## RESEARCH

# Peripheral Neutrophil to Lymphocyte Ratio (NLR), a cogent clinical adjunct for Ki-67 in breast cancer

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## Abstract

**Background**  Clinical utility of Ki-67 immunohistochemistry (IHC) in breast cancer (BC) is mainly limited to decide for the use of chemotherapy and estimate prognosis in patients with either Ki-67 index < 5% or > 30%; however, lacunae still exists pertaining to its analytical validity. Neutrophilia is common in cancer with accompanying lymphocytopenia. Neutrophil to lymphocyte ratio (NLR) captures the intricate balance between pro-tumor neutrophilia and anti-tumor lymphocyte immunity. This study aimed to correlate cellular proliferation in breast cancer with NLR.

**Methods**  An observational study was carried out including 73 cases of BC; pre-treatment NLR and Ki-67 grading were performed. NLR < 3 was considered low, while ≥ 3 was high. The Ki-67 expression was graded as low ≤ 5%, intermediate 6–29%, or high ≥ 30%. Various clinico-pathological variables were studied, and the association of categorical variables was analyzed using Pearson's chi-square test, and a *p*-value of < 0.05 was taken as significant.

**Results**  Ki-67 correlated significantly with modified Scarff-Bloom-Richardson (SBR) grade ($p < 0.01$), and tumor-node-metastasis (TNM) stage ($p < 0.001$). Correlation of NLR was not significant with SBR grade ($p > 0.05$) and molecular subtype ($p > 0.05$); however, NLR was found to be significantly correlated with TNM stage ($p < 0.001$) and Ki-67 ($p < 0.001$).

**Conclusion**  NLR is fast emerging as a personalized theranostic marker in breast cancer. Instead of determining a generalized cut-off value, individual baseline NLR and its dynamics with disease progression will help manage patients better, obviating some of the drawbacks associated with Ki-67.

**Keywords**  Ki-67, NLR, Breast cancer, Prognosis, Personalized biomarker

## Background

Ki-67 expression is a reliable predictor of proliferative activity of the tumor cells in colorectal, prostate, gastric, and other cancer types [1–4]. In addition, prognostic significance of Ki-67 expression has been documented, and presently systemic therapeutic strategies are being deduced from the expression levels of Ki-67 in certain malignancies [5]. In BC, Ki-67could signify responsiveness/ resistance to chemotherapy /endocrine therapy [6], estimate the residual risk in patients on standard therapy, and can also predict treatment effectiveness before, during, and after neoadjuvant therapy, especially neoadjuvant endocrine therapy [7, 8]. The 12th St. Gallen Expert Consensus panel in 2011 established that a Ki-67 expression < 14% in Estrogen Receptor (ER) positive, Human Epidermal Growth Factor Receptor 2 (Her 2/neu) negative patient represents the luminal A subtype, while ER positive Her 2 negative patients with Ki-67 expression > 14% represents the luminal B subtype [9]. However,

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in 2013, the St. Gallen consensus statement re-defined Ki-67 expression > 20% as the new threshold for classifying breast cancer luminal subtypes [10], since tumors with a higher Ki-67 expression were more likely to get benefitted from cytotoxic chemotherapy. Meanwhile, it was also seen that Ki-67 immuno-expression suffered from low reproducibility of results, principally in the intermediate immuno-expression group (ranging from 15 to 30%). So, in the last Saint Gallen Conference (March 2015), the consensus cut-off for Ki-67 had to be specifically made in the light of local laboratory reports [11].

