Prognostic value of neutrophil-to-lymphocyte ratio in breast cancer

Jie Chen a,1, Qiwen Deng b,1, Yuqin Pan b, Bangshun He b, Houqun Ying c, Huiling Sun a, Xian Liu b, Shukui Wang b,∗

a Department of Life Sciences, Nanjing Normal University, Nanjing, Jiangsu, China
b Central Laboratory, Nanjing First Hospital, Nanjing Medical University, Nanjing, Jiangsu, China
c Medical College, Southeast University, Nanjing, Jiangsu, China

A R T I C L E   I N F O

Article history:
Received 25 March 2015
Revised 4 May 2015
Accepted 7 May 2015

Keywords:
Breast cancer
Inflammation
NLR
Prognosis

A B S T R A C T

Inflammation is an essential component of pathogenesis and progression of cancer. A high neutrophil-to-lymphocyte ratio (NLR) is considered as a prognostic indicator for breast cancer. This meta-analysis was conducted to establish the overall accuracy of the NLR test in the diagnosis of breast cancer. A comprehensive search of the literature was conducted by using PubMed, Web of Science and China National Knowledge Infrastructure (CNKI). Published studies dating up to July 2014 and 4,293 patients were enrolled in the present study. In order to evaluate the association between NLR and overall survival (OS), disease-free survival (DFS), recurrence-free survival (RFS) or cancer specific survival (CSS), the hazard ratios (HRs) and their 95% confidence intervals (CIs) were extracted. OS was the primary outcome. The results suggested that increased NLR was a strong predictor for OS with HR of 2.28 (95% CI = 1.08–4.80, Pheterogeneity < 0.001). Stratified analyses indicated that a high NLR appeared to be a negative prognostic marker in Caucasian populations (HR = 4.53, 95% CI = 3.11–6.60, P heterogeneity = 0.096), multivariate analysis method (HR = 2.10, 95% CI = 1.52–2.89, P heterogeneity = 0.591), and mixed metastasis (HR = 4.53, 95% CI = 3.11–6.60, P heterogeneity = 0.096). Elevated NLR was associated with a high risk for DFS (HR = 1.38, 95% CI = 1.09–1.74, P heterogeneity = 0.050) and in subgroups of multivariate analysis (HR = 1.64, 95% CI = 1.25–2.14, P heterogeneity = 0.545) and mixed metastasis (HR = 1.99, 95% CI = 1.28–3.09, P heterogeneity = 0.992). In summary, NLR could be considered as a predictive factor for patients with breast cancer.

© 2015 The Authors. Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

Breast cancer is a common malignancy that affects the health of women worldwide. One in eight women will be diagnosed with breast cancer in their lifetime [1]. 5–7% of women are diagnosed before the age of 40, and the highest frequency is found in the age group 25 to 39 [2–5]. With the rapid advancement of early diagnosis and treatment in breast cancer, more than four fifths of patients are now successfully treated [4] and the mortality has recently declined in young women [6]. However, a large proportion of patients were still suffered from breast cancer due to heterogeneity of diagnosis and treatment. Therefore, it is crucial to understand causes contributing to breast carcinogenesis, invasion and metastasis and to identify effective early-diagnostic and prognostic biomarkers that help to diagnose, evaluate treatment efficacy and prognosis and follow-up schedule [7].

It has been demonstrated that the inflammatory response plays an important role in the development and progression of various cancers, including breast cancer [8–10]. The cancer-related inflammatory response helps proliferation and survival of malignant cells, angiogenesis and metastasis of breast cancer, and it subverts adaptive immune response, leading to an imbalance of immune response and malignant cancer to promote cancer progression and poor OS.

Biomarkers such as neutrophils, lymphocytes, neutrophil-to-lymphocyte ratio (NLR), mean platelet volume, red cell distribution width, circulating tumor cells and gamma-glutamyl transferase have been proposed as potential prognostic factors for cancer [11–15]. There is accumulating evidence for the association of NLR with survival of patients with many kinds of
cancers, including breast cancer [16–23]. However, the published results are inconsistent. Some studies reported that NLR was significantly associated with shorter DFS and OS in breast cancer patients [24,25], while others showed that NLR could not be considered as an independent prognostic factor for breast cancer [26,27].

In order to obtain an objective and consistent conclusion, we therefore conducted this comprehensive systematic review and meta-analysis of the association between NLR and survival of breast cancer.

