hypothesis that the increasing of PLR is correlated to the metastasis.

All this time authors know that the role of the platelet is to keep the integrity of the blood barrier and hemostasis, in fact, platelet also present at the cell surface molecule and secrete some of the protein, nucleotide, and lipid bioactive which also increased the inflammation and cancer cell development. The secondary development of far metastasis of the primary tumor involving complex mechanism starts from the cell adhesion changes, survival ability, invasion and access to the blood or lymphatic vessels which cause the tumor cell spread systemically.[24]

NKC and cytotoxic cell have an important role in solving the CLC threat in the circulation, but the ability will be inhibited by the platelet clot surrounding the CTC. In this situation, it's already explained that platelet or CTC will secrete tissue factor (TP), thrombin and ADP that will induce the formation and activated "platelet-cancer cells

aggregate" that will protect the CTC from rips and the body immune attack. As the function as CTC protector, platelet function also as a paracrine which will press the activation of NK-mediated cytolytic. In particular, transforming growth factor-β (TGF-β) which is secreted by active platelet will neutralize the character of NK mobilization, the toxicity, and interferon-γ secretion. Lymphocyte has a different character with platelet whereas lymphocyte will increasing cancer patient's immune surveillance, and a hypothesis said it will press the tumor maturation.[25] The increasing of CD8+ cytotoxic intra tumoral in breast cancer will suppress the recurrence number and the higher survival outcome.[26] A study which involved 1435 breast cancer patient in Malaysia measure PLR in the patient which never get any treatment (cut off 185) and get a significant relationship between PLR and bad prognosis in the patient.[9] But in this study, PLR result is different from the study before, in the study before with age average 49 get a higher PLR cut off which is 215.[9] It hasn't fully understood yet but based on the study before showed that Chinese have higher PLR than people in India, so ethnicity factor may be influenced the PLR number.[14]

Relationship between TIL with metastasis

TIL is one of the independent variables which is considered to have a relationship with metastasis, in the hypothesis before it is explained that the increasing of TIL is correlated to the low of metastasis. But after through the analysis process, the result is that the relationship is not significant. In the low TIL group, only 12.5% metastasis is found. The possibility of the local condition in primary tumor not always present the systemic condition. The spread of the tumor or the metastasis process needs systemic factors which can facilitate it such as neutrophil, meanwhile, TIL is a presentation of the local condition of a tumor. In a meta-analysis study, it is not proven that TIL is a prognosis factor in the breast cancer patient. But TIL can give advantages to the TNBC survival patient.[27]

The other study said that the condition of each type of cancer is affected by the immunogenic and nonimmunogenic character. Even breast cancer is grouped in the nonimmunogenic group, but breast cancer has multiple differential gene expression characteristics. As a proof of some type (TNBC) showed a high expression to the gene effectivity in the pathway which is involved the present of TIL and tumor microenvironment and the genetic instability which cause the high mutation level.[28]

This study found a non-significant result in TIL group with the relationship to metastasis, probably because of the reason that TIL is more express to the special group (TNBC) meanwhile looking to the spreading of the sample which the TNBC group only 14.7% from the total sample.[29]

TIL examination in biopsy histopathology result probably cannot present that TIL that is counted is the intratumor TIL or just stroma or the combination of both in detail. Intratumor TIL is placed in a collection or localized and direct contact with the tumor cell, meanwhile, stroma TIL is spreading in stroma and not direct contact with the tumor cell. Lymphocyte which direct contact with the tumor cell (intra tumoral TIL) is considered as the most representative. But the H and E-stained intratumor TIL examination is difficult to evaluate, so in the study, it is found that stroma TIL is more than intratumor TIL. This is one of the things that can be the lack of this study, but this lack of study can be solved by including the IHK/IHC data to support the results. But, in another study, CD3-immunohistochemistry or CD8-immunohistochemistry is used to examine the TIL and intra tumoral TIL to make it easier to identify as stroma TIL.[18,30]

The relationship between PLR, TIL, with metastasis after the age, subtype, histopathology grading and LVI variable analysis

Based on the multivariate regression analysis PLR is found to be the only variable that has a relationship to metastasis that is significant statistically. Even though, further multicenter study is needed with the homogenization of the sample and examine each variable to prove that another variable is not correlated to metastasis, which the data of each TIL group, age, grading, and LVI is uneven. This thing maybe can affect the validation of the data.[19,31,32]

CONCLUSION

Based on the study results above, authors can conclude something, such as there is a relationship between high PLR with higher metastasis in the breast cancer patient and there is no relationship between low TIL with breast cancer metastasis. Confounding factor in this study, after the analysis, is that there is no relationship with breast cancer metastasis.

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