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Circulating tumor cell with neutrophil to lymphocyte ratio in predicting efficacy of advanced gastric cancer

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Abstract: Objective To investigate the predictive effect of circulating tumor cell (CTC) combined with neutrophil to lymphocyte ratio (NLR) on advanced gastric cancer. **Methods** The clinicopathological data of 36 patients with advanced gastric cancer who received first-line treatment in Jiangning Hospital Affiliated to Nanjing Medical University and Jiangsu Cancer Hospital from June 2022 to January 2023 were collected. The optimal cutoff values of CTC and NLR were calculated using X-tile software, and the patients were divided into high CTC group (CTC>15, *n*=21) and low CTC group (CTC ⩽ 15, *n*=15), high NLR group (NLR>2.32, *n*=20) and low NLR group (NLR⩽2.32, *n*=16). Cox regression analysis and survival curve were used to analyze the relationship between CTC, NLR and progression free survival (PFS). Receiver operating characteristic (ROC) curves compared the predictive power of CTC, NLR, and the combination in predicting the outcome of advanced gastric cancer. **Results** Multivariate Cox regression analysis showed that CTC (*HR*=7.441, *P*=0.045) and NLR (*HR*=4.690, *P*=0.011) were associated with PFS in patients with advanced gastric cancer. Patients with low CTC and low NLR had longer PFS than those with high CTC and high NLR (*P*<0.01). Patients with both high CTC and high NLR had the shortest PFS (*P*<0.01). Compared with the single-factor prediction of CTC and NLR, the combination of CTC and NLR predicted the efficacy of first-line treatment for advanced gastric cancer best (AUC=0.746). **Conclusion** CTC and NLR are independent prognostic factors affecting PFS in patients with advanced gastric cancer, and CTC combined with NLR can better predict the efficacy of advanced gastric cancer.

Keywords: Circulating tumor cells; Neutrophil/lymphocyte ratio; Advanced gastric cancer; First-line treatment; Curative effect
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More than 80% of gastric cancer patients in China are already in advanced stage at the time of diagnosis. Currently, the first-line treatment regimens for advanced gastric cancer usually simple chemotherapy regimen (fluorouracil combined with cisplatinum and/or paclitaxel) and the combination therapy of chemotherapy and immunotherapy [3-4]. Despite the efficacy of these regimens, the prognosis of patients with advanced gastric cancer is still poor, so there is an urgent need to find more sensitive, convenient, and reliable tumor markers to predict the efficacy of patients with advanced gastric cancer receiving first-line treatment.

Circulating tumor cells (CTC) are a type of tumor cells that flow into the peripheral blood from primary or metastatic tumor sites, and their high heterogeneity can effectively evade immune monitoring and treatment, leading to distal metastasis of tumor cells [5-6]. Peripheral blood collection of CTC, as a non-invasive detection method, has been proved helpful for early diagnosis, recurrence monitoring, and prognosis prediction of malignant tumors, such as breast cancer, colon cancer, and gastric cancer [7-9]. Neutrophils and lymphocytes are the most common and available clinical indicators of immune inflammation, which are involved in tumor progression and immune response [10-11]. The neutrophil-to-lymphocyte ratio (NLR) can predict cancer prognosis and therapeutic response [12-13]. Recent studies have also shown that in malignant tumors such as prostate cancer, breast cancer, and colon cancer, combining circulating tumor cells with inflammatory indicators can predict patient prognosis better [14-16]. This paper will investigate the predictive role of CTC combined with NLR on the efficacy of patients with advanced gastric cancer by combining CTC and NLR.

1 Data and Methods

1.1 General data

Thirty-six patients with advanced gastric cancer who received first-line treatment from June 2022 to January 2023 in the Department of Oncology of Jiangning Hospital Affiliated to Nanjing Medical University and Jiangsu Provincial Cancer Hospital were selected.

Inclusion criteria: (1) Patients were diagnosed with stage IV gastric cancer by pathology; (2) Patients did not receive other previous treatment before receiving

first-line treatment; (3) The ECOG (Eastern Cooperative Oncology Group) score belongs to 0-1; (4) Patients' age >18 years old; (5) Patients received standard first-line chemotherapy as well as first-line immune-combination chemotherapy; (6) Patients could provide a complete medical record, including complete medical records, blood reports, tumor markers, inflammation indicators, and imaging reports.

Exclusion criteria: (1) Patients were combined with other malignant tumors; (2) Patients were combined with heart, brain, liver, kidney function and other serious organ function abnormalities; (3) Patients had autoimmune diseases and combined allergic diseases during treatment; (4) Patients were clearly defined acute infection or chronic infection before treatment; (5) Steroids and other treatments affecting the values of neutrophils and lymphocytes were used before treatment.