2. Materials and methods

2.1. Search strategy

This meta-analysis was conducted according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement and methods [28,29]. A comprehensive literature search was carried out using search terms of “neutrophil-to-lymphocyte ratio (NLR),” “breast cancer or tumor or carcinoma” and “prognosis or outcome or survival” in databases of PubMed, Web of Science and CNKI dating up to July 2014. Hand searches were performed to obtain substantial relevant study by reviewing all references within all relevant articles. All selected literatures were journal articles in Chinese and English. This study was approved by the institution ethics committee of Nanjing Normal University.

2.2. Selection criteria

In the meta-analysis, studies were considered eligible if they met the following criteria: (1) study investigated the association between NLR and clinical prognosis in patients with breast cancer; (2) study provided sufficient data for estimating hazard ratio (HR) with 95% confidence interval (CI). Meanwhile, studies were excluded based on the following criteria: (1) duplicate publications; (2) insufficient data for further analysis; (3) letters, reviews, meeting abstracts, editorials, and case reports; (4) other topics.

2.3. Data extraction

The following data, the first author, year of publication, name of journal, country of origin, ethnicity of the study population, type of specimen, metastasis, cut-off value, follow-up period, number of patients included in analysis, and HR with its 95% CI for overall survival (OS), disease-free survival (DFS), recurrence-free survival (RFS) or cancer specific survival (CSS) were extracted from each eligible study by two independent investigators (JC, QWD). If there was any disagreement, it solved by discussion to reach a consensus.

2.4. Statistical analysis

HR and its 95% CI were selected as common measurements to assess the strength of the association between NLR and prognosis in breast cancer. Cochran’s Q test was chosen to evaluate the heterogeneity and Higgins I-squared statistic was carried out to estimate the degree of heterogeneity of pooled results. The random-effect and fixed-effect models were used to calculate the pooled HR and its 95% CI. If \( P_H < 0.05 \), the random-effect model (DerSimonian–Laird method) was applied to calculate the pooled HRs [30]. Otherwise, the fixed-effect model (Mantel–Haenszel method) was employed [31]. The HR is commonly and conveniently estimated via a Cox proportional hazards model, which can include potential confounders as covariates. HR > 1 reflects that elevated NLR is associated with the corresponding variate, while HR < 1 has the opposite meaning. Furthermore, subgroup was performed to explore the heterogeneity among studies which stratified by ethnicity, analysis method and metastasis. Sensitivity analysis was conducted to check whether individual study influenced the results by sequential omission of each study in this meta-analysis. Additionally, Begg’s funnel plot and Egger’s linear regression test were used to assess the extent of publication bias in the meta-analysis and \( P_H < 0.05 \) was considered as statistically significant. Statistical analysis was performed by Stata 11.0 software (STATA Corporation, College Station, TX, USA).

3. Results

3.1. Included studies

A total of 45 potentially relevant articles were retrieved. 14 papers were defined duplicate publications according to their titles. Then 20 articles were excluded because of obvious lack of relevance. A careful review of the remaining 11 studies revealed that 3 studies did not provide sufficient information. Finally, 8 studies were included in the meta-analysis (Fig. 1) [16,22–25, 27,32,33].

3.2. Study characteristics

The main features of eligible studies were shown in Table 1. The eligible studies were published in a period of 2012 to 2014 and contained a total of 4,293 patients. In total, 8 studies were enrolled and 4 studies were conducted in Asian and Caucasian population, respectively. 5 studies were involved in mixed metastasis and the others without metastasis. The cut-off values applied in the studies were not consistent and it was not provided in one study [16]. Among them, 5, 4, 1 and 1 studies investigated the relationship of NLR and OS, DFS, RFS, and CSS, respectively. The useful data of HRs and 95% CIs were obtained from multivariate analysis in 5 studies and univariate analysis in 3 studies, respectively.

3.3. Overall survival

The pooled analysis was conducted in 5 studies including 3,350 patients that reported HR for OS. The main results of this meta-analysis were listed in Table 2 and Fig. 2. The results showed that elevated NLR was associated with a worse outcome for OS with the pooled HR of 2.28 (95% CI = 1.08–4.80, \( P_H < 0.001 \)). Subgroup analyses showed that the prognostic effect of NLR was found only in Caucasian population (HR = 4.53, 95% CI = 3.11–6.60, \( P_H = 0.096 \)) and it was examined to be was a strong prognostic factor in multivariate analysis (HR = 2.10, 95% CI = 1.52–2.89, \( P_H = 0.591 \)). When metastasis was taken into consideration, increased NLR was associated with a poor prognosis for OS in mixed metastasis (HR = 4.53, 95% CI = 3.11–6.60, \( P_H = 0.096 \)).

