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Role of neutrophil lymphocyte ratio in predicting lymphovascular invasion in carcinoma breast

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Abstract
Introduction: Inflammation is a protective response which involves immune cells, blood vessels, and molecular mediators. Neutrophils and lymphocytes help in healing and regeneration of damaged tissues and also fight infections. Neutrophil to lymphocyte ratio (NLR) is used as a pointer or marker for inflammation both clinical and subclinical. The presence of lymphovascular invasion (LVI) is understood as or points to increased risk of axillary lymph node and distant metastases.
Aims and Objectives: To compare neutrophil to lymphocyte ratio [NLR] with lymphovascular invasion in carcinoma breast.
Methodology: A descriptive study was conducted at JSS Hospital Mysuru over a period of two years comprising of 100 patients diagnosed as carcinoma breast and results were drawn. All demographic data was tabulated and measured as proportion, mean, standard deviation and the inferential statistics were obtained using Chi-square and Cramer's V tests.
Results: 65% of carcinoma breast cases in our study had lymphovascular invasion. The percentage of lymphovascular invasion increased with increase in NLR progressively with 22.2 percent in category A to 91.8 percent in category D.
Conclusion: Increase in neutrophil to lymphocyte ratio is associated with increased incidence of lymphovascular invasion in carcinoma breast.

Keywords: Neutrophils, Lymphocytes, Neutrophil Lymphocyte ratio (NLR) Lymphovascular invasion (LVI), Carcinoma breast

Introduction

Inflammation is derived from Latin word "inflammation" which is a part of the complex biological response of cells and tissues of body to advert stimuli, such as microbes, pathogens, foreign cells, or irritants, [1] and is a protective response which involves immune cells, blood vessels, and molecular mediators. The cells of the immune system are of several types as lymphocytes (T-cells, B-cells and NK cells), neutrophils, and monocytes/macrophages. These are all types of WBC's.

Neutrophils are a type of white blood cell that helps in healing and regeneration of damaged tissues and also fight infections. Blood levels of neutrophils increase naturally in response to infections, injuries, and other types of stress.

Lymphocytes are also one of the subtypes of a white blood cell in our immune system. Lymphocytes comprises of T cells, Natural killer cells and B cells (for humoral, antibody-driven adaptive immunity) which are mostly present in lymph, hence the name "lymphocyte".

Neutrophil to lymphocyte ratio (NLR) is used as a pointer or marker for inflammation both clinical and subclinical. It is calculated by dividing the number of neutrophils by number of lymphocytes, usually from peripheral blood sample but sometimes also from cells that infiltrate tissue, such as tumor [2].

Lymphovascular invasion (LVI) is described as the presence of tumor cells within a definite endothelial-lined space (lymphatics or blood vessels) in the breast surrounding the actual invasive lump. The presence of this is understood as or points to increased risk of axillary lymph node and distant metastases [3].

It is well known that systemic inflammatory factors play a significant role in progression of cancer [4, 5]. The micro-environment of the tumour regulated by these cells is clearly related to

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Cancer progression. Systemic inflammatory factors are reflected by changes of peripheral leukocyte, lymphocytes, neutrophils, monocytes and platelets and can reflect the tumor micro environment indirectly in cancer patients.

Breast cancer is a heterogeneous complex of diseases that has different subtypes which have different treatment responses and outcomes clinically.

Tumor characteristics and host response, including the inflammatory response, both contribute to the clinical outcomes of cancer patients [6]. Histopathological analysis of tumors has shown tumor infiltration by inflammatory and lymphocytic cells.

Objectives
Primary Objective
To compare neutrophil to lymphocyte [NLR] with lymphovascular invasion in carcinoma breast.

Methodology
a.   **Study Design:** Descriptive Study
b.   **Study place:** JSS Hospital Department of Surgery
c.   **Study Duration:** January 2017 to December 2019
d.   **Sample size:** Sample Size is considered as 100
e.   **Sampling technique and study population:** Convenient sample. All consecutive cases coming to surgical department.
f.   **Inclusion *criteria***
     All proven cases of Carcinoma Breast who have under gone modified radical mastectomy.

g.   **Exclusion *criteria***
1.   Benign breast disease
2.   Patients with bleeding diathesis
3.   Patients unwilling for modified radical mastectomy
4.   Patients not willing to be part of study.