The first-line chemotherapy regimens included SOX (oxaliplatin + S-1) and XELOX (oxaliplatin + capecitabine). The immunotherapy in the first-line immune-combination chemotherapy included nivolumab and sintilimab, and the combination chemotherapy included XELOX and FOLFOX (5-fluorouracil + oxaliplatin). The related drug dosage and the frequency of use were according to the package insert. This study was reviewed by the Ethics Committee of Jiangning Hospital of Nanjing Medical University (Grant No. 2023-03-029-K01).

1.2 Data collection

The baseline data of the patients obtained from the hospital's electronic medical record system included: (1) age, gender, ECOG score, pathology type, TNM stage, presence of vascular invasion, and treatment regimen; (2) the baseline peripheral blood indicators before treatment, including carcinoembryonic antigen (CEA), carbohydrate antigen 199 (CA199), blood routine, and CTC. Data of CTC at baseline and routine haematology analysis were collected before the patients received the first treatment, and NLR was obtained by comparing the absolute neutrophil value with the absolute lymphocyte value before treatment.

1.3 CTC isolation and counting

A total of 7.5 mL of peripheral blood was collected one week before patients received their first treatment. Cells were enriched and isolated using immunomagnetic bead adsorption combined with the microfluidic microarray method from Beigel Medical Testing Laboratory (Changzhou Beshol CTC Detection System). Cell staining was performed by immunofluorescence in situ hybridization (FISH), and the number of CTC was counted in the 7.5mL of blood samples. The CTC were defined as: CK (cytokeratin) positive, CD45 (leukocyte common antigen 45) negative, and DAPI (4',6-diamidino-2-phenylindole) positive. Counts of CTC of < 5 were considered negative, and $\geqslant 5$ were considered positive. **[Figure 1]**

1.4 Follow-up

Outpatient reviews or telephone follow-ups were conducted every three months on patients discharged from the hospital. All patients were followed up until September 30, 2023, and no patient was lost to follow-up. The efficacy was assessed by CT imaging every 2-3 months using the solid tumor efficacy assessment (RECIST1.1). Progression-free survival (PFS) is the time from the patient's first treatment to disease progression or death from any cause.

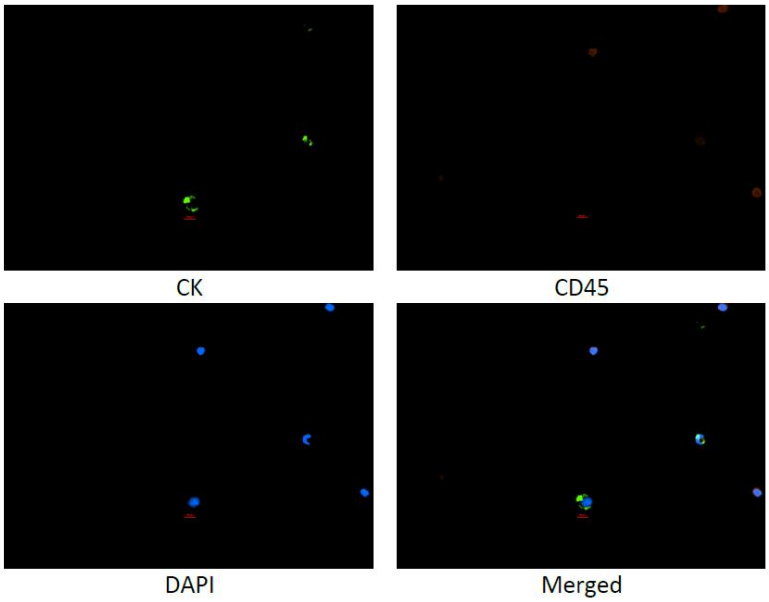

Fig.1 The picture of patient's CTC fluorescence staining

1.5 Statistical methods

Data were analyzed using SPSS 27.0 software and plotted using Graphpad-Prism 5.0 software. The optimal cut-off values of CTC and NLR were confirmed using X-tile software. Discrete data were expressed by cases, and comparisons were made using Fisher's exact test. Factors affecting PFS were analyzed using the Cox regression model, and significant factors in univariate Cox analysis were then included in multivariate Cox regression analysis. The Kaplan-Meier analysis was used to perform survival analysis and plot the curves. Differences in PFS between groups were assessed using the log-rank test. Receiver operating characteristic (ROC) curves were used to evaluate relevant indicators for predicting the prognosis of patients with advanced gastric cancer. P<0.05 was considered a statistically significant difference.

