Development of prognostic predictive model with neutrophil–lymphocyte ratio (NLR) in patients with gastric signet ring carcinoma

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Abstract
The risk factors have not been well-defined for prognosis in gastric signet ring cell carcinoma (GSRC) patients. This study is designed to prognosticate survival in GSRC patients by establishing and verifying a predictive model with neutrophil–lymphocyte ratio (NLR). A total of 147 GSRC patients from Department of Surgical Oncology, Neimenggu Baogang Hospital, Inner Mongolia Medical University were retrospectively reviewed. A predictive model was established using Cox proportional hazards. The performance of the model was evaluated by ROC curves.

In present study, we found that overall survival (OS) (P < .001, Fig. 1A) and tumor recurrence rate (P = .036, Fig. 1B) in the NLR < 2.8 group were significantly better than those in the NLR > 2.8 group. These results showed that NLR < 2.8 was significant prognostic factor related with both OS and tumor recurrence in patients with GSRC. After adjusting for competing risk factors, NLR < 2.8 (hazard ratio [HR]: 2.625, 95% confidence interval [CI]: 1.505–5.3166, P = .003), tumor size (HR: 3.024, 95% CI: 1.521–4.186, P = .005), and tumor metastasis (HR: 3.303, 95% CI: 1.25–4.525, P = .012) remained independent predictors of tumor recurrence rate and OS. Our results showed that comparing with the model without NLR (area under ROC curve: 0.798), the model with NLR (area under ROC curve: 0.826) had significant better predictive power than the model without NLR, which further confirmed the value of NLR in predicting prognosis of patients with GSRC.

In conclusion, a high NLR value independently predicts poor survival in patients with GSRC after surgery. The NLR may help oncologists evaluate outcomes of patients received surgical resection and chemotherapy in order to choose alternative therapies for patients with high NLR value.

Abbreviations: AUC = area under ROC curve, CI = confidence interval, EGC = early gastric cancer, ESD = endoscopic submucosal dissection, GC = gastric cancer, GSRC = gastric signet ring cell carcinoma, HR = hazard ratio, NLR = neutrophil–lymphocyte ratio, OS = overall survival, TTR = tumor recurrence rate.

Keywords: GSRC, neutrophil–lymphocyte ratio, prognosis

1. Introduction
Gastric cancer (GC), a high incidence and mortality disease, topped the public health problems worldwide. Signet ring cell carcinoma with abundant intracytoplasmic mucin in cells, which to be reported has more aggressive biological behavior and poor prognosis. Although the prevalence of GC has gradually declined in recent decades, the incidence of gastric signet ring cell carcinoma (GSRC) is still increasing.

Additionally, the associations of inflammation-based scores with the prognosis of PC have been actively explored. Inflammatory response plays a vital role in tumor progression including initiation, promotion, malignant conversion, invasion, and metastasis. Based on these factors, several inflations and immune-based prognostic scores such as lymphocyte count, platelet–lymphocyte ratio, and neutrophil–lymphocyte ratio (NLR) have been developed to predict the inflammatory response being associated with poor survival and recurrence in different types of cancer, including GSRC. An increasing body of evidence shows that systemic inflammation activation exerted by cancer cells anticipates tumor progression via inducing cancer proliferation and metastasis or promoting angiogenesis. The NLR, which has been considered as a member of the marker of the systemic inflammation response, is valuable for predicting the prognosis of various cancers. However, these indexes did not comprehensively reflect the balance of host inflammatory and immune status. Challenges remain in order to identify reliable, cost-effective biomarkers to identify which patients are most likely to receive therapeutic benefit from surgery for GCs.

Increasing evidence shows that systemic inflammatory activation caused by cancer cells can induce cancer cell proliferation, metastasis, or promote angiogenesis. NLR is considered to be one of the markers of systemic inflammatory response and is valuable for predicting the prognosis of various cancers. Studies have
shown that in patients with GSRC who have undergone chemotherapy or surgical resection, elevated NLR before treatment is associated with poor prognosis. In the present study, we evaluated the prognostic value of NLR in patients with GSRC who received curative resection. Moreover, we also analyzed the predictive values between the models with or without NLR.

