Neutrophil to Lymphocyte Ratio in Patients Who Received Neoadjuvant Treatment before Gastrectomy

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ABSTRACT **Background:** The neutrophil to lymphocyte ratio (NLR) has demonstrated prognostic value in various malignant conditions, including gastric adenocarcinoma. However, chemotherapy may affect NLR.

Objectives: To evaluate the prognostic value of NLR as an accessory decision-making tool in terms of operating patients after neoadjuvant chemotherapy in patients with resectable gastric cancer.

Methods: We collected oncologic, perioperative, and survival data of patients with gastric adenocarcinoma who underwent curative intent gastrectomy and D2 lymphadenectomy between 2009 and 2016. The NLR was calculated from preoperative laboratory tests and classified as high (> 4) and low (≤ 4). The *t*-test, chi-square, Kaplan-Meier analysis, and Cox multivariate regression models were used to assess associations of clinical, histologic, and hematological variables with survival.

Results: For 124 patients the median follow-up was 23 months (range 1–88). High NLR was associated with greater rate of local complication (r=0.268, $P < 0.01$). The rate of major complications (Clavien-Dindo ≥ 3) was higher in the high NLR group (28% vs. 9%, $P = 0.022$). Among the 53 patients who received neoadjuvant chemotherapy, those with low NLR had significantly improved disease-free survival (DFS) (49.7 vs. 27.7 months, $P = 0.025$). Low NLR was not significantly associated with overall survival (mean survival, 51.2 vs. 42.3 months, $P = 0.19$). Multivariate regression identified NLR group ($P = 0.013$), male gender ($P = 0.04$), and body mass index ($P = 0.026$) as independently associated with DFS.

Conclusions: Among gastric cancer patients planned for curative intent surgery who underwent neoadjuvant chemotherapy, NLR may have prognostic value, particularly regarding DFS and postoperative complications.

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KEY WORDS: chemotherapy, gastric adenocarcinoma (GAC), gastric cancer, neoadjuvant chemotherapy (NACT), neutrophil to lymphocyte ratio

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Gastric adenocarcinoma (GAC) is the fourth leading cancer type and the second cause of cancer-related mortality worldwide. The 5-year survival rate of GAC in the United States is about 26%, with surgical resection and chemotherapy generally being the mainstay of treatment for non-metastatic disease [1]. In recent decades, neoadjuvant chemotherapy (NACT) has gradually become a prominent method of care due to proven improvement in survival and R0 resection (microscopic resection free of tumor) rate, mostly in locally advanced disease [2,3]. Nonetheless, the response rate is only moderate [4] and carries a risk of significant adverse effects. Better modalities are needed to identify responders in advance [5]. Moreover, operation for gastric cancer may carry risks of morbidity and mortality even after NACT. Better predicting models are required to evaluate the benefit to risk ratio and the value of operating after NACT.

Hematologic-derived biomarkers are increasingly being studied as surrogate expressers of systemic inflammatory homeostasis. The neutrophil to lymphocyte ratio (NLR) is calculated as the ratio between neutrophil and lymphocyte counts in routine blood count analysis. Numerous studies have demonstrated NLR to be an efficient and potent prognostic marker in various medical and surgical conditions including benign as well as malignant disorders [6-9]. In GAC, the NLR biomarker was demonstrated to have prognostic significance [10], especially in patients who underwent curative-intent gastric resection [11-13]. However, the use of this marker may be confounded by other acute inflammatory or infectious conditions [14] and by chemotherapy [15]. Considering the growing use of neoadjuvant therapy in GAC, we determined whether NLR maintained its prognostic usefulness after administration of NACT in gastric cancer patients. Moreover, we investigated the value of NLR as an accessory decision-making tool in terms of operating patients after NACT.

PATIENTS AND METHODS

DATA SOURCE

We retrospectively reviewed the medical charts of all consecutive patients who underwent curative intent gastric resection

in our tertiary center institution from 2009 to 2016. Inclusion criteria included patients with gastric adenocarcinoma and gastroesophageal junction carcinoma without known metastatic disease at time of operation. Institutional review board approved this study (2201-15-SMC).