2 Results

2.1 Relationship between CTC, NLR and clinical characteristics of patients with advanced gastric cancer

The median progression-free time (mPFS) of 36 patients was 8.9 months, and the PFS of patients based on the RECIST1.1 standard was used as the state variable, and the CTC and NLR were used as the test variables, respectively. The X-tile software was used to draw the time-dependent ROC curves, which identified the optimal cut-off value of CTC as 15 and the optimal cut-off value of NLR as 2.32. Therefore, the patients were divided into high CTC group (CTC>15, n=21), low CTC group (CTC ≤15, n=15), high NLR group (NLR>2.32, n=20), and low NLR group (NLR ≤ 2.32, n=16) according to the optimal cut-off values. The differences in age, gender, and ECOG scores between the different CTC and NLR groups were not statistically significant (P>0.05). Differences in lymph node staging, CA199, and CEA were statistically significant between the low and high CTC groups (P<0.05), and differences in the degree of differentiation, presence or absence of vascular invasion, and therapies were statistically significant between the low and high NLR groups (P<0.05). **[Table 1]**

2.2 Cox regression analysis of PFS in patients with advanced gastric cancer receiving first-line treatment

Univariate Cox regression analysis showed that lymph node metastasis, vascular invasion, CTC, and NLR were the factors affecting PFS of advanced gastric cancer (P<0.05). The results of further multivariate Cox regression analysis showed that CTC (HR=7.441, P=0.045) and NLR (HR=4.690, P=0.011) were independent prognostic factors affecting PFS, and lymph node metastasis and vascular invasion were not significantly associated with PFS (P>0.05). **[Table 2].**

2.3 Survival curves for CTC, NLR and combined subgroups

Survival curves suggested that patients with high CTC had shorter PFS than those with low CTC (P<0.01). Patients with low NLR had longer PFS than those with high NLR (P<0.01). At the same time, further grouping was also performed by combining NLR and CTC, and patients were categorized into low CTC + low NLR group (n=10), low CTC + high NLR group (n=5), high CTC + low NLR group (n=6), and high CTC + high NLR group (n=15). Survival curves showed that patients in the high CTC + high NLR group had the shortest PFS(P<0.001). **[Figure 2]**

2.4 Combination of CTC and NLR to predict the prognosis of patients with advanced gastric cancer

The ROC curve suggested that the sensitivity of CTC in predicting the prognosis of patients was 70.59%, the specificity was 52.63%, and the area under the curve (AUC) was 0.695. The AUC of NLR was 0.693, with the sensitivity and the specificity of 64.71% and 57.89%, relatively. Both AUC values were greater than 0.5, suggesting an excellent predictive efficiency of a single indicator. The predictive efficacy of CTC combined with NLR was even higher, with an AUC of 0.746, a sensitivity of 76.47%, and a specificity of 63.16%. **[Figure 3]**

Tab.1 Relationship between CTC, NLR and clinical features in patients with advanced gastric cancer (case)

Item	Low CTC	High CTC	*P* value	Low NLR	High NLR	*P* value
Age (<60years /≥60 years)	7/8	10/11	1.000	7/9	10/10	0.749
Male/female	13/2	15/6	0.424	12/4	16/4	1.000
ECOG (0/1 point)	4/11	2/19	0.210	4/12	2/18	0.374
Degree of differentiation (high +medium/low)	5/10	1/20	0.063	6/10	0/20	0.004
Lymph node staging (N1/N2+N3)	11/4	4/17	0.002	9/7	6/14	0.175
Vascular invasion(yes/no)	9/6	6/15	0.090	11/5	4/16	0.006
Therapy (chemotherapy/ immune-based combination therapy)	9/6	12/9	1.000	13/3	8/12	0.019
CA199 (<27 u/mL/≥27 u/mL)	13/2	8/13	0.006	12/4	9/11	0.096
CEA (<5.2 ng/mL /≥5.2 ng/mL)	12/3	8/13	0.019	10/6	10/10	0.515

Tab.2 Univariate and multivariate COX analysis of PFS of advanced gastric cancer patients

