Original Research Article

Role of pre-treatment neutrophil to lymphocyte ratio as a diagnostic marker of malignancy in breast carcinoma patients: a study in a tertiary care centre

Shruti Srivastava, Chandrashekhar Indoria*

Department of Pathology, Govt. Medical College, Rajnandgaon, Chhattisgarh, India

Received: 22 October 2018
Accepted: 26 October 2018

*Correspondence:
Dr. Chandrashekhar Indoria,
E-mail: dr.csindoria@gmail.com

ABSTRACT

Background: Inflammation is closely associated with cancer. Inflammation maintains and promotes cancer growth by causing tumor tissue remodelling and angiogenesis. It also helps in metastasis and helps tumor cells to survive by suppressing anti-cancer innate immune response. Recent studies have suggested use of Neutrophil lymphocyte ratio (NLR) for evaluation of systemic inflammation. Present study was undertaken with an aim to compare NLR among patients with malignant breast neoplasm with and without metastasis.
Methods: This was a cross sectional observational study comprising of 179 women with breast neoplasm. Patients were categorized into two subgroups of benign proliferative breast mass and malignant breast mass (with or without metastasis). Data was collected from pre intervention complete blood count reports of all patients. To obtain NLR values neutrophil count were divided by lymphocyte counts. A comparison of NLR values in different subgroups of the patients was performed.
Results: Mean neutrophil values in benign and malignant subgroups were 64.02±7.9 and 70.75±9.2 respectively. Mean lymphocyte count in benign and malignant subgroups was 27.527±7.565 and 20.220±7.354 respectively. Difference in mean values for neutrophils and lymphocytes was not significant in benign and malignant subgroups. Significant difference was present in values of NLR among benign and malignant subgroups, but within malignant subgroup no significant difference was present among patients with or without metastasis.
Conclusions: Present study findings supported that pre-intervention Neutrophil Lymphocyte ratio is a promising biomarker of malignancy.

Keywords: Breast carcinoma, Benign, Neutrophil, Lymphocyte, Ratio

INTRODUCTION

Breast cancer is the most common cancer in women all over the world representing about 12% of all new cancer and 20% of all cancer in women.[1] Earlier breast cancer was thought to be more common in developed countries but now it is the most common cancer among Indian woman.[2] Inflammation is closely associated with cancer. Inflammation maintains and promotes cancer growth by causing tumor tissue remodelling and angiogenesis. It also helps in metastasis and helps tumor cells to survive by suppressing anti-cancer innate immune response.[3,4]

Many studies have supported role of Glasgow prognostic score (GPS) / modified Glasgow prognostic system (mGPS) as a prognostic indicator for malignancy. It is based on C-reactive protein and albumin levels, which are markers of inflammation. Recent studies have suggested use of Neutrophil lymphocyte ratio (NLR) instead of GPS/ mGPS for evaluation of systemic

inflammation. Many studies have indicated that a high neutrophil-to-lymphocyte ratio (NLR) can be considered as a prognostic indicator for breast cancer.[5,6]

Axillary lymph node status and histologic tumor grades are independent prognostic factors. Patients with lower histologic tumor grade and no axillary lymph node metastasis show better prognosis.[7] Advanced stages of cancer are associated with comparatively more inflammation and higher NLR can be used as a predictor for this. Therefore, present study was undertaken with an aim to compare NLR among patients with malignant breast neoplasm with and without metastasis. A comparison with NLR values in patients with benign breast neoplasm was also done.

METHODS

This was a cross sectional observational study conducted from June 2014 to May 2015. Data was collected from 179 women with breast neoplasm. The study had approval of institutional scientific committee. The study included patients presenting with breast mass. After preliminary cytological investigations patients were categorized into two subgroups of benign proliferative breast mass and malignant breast mass. Patients in malignant breast tumor mass group were further categorized into two subgroups of patients on the basis of presence or absence of histologically proven lymph node metastasis. Detailed clinico-pathological profile of all patients was collected. Patients with any inflammatory disease other than breast neoplasm were excluded from the study. Patients with ductal carcinoma in situ with or without micro invasion were also excluded. Other exclusion criteria were patients with inflammatory and cystic disease of the breast, pre-existing hematologic disease, patients on immunosuppressive/ anti-inflammatory drugs and recent blood transfusion. Data was collected from pre-intervention complete blood count reports of all patients. For complete blood counts blood was collected in EDTA vacutainers. Analysis of blood was performed in five parts automated cell counter (Beckmen Coulter cell counter). To obtain NLR values neutrophil count were divided by lymphocyte counts. A comparison of NLR values in different subgroups of the patients was performed. For statistical analysis SPSS software was used.