From sensitivity analysis we found that the result was not obviously impacted by an included study conducted by Cihan et al. [27]. The HR for it was 3.08 (95% CI = 1.59–5.96, \( P_H = 0.002 \)). The shape of funnel plots showed no evidence of publication bias in the analysis (Fig. 3) and the result was further supported by Egger’s tests (\( P_H = 0.896 \)).

3.4. Disease-free survival

4 studies comprising 2,764 patients were included to assess the association between NLR and DFS in breast cancer (Table 2). Overall, elevated NLR was associated with a high risk for DFS.
HR = 1.38, 95% CI = 1.09–1.74, \( P_H = 0.050 \) and in subgroups of multivariate analysis (HR = 1.64, 95% CI = 1.25–2.14, \( P_H = 0.545 \)) and mixed metastasis (HR = 1.99, 95% CI = 1.28–3.09, \( P_H = 0.992 \)). Interestingly, the same study [27] had no effect on sensitivity analysis by removing one study each time. The HR for it was 1.64 (95% CI = 1.25–2.14, \( P < 0.001 \)). The rest studies [24,25,27,33] might be source of heterogeneity. The Begg’s funnel plot (Fig. 4) and the Egger’s test (\( P = 0.762 \)) did not provide any obvious evidence of publication bias.

### 4. Discussion

Inflammation has been shown to be an important factor in the development of tumorigenesis [34]. Peripheral blood tests before treatment or at the time of diagnosis could reflect inflammatory conditions within the tumor. Inflammation-related markers such as absolute white blood cell count, C-reactive protein (CRP), cytokines, platelet-to-lymphocyte ratio (PLR), and NLR have been shown to be associated with specific outcomes in cancer patients [35]. NLR is a biomarker for inflammation and it can be more easily and conveniently measured than conventional markers and at a low cost. A meta-analysis recently reported by Templeton et al. [35] only included 3 original studies and did not show a significant correlation between NLR and survival of breast cancer. The current meta-analysis combined the outcomes of 4,293 cancer patients from 8 studies was to assess the prognostic effect of NLR in breast cancer. In this meta-analysis, we found that high level of NLR significantly affected OS and DFS in breast cancer.

### Table 1

Main characteristics of eligible studies.

| No. of studies | First author | Journal | Year | Country | Ethnicity | Specimens | Metastasis | Cut-off | Follow-up (month) | Number of patients | Analysis | Survival | HR estimation |
|----------------|--------------|---------|------|---------|-----------|-----------|------------|--------|-------------------|-------------------|----------|-----------|---------------|
| [16] Forget P  | Ann Surg Oncol | 2013 | Belgium | Caucasian | Blood | Mix | 3.4 | NA | 162.172 | Univariate | RFS | HR + 95% CI |
| [22] Azab B    | Ann Surg Oncol | 2012 | USA | Caucasian | Blood | Mix | 3.3 | 45.6(mean) | 316 | Multivariate | OS | HR + 95% CI |
| [23] Noh H     | J Breast Cancer | 2013 | Korea | Caucasian | Blood | Mix | 2.5 | 6.1(mean) | 442 | Multivariate | CSS | HR + 95% CI |
| [24] Forget P  | Br J Anaesth | 2014 | Belgium | Caucasian | Blood | Mix | 3.3 | 69.8(median) | 720 | Multivariate | OS,DFS | OS,DFS | HR + 95% CI |
| [25] Dirican A | Int J Clin Oncol | 2014 | Turkey | Asian | Blood | Mix | 4 | 30(median) | 1,527 | Multivariate | OS,DFS | OS,DFS | HR + 95% CI |
| [27] Cihan YB  | Asian Pac J Cancer Prev | 2014 | Turkey | Asian | Blood | No | 3 | Mean range 10 days–112 months | 350 | Univariate | OS,DFS | HR + 95% CI |
| [32] Azab B    | Med Oncol Anticancer Res | 2013 | USA | Caucasian | Blood | Mix | 3.3 | 60(mean) | 437 | Univariate | OS | HR + 95% CI |
| [33] Nakano K  | Anticancer Res | 2013 | Japan | Asian | Blood | Mix | 2.5 | 85.8(mean) | 167 | Multivariate | DFS | HR + 95% CI |

NA: not available; DFS: disease-free survival; OS: overall survival; RFS: recurrence-free survival; CSS: cancer-specific survival; HR: hazard ratio; CI: 95% confidence interval.
population, multivariate analysis, and mixed metastasis, respectively. Meanwhile, the significant association was observed in multivariate analysis, and mixed metastasis subgroups in DFS. These findings indicated that NLR was associated with ethnicity, analysis methods and metastasis and it could act as a prognostic biomarker in predicting clinical outcome for breast cancer.