Item	Case	Univariate Cox regression			Multivariate Cox regression		
		HR	*95%CI*	*P* value	*HR*	*95%CI*	*P* value
Gender							
Female	8	0.988	(0.357-2.736)	0.982	-	-	-
Male	28						
Age (Years)							
<60	17	0.974	(0.935-1.015)	0.205	-	-	
≥60	19						
Degree of differentiation							
High +medium	6	0.200	(0.027-1.500)	0.118	-	-	-
Low	30						
Lymph node staging							
N1	15	4.545	(1.447-14.278)	0.010	1.294	(0.31-5.397)	0.723
N2-N3	21						
Vascular invasion							
Yes	15	4.482	(1.583-12.691)	0.005	2.729	(0.878-8.482)	0.083
No	21						
Therapy							
Chemotherapy	21	1.767	(0.732-4.262)	0.205	-	-	-
Immune-based combination therapy	15						
CTC							
≤15	15	9.976	(2.257-44.099)	0.002	7.441	(1.048-52.847)	0.045
>15	21						
NLR							
≤2.32	16	6.915	(2.261-21.146)	0.001	4.690	(1.426-15.426)	0.011
>2.32	20						

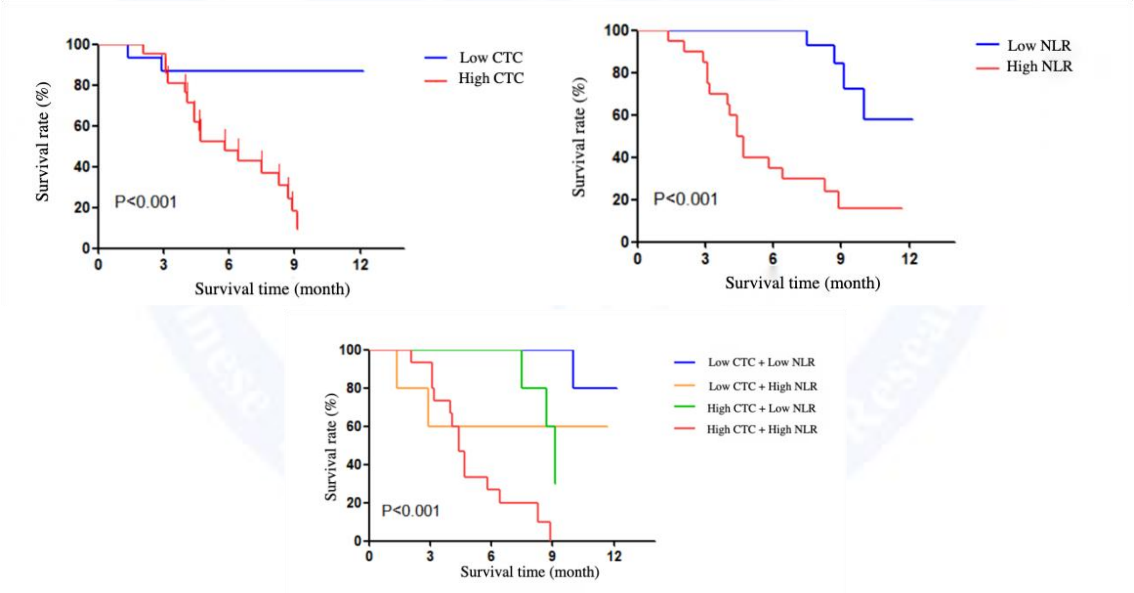

Fig 2 PFS survival curves of advanced gastric cancer patients in different CTC groups and NLR groups

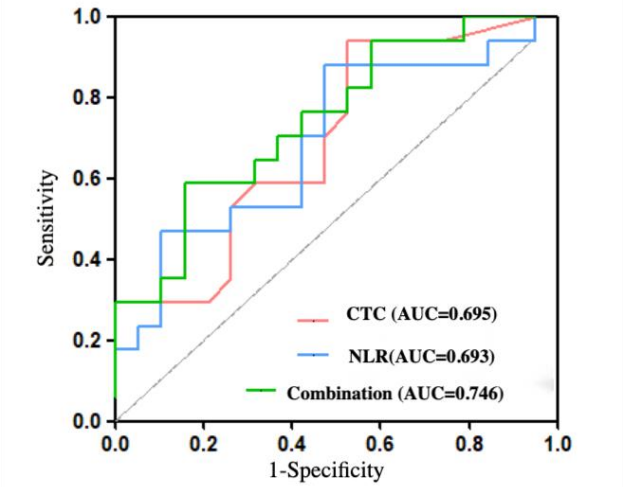

Fig.3 The ROC curves of CTC, NLR and their combination

3 Discussion

China is a country with a high incidence of gastric cancer, and the early diagnosis rate of patients is low. Many patients are in the advanced stage at the first diagnosis [17]. In recent years, newly developed immune checkpoint inhibitors such as nivolumab and pembrolizumab have been gradually incorporated into the first-line treatment regimen for advanced gastric cancer. However, the recurrence rate and metastasis rate of patients with advanced gastric cancer are still high after treatment, and the survival is still poor [18-19]. Therefore, searching for new peripheral blood markers plays a vital role in assessing the therapeutic efficacy of advanced gastric cancer and guiding subsequent treatment.