Triple-negative breast cancer (TNBC) is an aggressive malignancy with poor prognosis and does not benefit from either targeted therapy or endocrine therapy [12]. Newer biomarkers are continuously being searched for better patient management; Ki-67 IHC is also being investigated as a possible predictive and prognostic biomarker following neoadjuvant chemotherapy (NAC) in patients with TNBC [13]. Generally, TNBC shows a higher baseline Ki-67 immuno-expression than the luminal tumors. A meta-analysis of 35 studies with 7716 enrolled patients concluded that a high Ki-67 of ≥ 40% is associated with a greater risk of recurrence and death compared with lower expression rates [14]. Therefore, currently, the role of Ki-67 in therapeutic decision making still remains controversial, be it luminal A, luminal B, or the triple negative variant. Furthermore, inconsistencies in Ki-67 IHC are reported at the pre-analytical, analytical, interpretation, as well as the data analysis stages [15]. To standardize Ki-67 scoring, the International Ki-67 in Breast Cancer Working Group (IKWG) was established in 2011 [8]; in its last meeting held in October 2019, the IKWG recommended the following: (1) Pre-analytical handling considerations are critical in Ki-67 scoring. (2) A standardized visual method for estimating Ki-67 has to be adopted. (3) Analytical validity of Ki-67 score must be maintained by quality assurance and quality control programs. (4) Lastly, Ki-67 IHC identifies T1-2 and N0-1, ER + / HER2 − breast cancer patients who do not need (≤ 5% score) or need (≥ 30% score) adjuvant chemotherapy and thence signifies prognosis [16].

NLR is being increasingly used as a reliable and easy available marker of immune response to various infectious and non-infectious stimuli. Normally, NLR lies between 1 and 2; the values higher than 3.0 and below 0.7 in adults are definitely abnormal. The intermediate NLR (2.3–3.0) may serve as an early warning of the varied pathological processes like cancer, atherosclerosis, infection, inflammation, psychiatric disorders, and stress. NLR is used as a reliable and cheap marker of prognosis in solid tumors. Meta-analyses explored the prognostic significance of NLR in solid tumors and found a

cut-off value of NLR above 3.0 [17]. The negative impact of elevated NLR on BC patient's outcome has been documented [18] including the TNBC subtype [19]. During an inflammatory process (infective/allergic/tumoral), the hematopoietic stem cells (HSCs) in the bone marrow detect peripheral perturbations through danger signals [20]. Confronting the stress, HSCs undergo a myeloid-biased differentiation mediated via cytokines; this increased output of myeloid cells is called stress-induced myelopoiesis or emergency myelopoiesis [21]. This emergency myelopoiesis may have a role in host immunity, promoting disease development and progression [20]. The release of these stress-induced myeloid cells into the peripheral circulation is usually reflected as a raised NLR.

The present study aims to unravel any link between tumor proliferative potential (Ki-67 score) and tumor-induced emergency myelopoiesis (NLR).

## Methods

### Study outline

An observational study was carried out in the Department of Pathology in collaboration with the Department of Surgery, J.N Medical College, Aligarh Muslim University, India, from November 2019 to November 2021. Seventy-three consenting female patients with core needle biopsy-based newly diagnosed, early, or locally advanced BC were enrolled for the study. All the necessary details about the process of the study and the rationale behind the study were explicitly explained to the patients in their own language, and any doubts were clarified. Patients refusing consent, those who received any prior neoadjuvant chemotherapy and/ or radiotherapy, with known metastatic lesions or inflammatory subtype of breast cancer, were excluded from this study. NLR of study participants was noted prior to any surgical/ therapeutic intervention (even before performing the core needle biopsy). To calculate NLR, 2 ml peripheral venous blood was collected in Ethylenediaminetetraacetic acid (EDTA) anti-coagulant vial, and the cell counts were performed by Bene Sphera H33 3-part hematology analyzer. NLR was then calculated as a ratio of absolute neutrophil count to absolute lymphocyte count and was categorized as low < 3 or high ≥ 3. After the core needle primary Hematoxylin & Eosin (H&E) based histopathological diagnosis, the Ki-67 IHC was performed. The primary antibody used was Thermo Scientific Ki-67 purified rabbit polyclonal antibody, and the secondary antibody used was Horse Radish Peroxidase (HRP). Ki-67 IHC was performed by the fully automated slide preparation system, Ventana Benchmark GX. The slides were then examined under a light microscope (Magnüs MX21i LED) using 100 × and 400 × magnification. For routine IHC reporting

of BC patients, a Ki-67 cut-off of 14% was used to differentiate luminal A from luminal B [9]. However, for the purpose of this study, the Ki-67 was classified using the guidelines as recommended by the IKWG 2019 consensus meeting. Finally, the expression of Ki-67 was graded as low $\leq 5\%$, intermediate 6–29%, and high $\geq 30\%$ [16]. Clinical data was collected from the hospital archives, and pathological observational data was collected and recorded electronically.