EXPLANATORY AND OUTCOME VARIABLES

Preoperative laboratory results, the American Society of Anesthesiologists (ASA) physical status classification score, surgical notes, histological reports, and clinical follow-up data were recorded including data regarding neoadjuvant and adjuvant treatment regimens. Postoperative complications were recorded and classified according to the Clavien-Dindo grade [16]. Our primary explanatory variable was the last NLR value before surgery, calculated from a blood count analysis derived at least 3 days prior to the operation. Patients were dichotomously grouped into high NLR and low NLR groups using a threshold value of NLR 4.0 [9]. Primary outcomes were disease free survival (DFS) and overall survival (OS). All patients were staged (or restaged) according to the American Joint Committee on Cancer (AJCC) Eighth Edition AJCC Cancer Staging Manual [17] by senior pathologists.

STATISTICAL ANALYSIS

Groups were compared using the t-test for continuous normally distributed outcomes and the Wilcoxon rank-sum test for non-parametric continuous outcomes. Correlation studies used either Pearson's correlation test for normally distributed continuous variables or Spearman's correlation test for non-parametric variables. Categorical outcomes were compared using the chi-square test or the Fisher's exact test, when appropriate. Receiver operating characteristic (ROC) curves were plotted to measure the association between NLR status and mortality. Kaplan-Meier curves and log rank tests were utilized for survival analysis. Multivariate survival analysis was performed using Cox regression analysis. Patients were censored if the relevant events did not develop during follow-up. Significance level was set at $\alpha = 0.05$. Statistical analyses were performed using IBM Statistical Package for the Social Sciences statistics software, version 20 (SPSS, IBM Corp, Armonk, NY, USA).

RESULTS

A total of 174 patients underwent oncologic gastric resection during the study period. Complete preoperative, histological, and follow-up data were available for 139 (67.2%). We excluded 15 patients. Eleven had known metastasis before surgery and were not operated on with curative intent. Four had neuroendocrine tumors and not adenocarcinoma. The median follow-up duration was 23 months (range 1–88). Fifty-nine patients (50.4%) did not undergo neoadjuvant chemotherapy (NACT), 19 failed to complete their chemotherapeutic regimen (16.2%) and 34 underwent complete NACT (29.1%) primarily based on ECF/ECX [19] (5 missing 4.3%). Patient demographic characteristics are summarized in Table 1. Overall, 94 patients underwent open gastrectomy (75.8%), the rest underwent minimal invasive gastrectomy. All patients also underwent complete D2 lymphadenectomy as part of their index operation.

Six patients had an in-situ diagnosis (4.8%), 16 were with stage 1 cancer (12.9%), 22 stage 2 (17.7%), 37 stage 3 (29.8%), and 12 with stage 4 (9.7%) based on the AJCC Eighth Edition AJCC Cancer Staging Manual [17]. Data were insufficient for 31 patients (25%). Patients with stage 4 disease were not diagnosed with metastasis before the surgery, and metastasis was only detected during the surgery or later after the pathologic examination.

FULL COHORT ANALYSIS

High NLR correlated with higher rates of postoperative morbidity. High NLR was associated with local complications (r=0.268, $P < 0.01$) and with major complications (Clavien-Dindo ≥ 3) (chi-square 6.05, 28% vs. 9 %, $P = 0.022$). Older age (ρ coefficient = 0.29, $P = 0.001$) and the Clavien-Dindo score (ρ coefficient = 0.23, $P = 0.007$) correlated positively with NLR; and BMI (ρ coefficient = -0.27, $P = 0.003$) and NACT (ρ coefficient = -0.27, $P = 0.002$) correlated negatively with NLR. No correlation was found between NLR to ASA score ($P = 0.29$), gender ($P = 0.44$), or clinical staging ($P = 0.27$).

High NLR correlated with higher mortality rate. ROC curve analysis showed NLR to significantly correlate with death events (AUC = 0.64, $P = 0.019$, range 0.53–0.75) [Figure 1]. No significant association was found with recurrence events (AUC = 0.594, $P = 0.8$).