CTC has been applied to solid tumors with predominantly hematogenous metastasis for many years and has been approved by the US. FDA as a biomarker for monitoring the prognosis of patients with breast, lung, and colorectal cancers [20-21]. However, for malignant tumors whose metastatic modes are mainly lymph node metastasis and abdominopelvic metastasis rather than hematogenous metastasis, the number of gastric cancer cells entering the body circulation is less than that of other cancers with predominantly hematogenous metastasis. The result often shows a high rate of false-negative. With the development of detection, the CTC detection rate in gastric cancer patients' peripheral blood has significantly increased. In a latest study of 2023, the CTC-positive rate in patients with advanced gastric cancer can exceed 80% [22]. Our research took more than 5 CTCs per 7.5 mL of blood as the criterion for positivity. The positivity rate of CTC at baseline measured in patients with advanced gastric cancer was as high as 83.3% (30/36), which further supports the possibility of CTC being a potential marker in gastric cancer. A positive or high baseline CTC usually indicates poor efficacy or poor prognosis. This paper used 15 as the optimal cut-off value for CTC, and a high CTC was an independent factor in influencing PFS in patients receiving first-line treatment. Patients with a higher CTC had a shorter PFS and poorer efficacy.

In addition to exploring the role of CTC in predicting efficacy in advanced gastric cancer, this paper also explores the role of inflammatory indicators in predicting efficacy. As a classical indicator of peripheral blood inflammation, NLR can be obtained simply by performing routine blood tests, which are simple and easy. Sun *et al.* demonstrated that the NLR was correlated with patients' prognosis in gastric cancer, and patients with a normal NLR had a more prolonged overall survival (OS) than those with a high NLR [23]. In a study by Wu *et al.*, NLR was also correlated with response to treatment, with high NLR predicting poorer efficacy [24]. CTC can also interact with immune inflammatory cells to promote tumor metastasis and accelerate the death of patients [25]. In patients with breast cancer, CTC can form CTC-neutrophil clusters with neutrophils, which help tumor cells metastasize by regulating the cell cycle [26]. Therefore, many studies have also focused on combining inflammatory indicators with CTC to predict tumor efficacy and prognosis. The results of this paper showed that NLR, like CTC, could also be used as an independent prognostic factor to predict PFS in advanced gastric cancer. Survival analysis suggested that patients with high CTC and NLR had the shortest PFS and the poorest clinical efficacy. The ROC curve indicated that the accuracy of CTC combined with NLR for predicting the efficacy of patients with advanced gastric cancer was significantly higher than that of either index alone. This revealed that CTC combined with NLR could help better predict treatment efficacy in advance and guide the subsequent treatment.

NLR has a predictive role in malignant tumors undergoing immunotherapy. In colorectal cancer patients undergoing immunotherapy, both the low NLR at baseline and the decrease in NLR after treatment suggest a good prognosis and a significantly longer OS [27]. In recent years, some multi-center phase III clinical studies have demonstrated the advantages of immune-based combination chemotherapy as a first-line therapy in patients with advanced gastric cancer [28]. This paper also

included some patients who received immune-based combination chemotherapy as first-line treatment. Among all these patients, there was a difference in the treatments between the high and low NLR groups, while there was no difference between the high and low CTC groups. Unfortunately, the small sample size of patients receiving immunotherapy in this paper is not enough for relevant subgroup analyses, and it is expected that more patients receiving immunotherapy will be included in the future to explore further the relationship between CTC, NLR and the efficacy of immunotherapy in advanced gastric cancer.

In conclusion, CTC combined with NLR can be used as a potential predictor of the efficacy of first-line treatment in patients with advanced gastric cancer, and provide a reference in clinical work. However, the follow-up period of this study is relatively short, and it is hard to analyze the OS of the patients. Long-term follow-up is still needed to explore the predictive value of CTC combined with NLR in OS at a later stage. Meanwhile, as a retrospective analysis with a small sample size, this paper still needs to expand the sample size for further validation.