Study Assessments of end points - To assess lymphovascular invasion in carcinoma breast and compare the same with NLR.

Statistical methods applied
Data Analysis
All demographic data is summarized as proportion, mean, median, standard deviation and tabulated. All the measurements are done using SPSS version 21 and EPI-Info version 7

Descriptive statistics
The Descriptive procedure displays univariate summary statistics for several variables in a single table and calculates standardized values (z scores). Variables can be ordered by the size of their means (in ascending or descending order), alphabetically, or by the order in which the researcher specifies. Descriptive statistics included
Mean,
Median,
Standard deviation,
Frequency and
Percent

Inferential statistics
Chi-Square Test
The Chi-Square Test procedure tabulates a variable into categories and computes a chi-square statistic. This goodness-of-fit test compares the observed and expected frequencies in each category to test either that all categories contain the same

proportion of values or that each category contains a user-specified proportion of values.

Crosstabs (Cramer's V)
The Crosstabs procedure forms two-way and multiway tables and provides a variety of tests and measures of association for two-way tables. The structure of the table and whether categories are ordered determine what test or measure to use. Cramer's V test was employed in the present study.

Results
Neutrophil lymphocyte ratio [NLR] was obtained for all carcinoma breast cases and a median of 2.03 was taken for all values, the lowest value being 1.27 and maximum being 3.34. Based on this various categories of NLR were formed in this study-
1. Category A having NLR of less than equal to 1.5
2. Category B having NLR of 1.51-2.0
3. Category C having NLR of 2.01-2.5
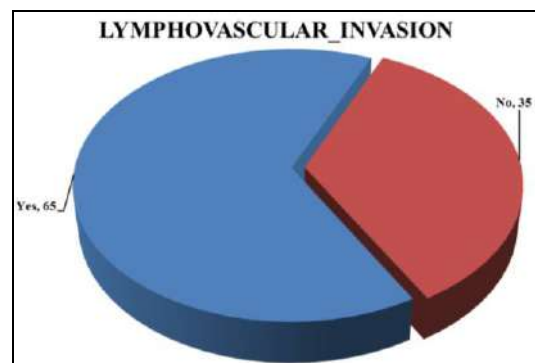4. Category D having NLR of more than 2.5

Platelet count was also taken into account in the study and a median value of 2.52 was obtained and accordingly various categories of platelets were formed
1. Category X- 1 to 2 lakhs/cumm
2. Category Y-2 to 2.52 lakhs/cumm
3. Category Z- more than 2.52 lakhs /cumm

Table 1: Platelets VS NLR

			NLR				Total
			A	**B**	**C**	**D**	
platelets	X	Count	1	0	1	2	4
		% within NLR	3.7%	0.0%	6.7%	4.1%	4.0%
	Y	Count	8	3	3	7	21
		% within NLR	29.6%	33.3%	20.0%	14.3%	21.0%
	Z	Count	18	6	11	40	75
		% within NLR	66.7%	66.7%	73.3%	81.6%	75.0%
Total		Count	27	9	15	49	100
		% within NLR	100.0%	100.0%	100.0%	100.0%	100.0%

In our study it was observed that a lower NLR eg in Category A -66.7 percent cases had value of platelets more than 2.5 lakhs/c mm, in category B this was again 66.7 percent, in category C it increased to 73.3 percent whereas in category D this further increased to 81.6 percent.

Pie Chart 1: Lymphovascular invasion

Out of a total 100 cases of carcinoma breast lymphovascular invasion was seen in 65 case while in 35 cases there was no lymphovascular invasion

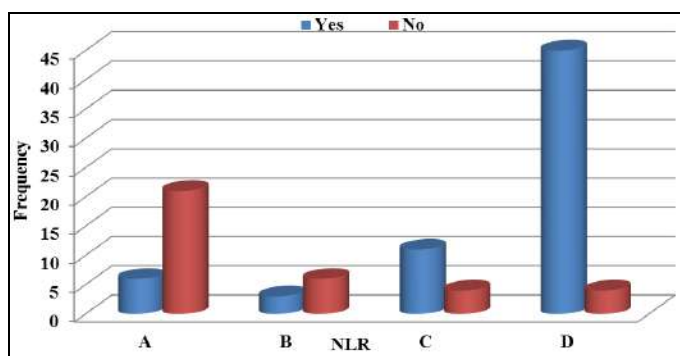**Table 2:** Lymphovascular invasion VS NLR