Figure 1. Association between neutrophil to lymphocyte ratio and postoperative mortality rate

ROC curve analysis results

ROC = receiver operating characteristic

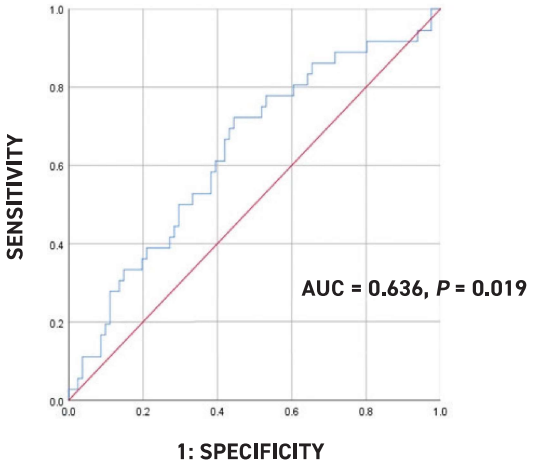


Table 1. Patient (n=124) demographics, characteristics, and basic clinical data

Demographics, clinical data, operative, and postoperative data are listed

Variable	Mean / median	
Age in years	65.09* ± 12.83	
Gender	Male	74 (59.7%)
	Female	50 (40.3%)
ASA score	General	3** (1–4)
	1	6 (4.8%)
	2	41 (33.1%)
	3	65 (52.4%)
	4	3 (2.3%)
	Missing	9 (7.3%)
Body mass index, kg/m^2		26.08* (4.59)
Co-morbidity, n (%)	Peptic ulcer disease	11 (8.9%)
	Hypertension	59 (47.6%)
	Diabetes mellitus	29 (22.6%)
	Ischemic heart disease	18 (14.5%)
	Chronic renal failure	10 (8.1%)
	Cerebrovascular accident	9 (7.3%)
Clavien-Dindo classification	No complication	39 (31.5%)
	1	34 (27.4%)
	2	30 (24.2%)
	3	9 (7.3%)
	4	6 (4.8%)
	5	1 (0.8%)
	Missing	5 (4%)
Surgical approach	Open	94 (75.8%)
	Minimal invasive	30 (24.2%)
NACT	No treatment	64 (51.6%)
	Partial	19 (15.3%)
	Complete	35 (28.82%)
	Missing	6 (4.8%)

***mean ± standard deviation**

****median ± range**

ASA = American Society of Anesthesiologists, NACT = neoadjuvant chemotherapy treatment

Among preoperative factors, the NLR group was shown to be an independent prognostic factor for DFS and OS. Cox multivariate regression identified NLR group ($P = 0.013$), male gender ($P = 0.041$), and BMI ($P = 0.026$) as independently associated with DFS. Age ($P = 0.402$), NACT ($P = 0.482$), and ASA ($P = 0.257$) were not independently associated with DFS. Cox multivariate regression also identified NLR group ($P = 0.013$) as the only factor to be independently associated with OS.

ANALYSIS FOR PATIENT TREATED WITH NEOADJUVANT CHEMOTHERAPY

Low NLR was associated with improved DFS after neoadjuvant chemotherapy [18]. Among patients who received NACT, those with low NLR showed improved DFS (mean DFS 49.7 vs. 27.7 months, $P = 0.025$) [Figure 2]; however, the advantage of OS was not significant (mean survival 51.2 vs. 42.3 months, $P = 0.1$) [Figure 3].

DISCUSSION

Among our patients who were treated with NACT, low NLR was associated with an almost twofold better DFS. Moreover, considering preoperative data, NLR was found to be an independent prognostic factor for both OS and DFS among other preoperative factors. To the best of our knowledge, this research is the first to evaluate NLR prognostic value in GAC patients treated with NAT outside of eastern Asia. Among the study's general population, our results validate previous findings in terms of NLR as a prognostic factor for postoperative complications and morbidity [12]. NACT was found to be negatively correlated with NLR, which suggests that NACT may change NLR; however, we did not have pre-NACT NLR.