Conflict of interest None

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循环肿瘤细胞联合中性粒细胞/淋巴细胞比值预测晚期胃癌疗效

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摘要：目的　探索循环肿瘤细胞（circulating tumor cell，CTC）联合中性粒细胞/淋巴细胞比值（neutrophil to lymphocyte ratio，NLR）对晚期胃癌疗效的预测作用。**方法**　收集 2022 年 6 月至 2023 年 1 月在南京医科大学附属江宁医院及江苏省肿瘤医院接受一线治疗的 36 例晚期胃癌患者的临床病理资料。通过 X-tile 软件计算 CTC 和 NLR 的最佳截断值，将患者分为高 CTC 组（CTC>15，$n=21$）和低 CTC 组（CTC≤15，$n=15$），高 NLR 组（NLR>2.32，$n=20$）和低 NLR 组（NLR≤2.32，$n=16$）。COX 回归分析和生存曲线用于分析 CTC，NLR 与无进展生存期（progression free survival，PFS）的关系。受试者工作特征（ROC）曲线比较 CTC、NLR 和两者联合对晚期胃癌疗效的预测效能。**结果**　多因素 COX 回归分析显示，CTC（$HR=7.441$，$P=0.045$）和 NLR（$HR=4.690$，$P=0.011$）与晚期胃癌患者的 PFS 有关。低 CTC 组和低 NLR 组患者的 PFS 分别长于高 CTC 组和高 NLR 组（$P<0.01$）。同时具有高 CTC 和高 NLR 的患者 PFS 最短（$P<0.01$）。与 CTC 和 NLR 单一因素预测相比，两者联合预测晚期胃癌一线治疗疗效最佳（AUC=0.746）。**结论**　CTC 和 NLR 是影响晚期胃癌患者 PFS 的独立预后因子，CTC 联合 NLR 能更好地预测晚期胃癌疗效。

关键词：循环肿瘤细胞；中性粒细胞/淋巴细胞比值；胃癌晚期；一线治疗；无进展生存期
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Abstract: Objective　To investigate the predictive effect of circulating tumor cell (CTC) combined with neutrophil to lymphocyte ratio (NLR) on efficacy of advanced gastric cancer. **Methods**　The clinicopathological data of 36 patients with advanced gastric cancer who received first-line treatment in Jiangning Hospital Affiliated to Nanjing Medical University and Jiangsu Cancer Hospital from June 2022 to January 2023 were collected. The optimal cutoff values of CTC and NLR were calculated using X-tile software, and the patients were divided into high CTC group (CTC>15, $n=21$) and low CTC group (CTC≤15, $n=15$), high NLR group (NLR>2.32, $n=20$) and low NLR group (NLR≤2.32, $n=16$). COX regression analysis and survival curve were used to analyze the relationship between CTC, NLR and progression free survival (PFS). Receiver operating characteristic (ROC) curves compared the predictive power of CTC, NLR, and the combination in predicting the outcome of advanced gastric cancer. **Results**　Multivariate COX regression analysis showed that CTC ($HR=7.441$, $P=0.045$) and NLR ($HR=4.690$, $P=0.011$) were associated with PFS in patients with advanced gastric cancer. Patients in CTC group and low NLR group had longer PFS than those in high CTC group and high NLR group, respectively ($P<0.01$). Patients with both high CTC and high NLR had the shortest PFS ($P<0.01$). Compared with the single-factor prediction, the combination of CTC and NLR predicted the

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efficacy of first-line treatment for advanced gastric cancer best (AUC = 0.746). **Conclusion** CTC and NLR are independent prognostic factors affecting PFS in patients with advanced gastric cancer, and CTC combined with NLR can better predict the efficacy of advanced gastric cancer.

Keywords: Circulating tumor cells; Neutrophil/lymphocyte ratio; Gastric cancer, advanced; First-line treatment; rogression free survival

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胃癌是世界上第四大导致癌症死亡的原因,也是我国第三大癌症死亡原因[1-2]。我国超过 80%胃癌患者诊断时便已经是晚期,目前晚期胃癌的一线治疗方案通常包括氟尿嘧啶辅以铂类和(或)紫杉醇的单纯化疗方案和应用免疫检查点抑制剂联合化疗的免疫+化疗方案[3-4]。尽管这些治疗方案有一定的疗效,但晚期胃癌患者的预后仍然较差,因此急需寻求更加灵敏、便捷、可靠的肿瘤标志物来预测接受一线治疗的晚期胃癌患者的疗效。