2. Patients and methods

2.1. Study design and participants

The cohort consisted of 147 consecutive patients with GSRC identified retrospectively from January 1, 2015 to July 30, 2019. The study was approved by the Regional Ethical Review Board for Department of Surgical Oncology, Neimenggu Baogang Hospital, Inner Mongolia Medical University. Patients were treated according to the Declaration of Helsinki's ethical principles for medical research involving human subjects. All patients provided an informed written consent prior to study entry. Patients were required to meet the following inclusion criteria: participants were aged 18 to <80 years; Eastern Cooperative Oncology Group performance status\(^{16}\) was evaluated; the primary procedure was surgical resection, histologically or cytologically confirmed GSRC. No prior chemotherapy or immunotherapy was allowed. Patients were excluded if they had a concurrent malignancy other than GSRC, a serious, uncontrollable medical condition, or a psychiatric disorder that would limit ability to comply with study requirements.

2.2. Pre-treatment evaluation

Medical history and physical findings were documented in each patient. Each patient also had an ECG, computed tomography of the abdomen and pelvis (and thorax, if needed), serum chemistry and CBC, and urine analysis.

2.3. Procedures

All patients received surgical resection, while 98 patients received adjuvant chemotherapy and number of previous lines of palliative intent chemotherapy were recorded. Adverse events were assessed according to the National Cancer Institute's Common Terminology Criteria for Adverse Events version 4.0 (NCI-CTCAE v4.0) and response to treatment was assessed by the Response Evaluation Criteria in Solid Tumors (www.cancer.gov/).

2.4. Survival assessment

Overall survival (OS) was calculated from the time of surgery until death from any cause or last follow-up. Tumor recurrence was calculated as the time from surgery until tumor progression as determined by the treating physician, death from any cause, or last follow-up, whichever occurred first.

2.5. Statistical methods

Continuous variables were expressed as mean ± standard deviation and compared using a two-tailed unpaired Student t test; categorical variables were compared using \(\chi^2\) or Fisher analysis. The predictive performance of NLR was measured using the area under ROC curve (AUC)\(^{17}\). Life-table estimates of survival time were calculated according to the Kaplan and Meier methodology\(^{18}\). The Greenwood formula was used for the standard deviation. A Cox proportional hazards regression approach\(^{19}\) was chosen for the evaluation of tumor recurrence rate (TTR) and OS as the primary end-point. Potential prognostic variables were analyzed both univariately with 1 factor taken at a time, and then in a multivariate model combining all factors. Results are reported as hazard ratios (HRs) and their 95% confidence intervals (CIs). A HR >1 indicated an elevated risk with respect to the reference category. A CI which did not include the value 1 indicated statistical significance at the 5% level. All statistical evaluations were carried out using SPSS software (Statistical Package for the Social Science, version 13.0, SPSS Inc., Chicago, IL). A value of \(P < 0.05\) was considered to be statistically significant in all the analyses.

3. Results

3.1. Patients' characteristics

The 147 patients GSRC patients were retrospectively enrolled in this study from the hospital cohorts with 66.3% LNM rates,
to predict the OS of patients with GSRC. We measured the AUC of the Cox proportional hazards models and found that the model with NLR (AUC: 0.798) had a significantly better predictive power than the model without NLR (AUC: 0.826) in predicting prognosis of patients with GSRC.

### 3.4. Prognosis predictive model for patients with GSRC

We then used the results from Cox proportional hazards models to predict the OS of patients with GSRC. We measured the AUC to confirm the predictive values of different models with/without the variable of NLR. Our results showed that comparing with the model without NLR (AUC: 0.798), the model with NLR (AUC: 0.826) had significant better predictive power than the model without NLR (Fig. 3), which further confirmed the value of NLR in predicting prognosis of patients with GSRC.

### 4. Discussion

Consistent with previous conclusions, GSRC patients preferred to be more advanced stages with a large proportion of T3 and T4 (SEER cohort: 71.3%; YJS cohort: 65.9%). Prior studies reported that GSRC histology was independently associated with LNM status, patients of which have a significantly worse 5-year survival outcome than other types of GC, and have larger lesions and deeper infiltration and higher LNM rates than non-GSRC in advanced stage. Consistent with previous conclusions, GSRC patients have a high LNM rate (94.1%, 25/34) in advanced stage. Zu et al reported that the LNM rate of advanced GC was 56.8% (25/44). In our study, the LNM rate of GC patients in T2–4 stages was 75.9% (1558/2052), which was similar to previous conclusions. Recent studies showed that patients with lesions ≥2cm account for the majority proportion in early GC patients. Our research found that 60.8% (293/482) and 80.3% (2036/2534) patients had lesions >2cm in T1 and all patients in SEER cohort, respectively. Deeper infiltration and larger lesions had been reported to be independent risk factors of LNM in early GC patients. Naruhiko et al demonstrated that depth of infiltration and NLNE were independent predictors of LNM in T1–2 GC patients. Chen et al reported that deeper infiltration and larger lesions were independent risk factors of LNM in advanced GC patients. Our findings were similar to above conclusions.