RESULTS

Among women presenting with breast neoplasm preliminary screening was done according to exclusion criteria and 179 women were included in the study for evaluation of data. Patients were divided into various subgroups according to cytological categorization. Patients were categorized into malignant subgroup only after histological confirmation. Out of 179 patients, 98 (54.74%) had benign proliferative breast disease including Fibroadenoma. Malignant breast mass was confirmed in 81 (45.25%) patients after histology.

Among patients with malignant breast mass metastasis was present in 24 (29.62) patients while 57 (70.37%) patients showed no metastasis. (Table 1).

Table 1: Distribution of patients into various subgroups.

Sub group		No. of cases
Benign		98
Malignant	Without metastasis	57
	With metastasis	24
Total		179

Percentile Neutrophil counts ranged from 51 to 82 in benign subgroup and from 44 to 86 in malignant subgroup. Mean neutrophil values in benign and malignant subgroups were 64.02±7.9 and 70.75±9.2 respectively (Table 2).

Table 2: Neutrophil values in patient subgroups.

Sub group	Min. count	Max. count	Mean neutrophil count	SD	P value (IDC Vs Benign)
Benign	51	82	64.02	7.9	0.791
Malignant	44	86	70.75	9.2	

Table 3: Lymphocyte values in patient subgroups.

Sub group	Min. count	Max. count	Mean lymphocyte count	SD	P value (IDC Vs Benign)
Benign	12	41	27.527	7.565	0.905
Malignant	8	44	20.220	7.354	

Values for lymphocytes in patient subgroups are shown in (Table 3). In benign subgroup Percentile lymphocyte counts ranged from 12 to 41 and in malignant subgroup ranged from 8 to 44. Mean lymphocyte count in benign and malignant subgroups was 27.527±7.565 and 20.220±7.354 respectively (Table 3).

Difference in mean values for neutrophils and lymphocytes was not significant between benign and malignant subgroups. NLR ranged from 1.4 to 6.5 in benign subgroup and from 1.1 to 10.6 in malignant subgroup. Mean NLR in benign and malignant subgroup was 2.6±1.2 and 4.1±2.07 respectively (Table 4). Significant difference was present in values of NLR among benign and malignant subgroups. In malignant subgroup without metastasis NLR ranged from 1.1 to 10.6 and in subgroup with metastasis NLR range was from 1.7 to 9.3.

In malignant subgroup no significant difference was present among patients with or without metastasis. In normal adults the percentage population of neutrophils and lymphocytes in WBCs is approximately 50-60% and

30-40% respectively. This gives an NLR of approximately 2 for normal adults. Increase in NLR was considerable in both benign and malignant groups as compared to normal adults.

Table 4: Mean neutrophil lymphocyte ratio (NLR) in patient subgroups.

Sub group		Mean NLR	Standard deviation	P value (IDC Vs Benign)	P value (metastasis vs without metastasis)
Benign		2.6	1.2		
Malignant	Without metastasis	4.2	2.1	P <0.0001	P = 0.6887
	With metastasis	4.0	1.9		

Mean NLR value with standard deviation for malignant subgroup was- 4.1±2.07

DISCUSSION

Present study on analysis of NLR in 179 women with breast mass demonstrated that NLR was significantly high in women with malignant breast mass. Within malignant subgroup our findings demonstrated no significant difference between subgroups with or without metastasis. Present study findings of significant difference in NLR between benign and malignant subgroups were in concurrence with the findings of Ozyalvacli G et al. However, their study demonstrated a positive correlation of high NLR with metastasis. This correlation of NLR with axillary lymph node metastasis was not present in our study.[8]

Several studies have suggested association between the inflammation and cancer. Inflammation plays a dual role in progression and development of cancer. Tumors are infiltrated by leucocytes. Neutrophils help in growth and progression of malignancy, whereas lymphocytic response against tumor cells help to control tumor growth and progression.[9-11] These infiltrating lymphocytes include Natural killer and T helper cells and secrete interferon gamma. Interferon gamma plays an important role in immunomodulation and anticancer activities, including cancer cell growth inhibition, cytotoxicity, and MHC class I expression.[11] Lymphocytopenia decreases innate immune response. Recent studies have suggested decreased number of tumor infiltrating T cells is associated with poorer prognosis in cancers of Pancreas, Rectum and other organs.[12]