The mechanism between the high level of NLR and poor outcome of breast cancer remained unclear. There were several possible explanations for the association between elevated NLR and poor prognosis in breast cancer. First of all, their relationship might be explained by means of an inflammation response caused by cancer cells. As is known, lymphocytes can reduce malignant progression as tumor infiltration via a series of subtypes of lymphocytes, CD3$^+$ T cells, CD8$^+$ T cells, Th1 CD4$^+$ T cell, and p46$^+$ natural killer cells, which has been shown to improve the survival of patients with malignancy\cite{36–39}. An important event of immune escape was T-lymphocyte dysfunction. T-lymphocytes were a common kind of tumor infiltrating lymphocytes (TILs). A study suggested that anergic CD8$^+$ T-lymphocytes were functionally unresponsive, unable to directly lyse melanoma target cells.

Table 2

| Survival Variables | Number of studies | Number of patients | $P$ value $P_H$ $P_Z$ $P_E$ | Regression model |
|-------------------|------------------|-------------------|-------------------|------------------|
|                   |                  |                   |                   | Random | Fixed |
| OS                | 5                | 3,350             | <0.001            | 0.031  | 0.894 | 2.28(1.08-4.80) | 2.36(1.85-3.02) |
| Ethnicity         | 2                | 1,877             | 0.007             | 0.723  | <0.001| 1.19(0.45-3.19) | 1.46(1.06-2.02) |
| Caucasian         | 3                | 1,473             | 0.096             | <0.001 | 2.85(2.05-7.39) | 4.53(3.11-6.60) |
| Analysis method   | 2                | 787               | <0.001            | 0.495  | <0.001| 2.11(0.25-17.88)| 2.79(1.91-4.07) |
| Univariate        | 3                | 2,563             | 0.263             | 0.806  | <0.001| 2.10(1.52-2.89) | 2.10(1.52-2.89) |
| No                | 2                | 1,877             | 0.007             | 0.723  | <0.001| 1.19(0.45-3.19) | 1.46(1.06-2.02) |
| Mix               | 3                | 1,473             | 0.096             | <0.001 | 2.85(2.05-7.39) | 4.53(3.11-6.60) |
| DFS               | 4                | 2,764             | 0.050             | 0.093  | 0.762 | 1.41(0.94-2.12) | 1.38(1.09-1.74) |
| Ethnicity         |                  |                   |                   | 0.141  |       |                   |                   |
| Asian             | 3                | 2,044             | 0.06              | 0.328  | <0.001| 1.27(0.78-2.06) | 1.27(0.98-1.64) |
| Caucasian         | 1                | 720               | 0.012             | <0.001 | 1.99(1.16-3.41) | 1.99(1.16-3.41) |
| Analysis method   |                  |                   |                   | 0.010  |       |                   |                   |
| Univariate        | 1                | 350               | 0.360             | <0.001 | 1.64(1.25-2.14) | 1.64(1.25-2.14) |
| Multivariate      | 3                | 2,414             | 0.545             | <0.001 | 1.64(1.25-2.14) | 1.64(1.25-2.14) |
| Metastasis        |                  |                   |                   | 0.053  |       |                   |                   |
| No                | 2                | 1,877             | 0.044             | 0.732  | <0.001| 1.11(0.62-1.99) | 1.20(0.91-1.57) |
| Mix               | 2                | 887               | 0.992             | 0.002  | 1.99(1.28-3.09) | 1.99(1.28-3.09) |

OS: overall survival; DFS: disease-free survival; $P_H$, $P$ value of heterogeneity test; $P_Z$, $P$ value of Z test; $P_E$, $P$ value of Egger’s test.