Several studies have investigated possible biomarkers (especially blood biomarkers such as NLR and PLR) for their prognostic ability in gastric cancer. The biological basis behind such markers assumes anti-tumorigenic/pro-tumorigenic homeostasis. A change in the balance between these forces, played out by increased secretion of cytokines and immune regulatory molecules, results in changes in immune infiltrates locally, and to some extent, systemically [19]. Neutrophils were described as a major source of growth and angiogenetic factors, whereas cytotoxic lymphocytes are known to dominate the host immune response via cytotoxic cell death and secretion of cytokines that are inhibitory to tumor cells [11].

The salient view has been that the anti-cancer effects of chemotherapy and radiotherapy are through DNA-damage and mitotic toxicity. However, multiple studies have demonstrated several other important mechanisms of action, such as affecting the tumor microenvironment, modifying metabolic pathways, and locoregional changing of immune signaling. These mechanisms include recruiting infiltrating B cells [20] and certain types of T cells such as cytotoxic T lymphocytes [21]. Our find-

Figure 2. Among patients who received neoadjuvant chemotherapy, low neutrophil to lymphocyte ratio (NLR ≤ 4) was associated with improved disease-free survival

The curves show Kaplan–Meier survival analysis of disease-free survival by NLR groups

NLR = neutrophil to lymphocyte ratio

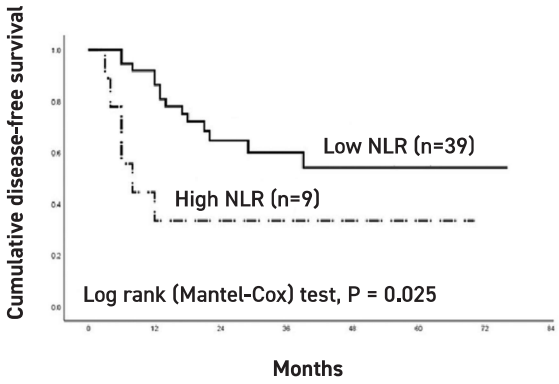
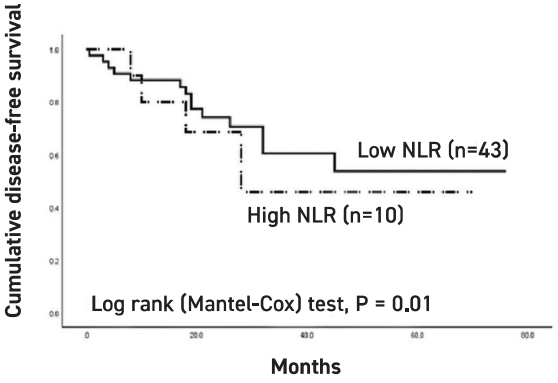


Figure 3. Among the patients who received neoadjuvant chemotherapy group, low NLR (NLR ≤ 4) was not associated with improved overall survival

The Kaplan–Meier survival curves show overall survival by NLR groups

NLR = neutrophil to lymphocyte ratio



ings confirmed our hypothesis that NLR is a useful biomarker after NACT for GAC.

Our results concur with studies that demonstrated the effectiveness of NLR as a prognostic factor for mortality [9,11] and postoperative complications [12] among individuals with GAC who did not receive NACT. Among 46 patients with stage 3 and 4 GAC, low NLR before NACT was associated with higher OS and progression-free survival [22]. Chen et el. [23] reported OS and DFS advantage among 91 patients treated with NACT before gastrectomy; however, NLR was not found as independent prognostic factor for OS and DFS. Their results resemble ours, although our study focused on assessing the value of NLR measurement post NACT and prior to surgery. Those studies were conducted in eastern Asia. Among women with breast cancer [24], low NLR was sampled after neoadjuvant treatment and before surgery, as in our study, and prognostic utility was demonstrated. Other studies have discussed the concept of NLR dynamics and changes between pre-NACT and post-NACT (and preoperative) values as a prognostic factor. For example, among 62 patients with borderline pancreatic ductal adenocarcinoma [25], those whose NLR increased after NACT had significantly worse overall survival.

The main limitations of our study are the lack of complete data due to retrospective design and the lack of immunohistochemical assays to measure the localized immune response surrounding and infiltrating the tumors. Another limitation is the lack of pre-NACT NLR measurements, which would have enabled assessing the impact of NACT on the NLR values. Further research is needed to elucidate the role of NLR in the management of GAC, including studies evaluating the dynamics of NLR after NACT and the prognostic value in terms of NACT response.