Endoscopic resection for early gastric cancer (EGC) with low recurrence rates, has been widely used in Asian countries, and endoscopic submucosal dissection (ESD) is increasingly being considered for EGC in America. Endoscopic resection for EGC in NCCN guideline meets following criteria: well or moderately differentiated, ≤2 cm in diameter, negative margins (lateral and deep margins), lacking lymphovascular invasion, and limited to
the superficial submucosa. Therefore, poor and undifferentiated GSR (account for 97.3%) in the SEER cohort is not suitable for endoscopic resection. Signet ring cell carcinoma is defined as an undifferentiated carcinoma in Japan. Association Japanese Gastric Cancer demonstrated that the LNM rate of T1a undifferentiated GC (<2 cm) without and with ulcer were 0% (0/310, 95% CI = 0%−0.96%) and 2.9% (8/271, 95% CI = 1.2%−5.7%) in Japanese cohort, respectively. The former subgroup met the expanded indication for ESD; however, the latter subgroup was not recommended for ESD. Pokala et al pointed out that the LNM rate of T1a GSR (<2 cm) was 5.4%, and proposed that endoscopic resection could be considered for this cohort.

The mechanism underlying the potential prognostic value of NLR is mainly due to the significance of the infiltrated neutrophils and lymphocytes. The systemic inflammatory response from cancer cells promotes the infiltration of neutrophils, which benefits cancer progression via secreting interleukin-2, interleukin-6, interleukin-10, tumor necrosis factor α, and vascular endothelial growth factor. Vascular endothelial growth factor is a proangiogenic factor contributes to cancer development especially through angiogenesis. Moreover, increased tumor necrosis factor α and interleukin-10 issue in lymphocyte count decrease and lymphocyte dysfunction also. It is well known that lymphocyte depletion is likely reflection of an impaired T-lymphocyte-mediated antitumor response, which represents an adverse prognostic trait. In general, the relative ratio of elevated neutrophils and decreased lymphocytes could be a scientific marker for evaluating the systemic inflammatory response and outcome of individuals. And so, NLR is valuable as a potential indicator of prognosis to some degree.

By the way, there were several limitations of this study: on one hand, this is a study with small sample size and retrospective design. On the other hand, the relationship between survival and change of NLR after treatment apart from pre-treatment can be investigated in future studies.

In conclusion, a high NLR value independently predicts poor survival in patients with GSR after surgery. The NLR may help oncologists evaluate outcomes of patients received surgical resection and chemotherapy in order to choose alternative therapies for patients with high NLR value.

**References**

[1] Li Y, Zhu Z, Ma F, Xue L, Tian Y. Improving survival of stage II-III primary gastric signet ring cell carcinoma by adjuvant chemoradiotherapy. Cancer Med 2020;9:6617–28.

[2] Lin CL, Zhu GW, Huang YJ, Zheng W, Yang SG, Ye JX. Operable gastric adenocarcinoma with different histological subtypes: cancerspecific survival in the United States. Saudi J Gastroenterol 2020;26:46–52.

[3] Choi BH, Song HS, An YS, Han SU, Kim JH, Yoon JK. Relation between fluorodeoxyglucose uptake and glucose transporter-1 expression in gastric signet ring cell carcinoma. Nucl Med Mol Imaging 2011;45:30–5.

[4] Tabouret T, Dhooge M, Rouquette A, et al. Gastric signet ring cell adenocarcinoma: a distinct entity. Predis Med 2014;43(4 Pt 1):353–7.

[5] Fourgeaud C, Derieux S, Mirshahi S, et al. PO-23 - expression of heparanase in cancer as biomarker of malignancies: overexpression in an aggressive, poor survival gastric cancer “gastric signet ring cell carcinoma” compared with that of other gastric cancers. Thromb Res 2016;140(Suppl 1):S184–5.

[6] Fung BM, Patel M, Patel N, Brown AF, Ostrzega NL, Tabibian JH. Signet ring cell gastric carcinoma: clinical epidemiology and outcomes in a predominantly Latino County hospital population. Int Dis Sci 2020;66:1240–8.