## Statistical analysis

The statistical analysis was done using Statistical Package for the Social Sciences (SPSS) version 25.0, categorical data were expressed as frequencies and continuous data as mean $\pm$ standard deviation, and the association of categorical variables was analyzed using Pearson's chi-square test. A $p$-value of $< 0.05$ was taken as significant.

## Results

In our study, 32.9% of patients were $\leq 40$ years of age, while 67.1% of patients were $> 40$ years of age. In the context of menopausal status, 42.5% of females were pre-menopausal, while 57.5% of them were menopausal. All the patients had a presenting complaint of a breast mass; in addition to it, 56.1% had concomitant axillary lymphadenopathy, 19.1% had fungating superficial ulcer, 17.8% had pain, and 7.0% had nipple discharge/retraction. The right side breast was involved in 56.2% of the cases, while the left side breast was involved in 43.8% of the cases. Location-wise, the upper inner quadrant was involved in 12.3% of the cases, and the upper outer quadrant was the most commonly affected accounting for 46.6% of the cases. The lower quadrant was less commonly involved with 2.7% of the cases in its outer part and 4.1% in its inner part. The central portion of the breast was involved in 15.1% of the patients and in 19.2% of the patients multiple quadrants of the breast were involved. Microscopic examination of the routine H&E stained slides identified that invasive breast cancer (IBC) of no special type (NST) was the commonest histologic variant with 74.0% of the cases, followed by IBC with medullary features 12.3%, metaplastic breast carcinoma 5.5%, and others 8.2% (mucinous carcinoma, invasive carcinoma with neuroendocrine differentiation, encapsulated papillary variant, etc.). The clinico-pathologic characteristics of the cases have been summarized in Table 1.

TNM staging of the cases showed that none of the case presented in stage I, 58.9% of patients were in stage II at the time of presentation, and 41.1% of patients were in stage III. Patients with distant metastasis were excluded from this study as mentioned earlier. The modified SBR grading of the cases was undertaken, and 11% of cases
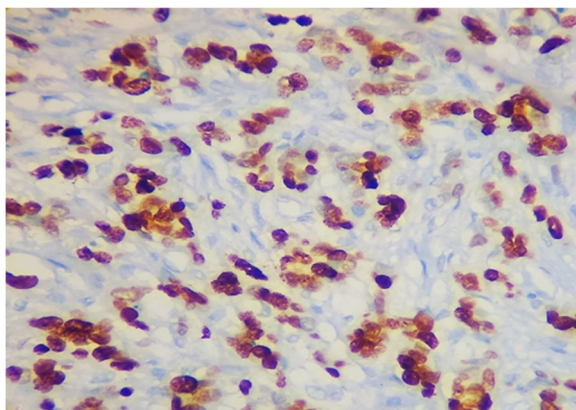
**Table 1** Distribution of clinico-pathologic characteristics of study cases

| Characteristic | No. of cases (total = 73) | % |
|---|---|---|
| 1. *Age (years)* | | |
| $\leq 40$ | 24 | 32.9 |
| $> 40$ | 49 | 67.1 |
| 2. *Menopausal status* | | |
| Pre-menopausal | 31 | 42.5 |
| Post menopausal | 42 | 57.5 |
| 3. *Presenting complaints* | | |
| Breast lump | 73 | 100 |
| Lump + Axillary lymphadenopathy | 41 | 56.1 |
| Lump + Fungating ulcer | 14 | 19.1 |
| Lump + Pain | 13 | 17.8 |
| Lump + others | 05 | 7.0 |
| 4. *Laterality* | | |
| Right | 41 | 56.2 |
| Left | 32 | 43.8 |
| 5. *Location* | | |
| Upper inner quadrant | 9 | 12.3 |
| Upper outer quadrant | 34 | 46.6 |
| Lower outer quadrant | 2 | 2.7 |
| Lower inner quadrant | 3 | 4.1 |
| Central | 11 | 15.1 |
| Multiple | 14 | 19.2 |
| 6. *Histological type* | | |
| Invasive Breast Cancer (IBC) NST | 54 | 74.0 |
| IBC with medullary features | 9 | 12.3 |
| Metaplastic breast carcinoma | 4 | 5.5 |
| Others | 6 | 8.2 |