循环肿瘤细胞(circulating tumor cell, CTC)是从原发或转移的肿瘤部位流入外周血的一类肿瘤细胞,因具有高度异质性,能有效躲避免疫监测和治疗,导致肿瘤细胞的远端转移[5-6]。外周血采集 CTC 作为一种非侵入性检测方法,已被许多研究证明可用于乳腺癌、结肠癌和胃癌等恶性肿瘤的早期诊断、复发监测和预后预测[7-9]。中性粒细胞及淋巴细胞是临床上最常见易得的免疫炎症相关指标,参与了肿瘤的发生发展和免疫应答[10-11]。中性粒细胞/淋巴细胞比值(neutrophil to lymphocyte ratio, NLR)可用于肿瘤疗效及患者预后的预测[12-13]。新近的研究也表明在前列腺癌、乳腺癌、结肠癌等恶性肿瘤中,将循环肿瘤细胞与炎症指标结合可更好地预测患者预后[14-16]。本文旨在结合 CTC、NLR 这两个易得的外周血指标,探讨 CTC 联合 NLR 对晚期胃癌患者疗效的预测作用。

1　资料与方法

1.1　一般资料　选取 2022 年 6 月至 2023 年 1 月在南京医科大学附属江宁医院及江苏省肿瘤医院肿瘤科接受一线治疗的 36 例晚期胃癌患者。纳入标准:(1) 经病理学确诊的Ⅳ期胃癌患者;(2) 接受一线治疗前既往未接受其他治疗;(3) 美国东部肿瘤协作组(ECOG)0~1 分;(4) 年龄>18 岁;(5) 患者接受标准的一线化疗以及一线免疫联合化疗;(6) 治疗过程中能提供完整的病历资料、血常规报告、肿瘤标志物、炎症指标、影像学报告。排除标准:(1) 合并其他恶性肿瘤;(2) 合并心、脑、肝、肾功能等严重器官功能异常;(3) 接受治疗时有自身免疫性疾病及合并过敏性疾病;(4) 治疗前有明确的急性感染或慢性感染;(5) 治疗前采用类固醇等影响中性粒细胞、淋巴细胞数值的治疗。一线化疗方案包括 SOX(奥沙利铂+替吉奥)及 XELOX(奥沙利铂+卡培他滨),一线免疫联合化疗中的免疫治疗包括纳武利尤单抗及信迪利单抗,联合化疗包括 XELOX 及 FOLFOX(5-氟尿嘧啶+奥沙利铂),相关药物剂量及使用频次均根据说明书推荐使用。本研究通过南京医科大学附属江宁医院伦理委员会审查(批号:2023-03-029-K01)。

1.2　资料收集　根据医院电子病历系统得到的患者基线数据包括:(1) 年龄、性别、ECOG 评分、病理类型、TNM 分期、有无脉管侵犯、治疗方案;(2) 治疗前的基线外周血指标包括癌胚抗原(CEA),糖类抗原 199(CA199),血常规以及 CTC 计数。基线外周血 CTC 及血常规为患者接受第一次治疗前采集,NLR 通过治疗前的血常规中性粒细胞绝对值与淋巴细胞绝对值相比得到。

1.3　CTC 分离及计数　患者接受初次治疗前 1 周采集 7.5 mL 外周血,采用来自贝格尔医学检验实验室的免疫磁珠吸附结合微流控芯片法富集分离细胞(常州贝烁尔 CTC 检测系统),免疫荧光原位杂交法(FISH)进行细胞染色,计数 7.5 mL 血样中 CTC 数目。CTC 的定义为:CK(细胞角蛋白)阳性、CD45(白细胞共同抗原 45)阴性、DAPI(4′,6-二脒基-2-苯基吲哚)阳性。CTC 数值<5 个为阴性,≥5 个为阳性。见图 1。

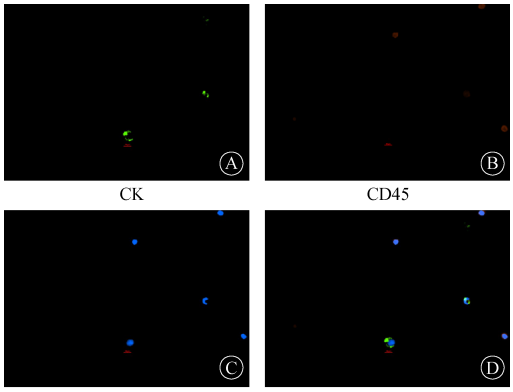

图 1　部分患者的 CTC 荧光染色图

Fig.1　The picture of patient's CTC fluorescence staining

1.4　随访　患者出院后每 3 个月门诊复查或电话随访 1 次，所有患者均随访至 2023 年 9 月 30 日，无患者失访。疗效采用实体瘤疗效评估（RECIST1.1），通过 CT 影像学每 2～3 个月进行一次疗效评估。无进展生存期（progression free survival，PFS）定义为患者从第一次接受治疗至疾病进展或因任何原因死亡的时间。