On review of literature authors were unable to find any definite mechanism for association of high NLR with breast cancers. Suggested hypothesis is of multifactorial association. One mechanism suggests that neutrophils inhibit the immune system by suppression of cytolytic activity of lymphocytes, NK cells and activated T cells and thereby alter immune response against tumor cells.[13-14] Neutrophils can promote metastasis by increasing secretion of various cytokines, chemokines, proteases and growth factors, including vascular endothelial growth factor, platelet-derived growth factor, fibroblast growth factor, matrix metalloproteinase and IL-6.[15-17] Overall effect of these mechanisms suggested is change in tumor microenvironment, helping in extracellular remodelling, endothelial cell migration and tumor cell dissociation leading to tumor growth and progression by angiogenesis and metastasis.

There are variable reports on association of NLR with different breast cancer subtypes.[18-21] Several studies have indicated that high neutrophil count has an independent prognostic value and is inversely associated with cancer free survival.[20] Several researchers have supported association of high NLR with clinicopathologic features.[20] Yersal O et al, did not found any association between NLR and clinicopathologic features.[21-22] Studies have suggested cut-off value of INR ranging from 2.2 to 3 as optimal threshold for survival.[23] Faria SS et al, in their narrative review have demonstrated that pretreatment NLR between 2 and 4 has shown an association with a significant increase in all-cause mortality and to a lesser extent with disease free survival.[20] Chen J, also suggested high NLR as a strong predictor for overall survival.[24]

In updated review and systemic meta-analysis Wei B et al, have concluded that NLR is a good prognostic marker for breast cancer and patients with a high NLR have poorer prognosis.[25]

In a systematic review and meta-analysis by Ethier JL et al, fifteen studies comprising a total of 8563 patients were included. The median cut-off value for high NLR used in these studies was 3.0 amongst 13 studies reporting a hazard ratio for overall survival, and 2.5 in 10 studies reporting Disease-free survival. NLR greater than the cut-off value was associated with worse OS.[26]

Pre-intervention neutrophil and lymphocyte count, especially when no other cause of inflammation is present, can be an indicator of cancer associated inflammation. These observations and hypothesis strongly support our result that an elevated NLR may be an indicator of malignancy. Present study results showed no significant difference between malignant tumor groups with or without metastasis and thus were not supporting

hypothesis of NLR being an indicator of advanced stage of tumor.

Present study had some limitations. It was a short-term observational study with a small sample size. Patients were not followed for long term to know prognosis of patients presenting with breast mass and high NLR. We suggest a prospective long-term study with larger sample size for studies on role of NLR as a predictive marker in breast neoplasia

CONCLUSION

Present study findings supported that pre-intervention neutrophil lymphocyte ratio is a promising biomarker of malignancy. Prognosis prediction is a part of effective treatment planning for any disease. Its validation as a prognostic biomarker will be helpful both for clinicians and patients due to its low cost, wider availability, and easy calculation.