were of grade 1, 76.8% of grade 2, and 12.2% were of grade 3. Molecular sub-typing of all the cases was done by ER, PR, Her 2 Neu, and Ki-67 IHC as a standard procedure. Upon interpretation of the IHC results, luminal A was present in 20.5% of the cases, 19.2% of patients were of luminal B, 38.3% cases were of triple negative, and the remainder 22.0% of cases were of Her 2 enriched subtype. Subsequently, Ki-67 IHC classification was done, and it showed that 15.0% of the cases were of low grade (Fig. 1), 28.8% of intermediate grade, and 56.2% of high grade (Fig. 2). Similarly, NLR was calculated, and 64.4% cases had high ($\geq 3$) NLR, while 35.6% of the cases had low ($< 3$) NLR. On applying Pearson's chi-square test, Ki-67 grade was significantly correlated with TNM stage of the tumor (Ch$^2$-value 38.124, $p < 0.001$), and SBR grade (Ch$^2$-value 16.595, $p < 0.01$). NLR showed a statistically significant correlation with TNM stage (Ch$^2$-value 36.948, $p < 0.001$); however, NLR values were not found to be significantly correlated with either the SBR grade
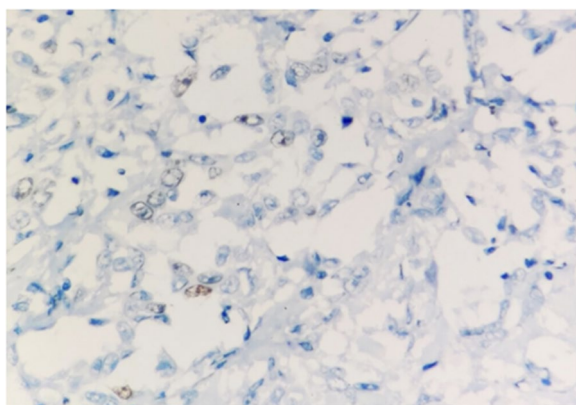
**Fig. 1** Tumor with low-grade Ki-67 expression < 5% (IHC × 400)



**Fig. 2** Highly proliferating tumor with high-grade Ki-67 expression > 30% (IHC × 400)

**Table 2** Correlation of clinico-pathologic variables and Ki-67 with NLR

| Cases ($n = 73$) | NLR | | $Ch^2$-value | $p$-value |
|---|---|---|---|---|
| | Low ($n = 26$) | High ($n = 47$) | | |
| **SBR grade** | | | | |
| 1 ($n = 8$) | 5 (62.5%) | 3 (37.5%) | 4.878 | > 0.05 |
| 2 ($n = 56$) | 20 (35.7%) | 36 (64.3%) | | |
| 3 ($n = 9$) | 1 (11.1%) | 8 (88.9%) | | |
| **Molecular subtype** | | | | |
| Luminal A ($n = 15$) | 8 (53.3%) | 7 (46.7%) | 4.856 | > 0.05 |
| Luminal B ($n = 14$) | 6 (42.9%) | 8 (57.1%) | | |
| Triple negative ($n = 28$) | 6 (21.4%) | 22 (78.6%) | | |
| Her2 enriched ($n = 16$) | 6 (37.5%) | 10 (62.5%) | | |
| **TNM stage** | | | | |
| I ($n = 0$) | 0 | 0 | 36.948 | < 0.001 |
| II ($n = 43$) | 25 (58.1%) | 18 (41.9%) | | |
| III ($n = 30$) | 1 (3.3%) | 29 (96.7%) | | |
| **Ki-67 expression** | | | | |
| Low ($n = 11$) | 11 (100%) | 0 | 43.107 | < 0.001 |
| Intermediate ($n = 21$) | 13 (61.9%) | 08 (38.1%) | | |
| High ($n = 41$) | 02 (4.9%) | 39 (95.1%) | | |