CONCLUSIONS

NLR is a well-established prognostic marker for GAC patients who undergo gastrectomy. Our results suggest that NLR may have prognostic value among candidates for curative intent surgery who underwent NACT. NLR may be an accessory tool to determine the value of surgery after NACT. Larger prospective studies are warranted to validate our results.

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References

1. Chang WJ, Du Y, Zhao X, Ma LY, Cao GW. Inflammation-related factors predicting prognosis of gastric cancer. *World J Gastroenterol* 2014; 20 (16): 4586-96.
2. Cunningham D, Allum WH, Stenning SP, et al; MAGIC Trial Participants. Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. *N Engl J Med* 2006; 355 (1): 11-20.
3. Shapiro J, van Lanschot JJB, Hulshof MCCM, et al; CROSS study group. Neoadjuvant chemoradiotherapy plus surgery versus surgery alone for oesophageal or junctional cancer (CROSS): long-term results of a randomised controlled trial. *Lancet Oncol* 2015; 16 (9): 1090-8.
4. Schuhmacher C, Gretschel S, Lordick F, et al. Neoadjuvant chemotherapy compared with surgery alone for locally advanced cancer of the stomach and cardia: European Organisation for Research and Treatment of Cancer randomized trial 40954. *J Clin Oncol* 2010; 28 (35): 5210-8.
5. Sánchez de Molina ML, Díaz Del Arco C, Vorwald P, García-Olmo D, Estrada L, Fernández-Aceñero MJ. Histopathological factors predicting response to neoadjuvant therapy in gastric carcinoma. *Clin Transl Oncol* 2018; 20 (2): 253-7.
6. Zager Y, Horesh N, Dan A, et al. Associations of novel inflammatory markers with long-term outcomes and recurrence of diverticulitis. *ANZ J Surg* 2020; 90 (10): 2041-5.