ACKNOWLEDGEMENTS

REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015 Mar 1;136(5):E359-86.
2. Gupta S. Breast cancer. Indian experience, data, and evidence. South Asian J Cancer. 2016;5(3):85-6.
3. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002;420(6917):860-7.
4. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet. 2001;357(9255):539-45.
5. McMillan DC. The systemic inflammation-based Glasgow Prognostic Score: a decade of experience in patients with cancer. Cancer Treatment Reviews. 2013 Aug 1;39(5):534-40.
6. Guthrie GJ, Charles KA, Roxburgh CS, Horgan PG, McMillan DC, Clarke SJ. The systemic inflammation-based neutrophil–lymphocyte ratio: experience in patients with cancer. Critical Reviews Oncol Hematol. 2013 Oct 1;88(1):218-30.
7. Cianfrocca M, Goldstein LJ. Prognostic and predictive factors in early-stage breast cancer. Oncologist. 2004 Nov 1;9(6):606-16.
8. Ozyalvacli G, Yesil C, Kargi E, Kizildag B, Kilitci A, Yilmaz F. Diagnostic and prognostic importance of the neutrophil lymphocyte ratio in breast cancer.
Asian Pac J Cancer Prev. 2014 Jan 1;15(23):10363-6.
9. Galdiero MR, Varricchi G, Loffredo S, Mantovani A, Marone G. Roles of neutrophils in cancer growth and progression. J Leukocyte Biol. 2018 Mar;103(3):457-64.
10. Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. Immunity. 2004 Aug 1;21(2):137-48.
11. Lin CF, Lin CM, Lee KY, Wu SY, Feng PH, Chen KY, et al. Escape from IFN-γ-dependent immunosurveillance in tumorigenesis. J Biomed Sci. 2017 Dec;24(1):10.
12. Hadrup S, Donia M, thor Straten P. Effector CD4 and CD8 T cells and their role in the tumor microenvironment. Cancer Microenvironment. 2013 Aug 1;6(2):123-33.
13. El-Hag A, Clark RA. Immunosuppression by activated human neutrophils. Dependence on the myeloperoxidase system. J Immunol. 1987 Oct 1;139(7):2406-13.
14. Shau HY, Kim A. Suppression of lymphokine-activated killer induction by neutrophils. J Immunol. 1988 Dec 15;141(12):4395-402.
15. Di Carlo E, Forni G, Musiani P. Neutrophils in the antitumoral immune response. Chem Immunol Allergy. 2003;83:182-203.
16. Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. Carcinogenesis. 2009 Jul 1;30(7):1073-81.
17. Deryugina EI, Zajac E, Juncker-Jensen A, Kupriyanova TA, Welter L, Quigley JP. Tissue-infiltrating neutrophils constitute the major in vivo source of angiogenesis-inducing MMP-9 in the tumor microenvironment. Neoplasia. 2014 Oct 1;16(10):771-88.
18. Malorni L, Shetty PB, Dc Angelis C, Hilsenbeck S, Rimawi MF, Elledge R, et al Clinical and biologic features of triple-negative breast cancers in a large cohort of patients with long-term follow-up Breast Cancer Res Treatment. 2012;136(3):795-804.
19. Asano Y, Kashiwagi S, Onoda N, Noda S, Kawajiri H, Takashima T, et al. Predictive value of neutrophil/lymphocyte ratio for efficacy of preoperative chemotherapy in triple-negative breast cancer. Ann Surgical Oncol. 2016 Apr 1;23(4):1104-10.
20. Faria SS, Fernandes Jr PC, Silva MJ, Lima VC, Fontes W, Freitas-Junior R, et al. The neutrophil-to-lymphocyte ratio: a narrative review. Ecancer Med Sci. 2016;10.
21. Yersal Ö, Çetinkünar S, Aktimur R, Aziret M, Özdaş S, Erdem H, et al. Neutrophil/Lymphocyte and Platelet/Lymphocyte Ratios are Not Different among Breast Cancer Subtypes. Asian Pacific J Cancer Prevention. 2017;18(8):2227.

22. Ulas A, Avci N, Kos T, Cubukcu E, Olmez OF, Bulut N, et al. Are neutrophil/lymphocyte ratio and platelet/lymphocyte ratio associated with prognosis in patients with HER2-positive early breast cancer receiving adjuvant trastuzumab?. Lung Cancer. 2015;18:20.
23. Chae S, Kang KM, Kim HJ, Kang E, Park SY, Kim JH, et al. Neutrophil-lymphocyte ratio predicts response to chemotherapy in triple-negative breast cancer. Current Oncology. 2018;25(2):e113-9.
24. Chen J, Deng Q, Pan Y, He B, Ying H, Sun H, et al. Prognostic value of neutrophil-to-lymphocyte ratio in breast cancer. FEBS Open Bio. 2015 Jan 1;5(1):502-7.
25. Wei B, Yao M, Xing C, Wang W, Yao J, Hong Y, et al. The neutrophil lymphocyte ratio is associated with breast cancer prognosis: an updated systematic review and meta-analysis. Onco Targets Therapy. 2016;9:5567.
26. Ethier JL, Desautels D, Templeton A, Shah PS, Amir E. Prognostic role of neutrophil-to-lymphocyte ratio in breast cancer: a systematic review and meta-analysis. Breast Cancer Res. 2017 Dec;19(1):2.