(Ch$^2$-value 4.878, $p > 0.05$) or the molecular subtype of breast cancer (Ch$^2$-value 4.856, $p > 0.05$). Lastly, the Ki-67 grade showed the strongest statistical correlation with NLR (Ch$^2$-value 43.107, $p < 0.001$) as mentioned in Table 2.

## Discussion

Proliferation at the cellular level decides the clinical behavior of BC; Ki-67 labeling index and mitotic count are known to identify cell proliferation in a tumor. Ki-67 labeling index seems to be a better guide of cell proliferation than mitotic figure counts as all the cells that are in active phases of the cell cycle can be recognized. Also, counting colored Ki-67 positive nuclei on IHC is easier than assessing mitotic figures [22, 23]. Ki-67 grade was higher in SBR grade 3 tumors as compared to grade 2 or and grade 1 tumors in our study. Similar results were discerned by Inwald et al. who reported an association between Ki-67 and tumor grade [24]. We found a significant correlation between Ki-67 and TNM stage (excluding metastatic/ stage IV) as demonstrated earlier by

Kamranzadeh et al. [25] and Thangarajah et al. [26] that there is a significant positive correlation of Ki-67 index with clinical stage.

The TNM classification has conventionally been used as an indicator of cancer prognosis, but cancers at the same stage of progression vary in their prognosis because of the varied and unique host factors such as tumor microenvironment, nutritional status, and immune status among others. Recently, neutrophils are proven to affect tumor initiation, progression, and metastasis, and a raised NLR is emerging as a key indicator of cancer development and progression in several cancers, including breast cancer [27]. Its prognostic significance has been well defined in advanced tumors under the influence of tumoral cytokines and growth factors [28]. In our study, the statistical correlation between NLR and tumor SBR grade was not significant, and an extensive study conducted by Dirican et al. also showed similar results [29]. Different molecular subtypes of breast cancer also showed a non-significant correlation with NLR in our study. Earlier, it has been shown that no significant correlation is present between NLR and different molecular subtypes [30]. We found a significant correlation between NLR and tumor stage, similar to what has been documented by Elyasinia et al. in 2017 [31]. Few studies had already shown that NLR correlates well with

Ki-67 expression in breast cancer. With a cut-off of 14%, Ki-67 was significantly correlated with NLR in the study of HER 2 negative breast cancer patients done by Bae et al. [32]. Low NLR along with platelet lymphocyte ratio (PLR) is an independent predictive factor for pathological complete response (pCR) in patients with early or locally advanced BC treated with NAC as this signifies a robust immune status [33]. Not only patient stratification and chemotherapeutic response, NLR is also associated with favorable prognosis in BC. Recently, NLR has been identified as an independent prognostic factor associated with better overall survival in oligometastatic BC [34]. Moving further with cancer progression, NLR can be used to predict metastatic spread and detect early recurrence in BC [35]. Growing body evidence correlating NLR with several biological aspects of BC prompted us to utilize the latest guidelines by the IKWG 2019 stratifying Ki-67 into low $\leq 5\%$, intermediate 6–29%, and high $\geq 30\%$ grades, and positively correlating them with NLR. A simple, rapid, cheap, and one of the most commonly performed investigation globally, the hemogram, can be used to calculate NLR which can have much profound ramifications than what had been thought for decades. This will go a long way in managing patients of breast cancer.