[7] Li Y, Zhu Z, Ma F, Xue L, Tian Y. Gastric signet ring cell carcinoma: current management and future challenges. Cancer Manag Res 2020;12:7973–81.
[8] Vartolomei MD, Ferro M, Cantiello F, et al. Validation of neutrophil-to-lymphocyte ratio in a multi-institutional cohort of patients with T1G3 non-muscle-invasive bladder cancer. Clin Genitourin Cancer 2018;16:445–52.
[9] Vartolomei MD, Porav-Hodade D, Ferro M, et al. Prognostic role of pretreatment neutrophil-to-lymphocyte ratio (NLR) in patients with non-muscle-invasive bladder cancer (NMIBC): a systematic review and meta-analysis. Urol Oncol 2018;36:389–99.
[10] Vartolomei MD, Mathieu R, Margulis V, et al. Promising role of preoperative neutrophil-to-lymphocyte ratio in patients treated with radical nephroureterectomy. World J Urol 2017;35:121–30.
[11] Hainaut P, Plymoth A. Targeting the hallmarks of cancer: towards a rational approach to next-generation cancer therapy. Curr Opin Oncol 2013;25:50–1.
[12] Jaiswal M, LaRusso NF, Burgart LJ, Gores GJ. Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by a nitric oxide-dependent mechanism. Cancer Res 2000;60:184–90.
[13] He W, Yin C, Guo G, et al. Initial neutrophil lymphocyte ratio is superior to platelet lymphocyte ratio as an adverse prognostic and predictive factor in metastatic colorectal cancer. Med Oncol 2013;30:439.
[14] Kobayashi N, Usui S, Kikuchi S, et al. Preoperative lymphocyte count is an independent prognostic factor in node-negative non-small cell lung cancer. Lung Cancer 2012;75:223–7.
[15] Jung MR, Park YK, Jeong O, et al. Elevated preoperative neutrophil to lymphocyte ratio predicts poor survival following resection in late stage gastric cancer. J Surg Oncol 2011;104:504–10.
[16] Oken MM, Creech RH, Torrey DC, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982;5:649–55.
[17] Hanley JA. Receiver operating characteristic (ROC) methodology: the state of the art. Crit Rev Diagn Imaging 1989;29:307–35.
[18] Kaplan EL, Meier P. Nonparametric estimations from incomplete observations. J Am Stat Assoc 1958;53:457–81.
[19] Cox DR. Regression models and life-tables. J Royal Stat Soc B 1972;34:187–220.
[20] Bleaney CW, Barrow M, Hayes S, Ang Y. The relevance and implications of signet-ring cell adenocarcinoma of the oesophagus. J Clin Pathol 2018;71:201–6.
[21] de Aguiar VG, Segatelli V, Macedo ALV, et al. Signet ring cell component, not the Lauren subtype, predicts poor survival: an analysis of 198 cases of gastric cancer. Future Oncol 2019;15:401–8.
[22] Khan MR, Farooqi NB, Shahzad N. Is proximal gastric cancer a different entity from distal gastric cancer? Anatomical site distribution of signet ring cell carcinoma and its association with Helicobacter pylori infection. J Ayub Med Coll Abbottabad 2020;32:194–7.
[23] Lee YM, Kang SH, Kim JS, et al. Subepithelial spread of early gastric signet ring cell carcinoma: how far they can reach? Dig Dis 2020;38:442–8.
[24] He CL, Chen P, Xia BL, Xiao Q, Cai FL. Breast metastasis of gastric signet-ring cell carcinoma: a case report and literature review. World J Surg Oncol 2015;13:120.
[25] Schröder W, Bruns C. Gastric signet ring cell carcinoma: new aspects for long-range prognosis. Chirurg 2017;88:710.
[26] Xiong ZF, Shi J, Fu ZH, Wan HP, Tu LX. Phenotypic classification of gastric signet ring cell carcinoma and its relationship with K-ras mutation. Genet Mol Res 2017;16.
[27] Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet 2001;357:539–45.
[28] Kusumanto YH, Dam WA, Hospers GA, Meijer C, Mulder NH. Platelets and granulocytes, in particular the neutrophils, form important compartments for circulating vascular endothelial growth factor. Angiogenesis 2003;6:283–7.
[29] Salazar-Onfray F, Lopez M N, Mendoza-Naranjo A. Paradoxical effects of cytokines in tumor immune surveillance and tumor immune escape. Cytokine Growth Factor Rev 2007;18:171–82.
[30] Kobayashi N, Hiraoka N, Yamagami W, et al. FOXP3+ regulatory T cells affect the development and progression of hepatocarcinogenesis. Clin Cancer Res 2007;13:902–11.