7. Zager Y, Hoffman A, Dreznik Y, et al. Cytoreductive surgery plus hyperthermic intraperitoneal chemotherapy in patients with peritoneal carcinomatosis from colorectal cancer: The prognostic impact of baseline neutrophil-lymphocyte, platelet-lymphocyte and lymphocyte-monocyte ratios. *Surg Oncol* 2020; 35: 321-7.
8. Cannon NA, Meyer J, Iyengar P, et al. Neutrophil-lymphocyte and platelet-lymphocyte ratios as prognostic factors after stereotactic radiation therapy for early-stage non-small-cell lung cancer. *J Thorac Oncol* 2015; 10 (2): 280-5.
9. Koh CH, Bhoo-Pathy N, Ng KL, etal. Utility of pre-treatment neutrophil-lymphocyte ratio and platelet-lymphocyte ratio as prognostic factors in breast cancer. *Br J Cancer* 2015; 113 (1): 150-8.
10. Cho IR, Park JC, Park CH, et al. Pre-treatment neutrophil to lymphocyte ratio as a prognostic marker to predict chemotherapeutic response and survival outcomes in metastatic advanced gastric cancer. *Gastric Cancer* 2014; 17 (4): 703-10.
11. Sun J, Chen X, Gao P, Song Y, Huang X, et al. Can the neutrophil to lymphocyte ratio be used to determine gastric cancer treatment outcomes? A systematic review and meta-analysis. *Dis Markers* 2016; 2016: 7862469.
12. Miyamoto R, Inagawa S, Sano N, Tadano S, Adachi S, Yamamoto M. The neutrophil-to-lymphocyte ratio (NLR) predicts short-term and long-term outcomes in gastric cancer patients. *Eur J Surg Oncol* 2018; 44 (5): 607-12.
13. Zahorec R. Ratio of neutrophil to lymphocyte counts--rapid and simple parameter of systemic inflammation and stress in critically ill. *Bratisl Lek Listy* 2001; 102 (1): 5-14. [Slovak].
14. Luo G, Guo M, Liu Z, et al. Blood neutrophil-lymphocyte ratio predicts survival in patients with advanced pancreatic cancer treated with chemotherapy. *Ann Surg Oncol* 2015; 22 (2): 670-6.
15. Wang F, Liu ZY, Xia YY, et al. Changes in neutrophil/lymphocyte and platelet/lymphocyte ratios after chemotherapy correlate with chemotherapy response and prediction of prognosis in patients with unresectable gastric cancer. *Oncol Lett* 2015; 10 (6): 3411-18.
16. Clavien PA, Barkun J, de Oliveira ML, et al. The Clavien-Dindo classification of surgical complications: five-year experience. Ann Surg 2009; 250 (2): 187-96.
17. Amin MB, Greene FL, Edge SB, et al. The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. *CA Cancer J Clin* 2017; 67 (2): 93-99.
18. Quéro L, Guillerm S, Hennequin C. Neoadjuvant or adjuvant therapy for gastric cancer. *World J Gastrointest Oncol* 2015; 7 (8): 102-10.
19. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; 144 (5): 646-74.
20. Goeppert B, Frauenschuh L, Zucknick M, et al. Prognostic impact of tumour-infiltrating immune cells on biliary tract cancer. *Br J Cancer* 2013; 109 (10): 2665-74.
21. Mignot G, Hervieu A, Vabres P, et al. Prospective study of the evolution of blood lymphoid immune parameters during dacarbazine chemotherapy in metastatic and locally advanced melanoma patients. *PLoS One* 2014; 9 (8): e105907.
22. Jin H, Zhang G, Liu X, et al. Blood neutrophil-lymphocyte ratio predicts survival for stages III-IV gastric cancer treated with neoadjuvant chemotherapy. *World J Surg Oncol* 2013; 11: 112.
23. Chen L, Zuo Y, Zhu L, et al. Peripheral venous blood neutrophil-to-lymphocyte ratio predicts survival in patients with advanced gastric cancer treated with neoadjuvant chemotherapy. *Onco Targets Ther* 2017; 10: 2569-80.
24. Chen Y, Chen K, Xiao X, et al. Pretreatment neutrophil-to-lymphocyte ratio is correlated with response to neoadjuvant chemotherapy as an independent prognostic indicator in breast cancer patients: a retrospective study. *BMC Cancer* 2016; 16: 320.
25. Glazer ES, Rashid OM, Pimiento JM, Hodul PJ, Malafa MP. Increased neutrophil-to-lymphocyte ratio after neoadjuvant therapy is associated with worse survival after resection of borderline resectable pancreatic ductal adenocarcinoma. *Surgery* 2016; 160 (5): 1288-93.

Hofstadter's Law: It always takes longer than you expect, even when you take into account Hofstadter's Law.

Douglas Hofstadter (born 1945), American professor of cognitive science, physics, and comparative literature

We're here to put a dent in the universe.

Steve Jobs (1955–2011), American entrepreneur, businessman, inventor, and industrial designer.
He was the co-founder, chairman and chief executive officer (CEO) of Apple Inc.

Capsule

Neutrophils and emergency granulopoiesis drive immune suppression and an extreme response endotype during sepsis

Sepsis arises from diverse and incompletely understood dysregulated host response processes following infection that leads to life-threatening organ dysfunction. **Kwok** et al. showed that neutrophils and emergency granulopoiesis drove a maladaptive response during sepsis. The authors generated a whole-blood single-cell multiomic atlas (272,993 cells, n=39 individuals) of the sepsis immune response that identified populations of immunosuppressive mature and immature neutrophils. In co-culture, CD66b+ sepsis neutrophils inhibited proliferation and activation of CD4+ T cells. Single-cell multiomic mapping of circulating hematopoietic stem and progenitor cells (HSPCs) (29,366 cells, n=27) indicated altered granulopoiesis in patients with sepsis. These features were enriched in a patient subset with poor outcome and a specific sepsis response signature that displayed higher frequencies of *IL1R2*+ immature neutrophils, epigenetic and transcriptomic signatures of emergency granulopoiesis in HSPCs and STAT3-mediated gene regulation across different infectious etiologies and syndromes. These findings offer potential therapeutic targets and opportunities for stratified medicine in severe infection.

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Eitan Israeli