## Conclusion

The therapeutic and prognostic relevance of Ki-67 which in part reflects the proliferative capabilities of breast cancer can be reinforced by peripheral NLR. Baseline NLR and its dynamics with unpredictable clinical behavior of breast cancer will serve as a minimally invasive personalized marker for early diagnosis, guiding therapy, prognosis, and follow-up of patients.

## Abbreviations

| | |
|---|---|
| NLR | Neutrophil to lymphocyte ratio |
| BC | Breast cancer |
| IHC | Immunohistochemistry |
| ER | Estrogen receptor |
| PR | Progesterone receptor |
| HER 2/neu | Human epidermal growth factor receptor 2 |
| TNBC | Triple-negative breast cancer |
| NAC | Neoadjuvant chemotherapy |
| IKWG | International Ki-67 in Breast Cancer Working Group |
| HSCs | Hematopoietic stem cells |
| EDTA | Ethylenediaminetetraacetic acid |
| H&E | Hematoxylin & Eosin |
| SBR | Scarff-Bloom-Richardson |
| SPSS | Statistical Package for the Social Sciences |
| IBC | Invasive breast cancer |
| NST | No special type |
| TNM | Tumor/nodes/metastasis |
| pCR | Pathological complete response |

## Authors' contributions
FA: concept, design, data analysis, drafting of article. RA: collection, analysis and interpretation of data. VM, KA, MH: supervision, critical revision, and final approval. AZ: provision of study material, interpretation, and supervision.

## Availability of data and materials
All the data has been supplied in the manuscript itself.

## Declarations

### Ethics approval and consent to participate
The study was performed in accordance with the Declaration of Helsinki. The study was approved by the Institutional Ethics Committee, J.N Medical College, AMU, Aligarh on 24.12.2019. , the registration no. of the certificate was 161/FM/IEC. Informed consent was obtained from the patients for obtaining the biopsy tissue and peripheral blood.

### Consent for publication
Not applicable.

### Competing interests
The authors declare that they have no competing interests.

## References
1. Heidari Z, Mahmoudzadeh- Sageb H, Jahantigh M, Charkhat Gorgich EA. Immunohistochemical expression of Ki 67 and HER2 in colorectal cancer compared to adenomatous and normal samples. Int J Cancer Manag. 2017;10:e12252. https://doi.org/10.5812/ijcm.12252.
2. Hammarsten P, Josefsson A, Thysell E, Lundholm M, Hägglöf C, Iglesias-Gato D, et al. Immunoreactivity for prostate specific antigen and Ki67 differentiates subgroups of prostate cancer related to outcome. Mod Pathol. 2019;32:1310–9. https://doi.org/10.1038/s41379-019-0260-6.
3. Wei Z, Huang L, Zhang X, Xu A. Expression and significance of Her2 and Ki-67 in gastric adenocarcinoma without distant metastasis: a cohort study. BMC Gastroenterol. 2020;20:343. https://doi.org/10.1186/s12876-020-01484-9.
4. Miller I, Min M, Yang C, Tian C, Gookin S, Carter D, et al. Ki67 is a graded rather than a binary marker of proliferation versus quiescence. Cell Rep. 2018;24:1105–12.e5.
5. Kim KI, Lee KH, Kim TR, Chun YS, Lee TH, Park HK. Ki-67 as a predictor of response to neoadjuvant chemotherapy in breast cancer patients. J Breast Cancer. 2014;17:40–6.
6. Fasching PA, Heusinger K, Haeberle L, Niklos M, Hein A, Bayer CM, et al. Ki67, chemotherapy response, and prognosis in breast cancer patients receiving neoadjuvant treatment. BMC Cancer. 2011;11:1–3.
7. Harbeck N, Gluz O, Christgen M, Kates RE, Braun M, Kueemmel S, et al. De-escalation strategies in human epidermal growth factor receptor 2 (HER2)-positive early breast cancer (BC): final analysis of the West German Study Group adjuvant dynamic marker-adjusted personalized therapy trial optimizing risk assessment and therapy response prediction in early BC HER2-and hormone receptor-positive phase II randomized trial-efficacy, safety, and predictive markers for 12 weeks of neoadjuvant trastuzumab emtansine with or without endocrine therapy (ET) versus trastuzumab plus ET. J Clin Oncol. 2017;35:3046–57.
8. Dowsett M, Nielsen TO, A'Hern R, Bartlett J, Coombes RC, Cuzick J, et al. Assessment of Ki67 in breast cancer: recommendations from the International Ki67 in Breast Cancer working group. J Natl Cancer Inst. 2011;103:1656–64.
9. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thürlimann B, Senn HJ. Strategies for subtypes—dealing with the diversity of breast cancer:

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highlights of the St. Gallen international expert consensus on the primary therapy of early breast cancer 2011. Ann Oncol. 2011;22:1736–47.

10. Untch M, Gerber B, Harbeck N, Jackisch C, Marschner N, Möbus V, et al. 13th St. Gallen international breast cancer conference 2013: primary therapy of early breast cancer evidence, controversies, consensus - opinion of a German team of experts (Zurich 2013). Breast Care. 2013;8:221–9. https://doi.org/10.1159/000351692.

11. Bustreo S, Osella-Abate S, Cassoni P, Donadio M, Airoldi M, Pedani F, et al. Optimal Ki 67 cut-off for luminal breast cancer prognostic evaluation: a large case series study with a long-term follow-up. Breast Cancer Res Treat. 2016;157:363–71. https://doi.org/10.1007/s10549-016-3817-9.

12. Zhu X, Chen L, Huang B, Wang Y, Ji L, Wu J, et al. The prognostic and predictive potential of Ki-67 in triple-negative breast cancer. Sci Rep. 2020;10:225. https://doi.org/10.1038/s41598-019-57094-3.

13. Arafah MA, Ouban A, Ameer OZ, Quek KJ. KI-67 LI expression in triple-negative breast cancer patients and its significance. Breast Cancer. 2021. https://doi.org/10.1177/11782234211016977.

14. Wu Q, Ma G, Deng Y, Luo W, Zhao Y, Li W, et al. Prognostic value of Ki-67 in patients with resected triple-negative breast cancer: a meta-analysis. Front Oncol. 2019;9:1068. https://doi.org/10.3389/fonc.2019.01068.

15. Davey MG, Hynes SO, Kerin MJ, Miller N, Lowery AJ. Ki-67 as a prognostic biomarker in invasive breast cancer. Cancers (Basel). 2021;13:4455. https://doi.org/10.3390/cancers13174455.

16. Nielsen TO, Leung SCY, Rimm DL, Dodson A, Acs B, Badve S, et al. Assessment of Ki67 in breast cancer: updated recommendations from the international Ki67 in breast cancer working group. J Natl Cancer Inst. 2021;113:808–19. https://doi.org/10.1093/jnci/djaa201.

17. Zahorec R. Neutrophil-to-lymphocyte ratio, past, present and future perspectives. Bratisl Lek Listy. 2021;122:474–88. https://doi.org/10.4149/BLL_2021_078.

18. 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;19:2.

19. Pistelli M, De Lisa M, Ballatore Z, Caramanti M, Pagliacci A, Battelli N, et al. Pre-treatment neutrophil to lymphocyte ratio may be a useful tool in predicting survival in early triple negative breast cancer patients. BMC Cancer. 2015;15:195.

20. Pu S, Qin B, He H, Zhan J, Wu Q, Zhang X, et al. Identification of early myeloid progenitors as immunosuppressive cells. Sci Rep. 2016;6:1–9.

21. Zhao JL, Ma C, O'Connell RM, Mehta A, DiLoreto R, Heath JR, et al. Conversion of danger signals into cytokine signals by hematopoietic stem and progenitor cells for regulation of stress-induced hematopoiesis. Cell Stem Cell. 2014;14:445–59.

22. Kontzoglou K, Palla V, Karaolanis G, Karaiskos I, Alexiou I, Pateras I, et al. Correlation between Ki67 and breast cancer prognosis. Oncology. 2013;84:219–25.