			NLR				Total
			A	**B**	**C**	**D**	
Lymphovascular invasion	Yes	Count	6	3	11	45	65
		% within NLR	22.2%	33.3%	73.3%	91.8%	65.0%
	No	Count	21	6	4	4	35
		% within NLR	77.8%	66.7%	26.7%	8.2%	35.0%
Total		Count	27	9	15	49	100
		% within NLR	100.0%	100.0%	100.0%	100.0%	100.0%

In category A i.e. NLR less than or equal to 1.5 Lymphovascular invasion was present in 22.2% cases whereas in 77.8% cases there was no lymphovascular invasion

In category B 33.3% cases had lymphovascular invasion while in 66.7% cases there was no lymphovascular invasion

In category C 73.7% cases had lymphovascular invasion while 26.7 percent cases of carcinoma breast had no lymphovascular invasion.

In category D 91.8% cases had lymphovascular invasion whereas in 8.2% cases there was no lymphovascular invasion.

Fig 1: Bar diagram – NLR VS Lymphovascular invasion

Discussion

The NLR, obtained by the number of neutrophils and lymphocytes, is frequently used as a tool in evaluating systemic inflammation. Many studies have corroborated that NLR is related with the prognosis in breast, colorectal, gastric, pancreas, hepatocellular, and renal cancers which is evident by the fact that neutrophils promote tumor cell proliferation, angiogenesis, and distant metastasis. Lymphocytes also play a central role in the immune response to various tumor cells. This leads one to predict that NLR is associated with prognosis [7, 8, 9].

Platelets are essential components of hemostasis and also play a pivotal role in the tumor microenvironment. Platelet count has been reported to have predictive value in different cancer types.

Increased risk of thrombosis as well as increased platelet activation has been observed particularly in women with breast cancer, but until recently, these clinical observations have been considered a paraneoplastic phenomenon [10, 11, 12, 13, 14].

Thrombocytosis can be an early indicator of cancer as a generalized entity which is evident by the fact that in women with ovarian adenocarcinoma prevalence of thrombocytosis (average platelet count=558×106/l) at the time of diagnosis is more. More importantly, paraneoplastic thrombocytosis in these patients is found to be associated with an advanced stage and shortened survival [15].

However according to some studies, preoperative thrombocytosis is not a prognostic indicator of survival in colorectal cancer [16].

The literature on thrombocytosis and breast cancer is limited and has high controversies. In breast cancer particularly high interleukin-6 (IL-6) levels and platelet counts have been reported, in patients with metastatic breast cancer as compared to those with localized disease [17].

"A recent publication has shed some light to the pathogenesis of thrombocytosis in cancer [8] and confirmed previous reports on the role of cytokines and in particular of IL-6." [18]

According to another study "Thrombocytosis (more than 400 × 109/L) was evaluated as a prognostic factor in an extensive series of 1513 patients with localized colorectal cancer that had undergone surgery" [19] which signified its importance in predicting the prognosis.

In our study 100 cases of carcinoma breast were included in the study and modified radical mastectomy is done for all cases as per standard guidelines. After the surgery all the specimens were subjected to histopathology and presence of lymphovacular invasion was noted for all cases which was compared to various categories of NLR formed considering 2.03 as median value.

65 percent of cases had lymphovascular invasion, the results were tabulated and compared. Lymphovascular invasion progressively increased from 22.2 percent in category A to 33.3 percent in category B to 73.7 percent in category C to 91.8 percent in category D. A value of more than the median value i.e. NLR more than 2.03 was found to be significantly associated with lymphovascular invasion with a p value of 0.03.

Also it was noted in the study that increase in number of platelets is associated with a corresponding increase in NLR, thereby making thrombocytosis a significant marker of lymphovascular invasion.

Conclusion
- Increase in platelets is observed in with an increase in NLR.
- Thrombocytosis is a marker of lymphovascular invasion.
- Increase in NLR signifies lymphovascular invasion and thus an advance stage of carcinoma breast.

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