Fig. 2. Forest plots of studies evaluating hazard ratios (HRs) of NLR for overall survival. The solid diamond represents each individual study and the hollow diamond represents overall studies. Error bars are 95% confidence intervals.
studies, and these differences were also a potential source of heterogeneity; finally, due to lack of appropriate data, the association of NLR and other clinical parameters, such as mean platelet volume, red cell distribution width, circulating tumor cells and gamma-glutamyl transferase was not explored. Thus, more worldwide studies are required to confirm the value of the NLR test for breast cancer diagnosis in the future.

In conclusion, elevated NLR is strongly associated with poor survival of breast cancer patients, and it can be regarded as a predictive and prognostic factor for patients with breast cancer. Further well designed prospective studies with multi-central and a large sample size are warrant to verify our findings.

Conflict of interest statement

The authors declare no conflict of interest in this work.

Author contributions

JC and QWD conceived and designed the experiments; YQP and BSH performed the experiments; HQY analyzed the data and helped in manuscript writing; HLS and XL contributed reagents and analysis tools; SKW and JC wrote the paper.

Acknowledgments

This project was supported by grants from the National Natural Science Foundation of China (nos. 81172141, 81200401), Nanjing Science and Technology Committee Project (no. 201108025), Nanjing Medical Technology Development Project (no. ZKX11025), Nanjing Health Young Talent Project, Jiangsu Provincial Key Medical Talents to S.K.W., Nanjing Medical Science and Technique Development Foundation to Y.Q.P. (no. QRX11255) and B.S.H. (no. QRX11254).

References

[1] Siegel, R., Naishadham, D. and Jemal, A. (2013) Cancer statistics, 2013. CA Cancer J. Clin. 63, 11–30.
[2] Anders, C.K., Johnson, R., Litton, J., Phillips, M. and Bleyer, A. (2009) Breast cancer before age 40 years. Semin. Oncol. 36, 237–249.
[3] Assi, H.A., Khoury, K.E., Dhok, K., Khalil, L.E., Mouhiddine, T.H., et al. (2013) Epidemiology and prognosis of breast cancer in young women. J. Thorac. Dis. 5 Suppl. 1, S5–8.
[4] DeSantis, C., Ma, J., Bryan, L. and Jemal, A. (2014) Breast cancer statistics, 2013. CA Cancer J. Clin. 64, 52–62.
[5] Gabriel, C.A. and Domchek, S.M. (2010) Breast cancer in young women. J. Thorac. Dis. 5, 217–227.
[6] Guthrie, G.J., Charles, K.A., Roxburgh, C.S., Horgan, P.G., McMillan, D.C., et al. (2010) The systemic inflammation-based neutrophil-lymphocyte ratio: experience in patients with cancer. Crit. Rev. Oncol. Hematol. 88, 218–230.
[7] Colotta, F., Allavena, P., Sica, A., Garlanda, C. and Mantovani, A. (2009) Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. Carcinogenesis 30, 1073–1081.
[8] Colotta, F., Allavena, P., Sica, A., Garlanda, C. and Mantovani, A. (2009) Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. Carcinogenesis 30, 1073–1081.
[9] Mantovani, A., Allavena, P., Sica, A. and Balkwill, F. (2008) Cancer-related inflammation. Nature 454, 436–444.
[10] Paul, D., Kumar, A., Gajbhiye, A., Santra, M.K. and Srikanth, R. (2013) Mass spectrometry-based proteomics in molecular diagnostics: discovery of cancer biomarkers using tissue culture. Biomed. Res. Int. 2013, 783131.
[11] Mantovani, A., Romero, P., Palauka, A.K. and Marincola, F.M. (2008) Tumour immunity: effector response to tumour and role of the microenvironment. Lancet 371, 771–783.
[12] Balkwill, F. and Mantovani, A. (2010) Cancer and inflammation: implications for pharmacology and therapeutics. Clin. Pharmacol. Ther. 87, 401–406.
[13] Guthrie, G.J., Charles, K.A., Roxburgh, C.S., Horgan, P.G., McMillan, D.C., et al. (2013) The systemic inflammation-based neutrophil-lymphocyte ratio: experience in patients with cancer. Crit. Rev. Oncol. Hematol. 88, 218–230.
[14] Satelli, A., Brownlie, Z., Mitra, A., Meng, Q.H. and Li, S. (2015) Circulating tumor cell enumeration with a combination of epithelial cell adhesion molecule- and cell-surface vimentin-based methods for monitoring breast cancer therapeutic response. Clin. Chem. 61, 259–266.