23. Jurikova M, Danihel Ľ, Polák Š, Varga I. Ki67, PCNA, and MCM proteins: markers of proliferation in the diagnosis of breast cancer. Acta Histochem. 2016;118:544–52.

24. Inwald EC, Klinkhammer-Schalke M, Hofstädter F, Zeman F, Koller M, Gerstenhauer M, et al. Ki-67 is a prognostic parameter in breast cancer patients: results of a large population-based cohort of a cancer registry. Breast Cancer Res Treat. 2013;139:539–52. https://doi.org/10.1007/s10549-013-2560-8.

25. Kamranzadeh H, Ardekani RM, Kasaeian A, Sadighi S, Maghsudi S, Jahanzad I, et al. Association between Ki-67 expression and clinicopathological features in prognosis of breast cancer: a retrospective cohort study. J Res Med Sci. 2019;24:30. https://doi.org/10.4103/jrms.JRMS_553_18.

26. Thangarajah F, Enninga I, Malter W, Hamacher S, Markiefka B, Richters L, et al. A retrospective analysis of Ki-67 index and its prognostic significance in over 800 primary breast cancer cases. Anticancer Res. 2017;37:1957–64. https://doi.org/10.21873/anticanres.11536.

27. Templeton AJ, McNamara MG, Šeruga B, Vera-Badillo FE, Aneja P, Ocaña A, et al. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. J Natl Cancer Inst. 2014;106:dju124. https://doi.org/10.1093/jnci/dju124.

28. Guthrie GJK, Charles KA, Roxburgh CSD, Horgan PG, McMillan DC, Clarke SJ. The systemic inflammation-based neutrophil-lymphocyte ratio: experience in patients with cancer. Crit Rev Oncol Hematol. 2013;88:218. https://doi.org/10.1016/j.critrevonc.2013.03.010.

29. Dirican A, Kucukzeybek BB, Alacacioglu A, Kucukzeybek Y, Erten C, Varol U, et al. Do the derived neutrophil to lymphocyte ratio and the neutrophil to lymphocyte ratio predict prognosis in breast cancer? Int J Clin Oncol. 2015;20:70–81. https://doi.org/10.1007/s10147-014-0672-8.

30. 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 Pac J Cancer Prev. 2017;18:2227–31. https://doi.org/10.22034/APJCP.2017.18.8.2227.

31. Elyasinia F, Keramati MR, Ahmadi F, Rezaei S, Ashouri M, Parsaei R, et al. Neutrophil-lymphocyte ratio in different stages of breast cancer. Acta Med Iran. 2017;55:228–32. https://acta.tums.ac.ir/index.php/acta/article/view/5704.

32. Bae SJ, Cha YJ, Yoon C, Kim D, Lee J, Park S, et al. Prognostic value of neutrophil-to-lymphocyte ratio in human epidermal growth factor receptor 2-negative breast cancer patients who received neoadjuvant chemotherapy. Sci Rep. 2020;10:13078. https://doi.org/10.1038/s41598-020-69965-1.

33. Graziano V, Grassadonia A, Iezzi L, Vici P, Pizzuti L, Barba M, et al. Combination of peripheral neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio is predictive of pathological complete response after neoadjuvant chemotherapy in breast cancer patients. Breast. 2019;44:33–8. https://doi.org/10.1016/j.breast.2018.12.014.

34. Inoue Y, Fujishima M, Ono M, Masuda J, Ozaki Y, Maeda T, et al. Clinical significance of the neutrophil-to-lymphocyte ratio in oligometastatic breast cancer. Breast Cancer Res Treat. 2022;196:341–8. https://doi.org/10.1007/s10549-022-06726-w.

35. Ren Z, Yang J, Liang J, Xu Y, Lu G, Han Y, et al. Monitoring of postoperative neutrophil- to- lymphocyte ratio, D-dimer, and CA 153 in: diagnostic value for recurrent and metastatic breast cancer. Front Surg. 2022;9:927491. https://doi.org/10.3389/fsurg.2022.927491.

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