1.5　统计学方法　采用 SPSS 27.0 软件分析数据，Graphpad-Prism5.0 软件绘图。根据 X-tile 软件获取 CTC、NLR 的最佳截断值。计数资料以例表示，比较采用 Fisher 确切概率法。COX 风险回归模型法分析影响 PFS 的因素，将单因素 COX 分析有意义的因素纳入多因素分析。使用 Kaplan-Meier 法进行生存分析，绘制生存曲线，用对数秩检验（log-rank）分析组间 PFS 的差异。受试者工作特征曲线（ROC）用于判断相关指标预测晚期胃癌患者预后的能力。$P<0.05$ 为差异有统计学意义。

2　结　果

2.1　CTC、NLR 与晚期胃癌患者临床特征的关系　36 例患者的中位无进展时间（mPFS）为 8.9 个月，以依据 RECIST1.1 标准制定的患者的 PFS 作为状态变量，分别以 CTC 和 NLR 作为检验变量，运用 X-tile 软件绘制时间-ROC 曲线，得出 CTC 的最佳截断值为 15，NLR 的最佳截断值为 2.32，根据最佳截断值将患者分为高 CTC 组（CTC>15，$n=21$）和低 CTC 组（CTC≤15，$n=15$），高 NLR 组（NLR>2.32，$n=20$）和低 NLR 组（NLR≤2.32，$n=16$）。不同 CTC 和 NLR 组年龄、性别、ECOG 评分差异无统计学意义（$P>0.05$）。低 CTC 和高 CTC 组淋巴结分期、CA199、CEA 差异有统计学意义（$P<0.05$），低 NLR 和高 NLR 组分化程度、有无脉管侵犯和治疗方法差异有统计学意义（$P<0.05$）。见表 1。

2.2　晚期胃癌一线治疗患者 PFS 的 COX 回归分析　单因素 COX 回归分析显示淋巴结转移、脉管侵犯、CTC、NLR 为影响晚期胃癌 PFS 的因素（$P<0.05$），进一步行多因素 COX 分析结果显示，CTC（$HR=7.441$，$P=0.045$）和 NLR（$HR=4.690$，$P=0.011$）是影响 PFS 的独立预后因素，淋巴结转移、脉管侵犯与 PFS 无显著关联（$P>0.05$）。见表 2。

2.3　CTC、NLR 及联合分组的生存曲线　生存曲线提示，高 CTC 的患者比低 CTC 的患者生存期明显缩短（$P<0.01$）。与高 NLR 的患者相比，低 NLR 比值的患者的 PFS 更长（$P<0.01$）。同时结合 NLR 和 CTC 进行进一步分组，将患者分为低 CTC 低 NLR 组（$n=10$），低 CTC 高 NLR 组（$n=5$），高 CTC 低 NLR 组（$n=6$）以及高 CTC 高 NLR 组（$n=15$）。生存曲线显示高 CTC 高 NLR 组患者 PFS 最短（$P<0.01$）。见图 2。

2.4　CTC 与 NLR 联合预测晚期胃癌患者的预后　ROC 曲线提示，CTC 计数单独预测患者预后的灵敏度为 70.59%、特异度为 52.63%，曲线下面积（AUC）为 0.695；NLR 单独预测患者预后的灵敏度度 64.71%、特异度 57.89%，AUC 为 0.693，均大于 0.5，提示单一指标的预测效能良好。CTC 联合 NLR 的预测效能更高，AUC 为 0.746，灵敏度为 76.47%、特异度为 63.16%。见图 3。

表 1　CTC、NLR 与晚期胃癌患者临床特征的关系　（例）
Tab. 1　Relationship between CTC, NLR and clinical features in patients with advanced gastric cancer　(case)

项目	低 CTC 组	高 CTC 组	P 值	低 NLR 组	高 NLR 组	P 值
年龄（<60 岁/≥60 岁）	7/8	10/11	1.000	7/9	10/10	0.749
男/女	13/2	15/6	0.424	12/4	16/4	1.000
ECOG（0/1 分）	4/11	2/19	0.210	4/12	2/18	0.374
分化程度（中高分化/低分化）	5/10	1/20	0.063	6/10	0/20	0.004
淋巴结分期（N1/N2+N3）	11/4	4/17	0.002	9/7	6/14	0.175
脉管侵犯（无/有）	9/6	6/15	0.090	11/5	4/16	0.006
治疗方法（化疗/免疫联合化疗）	9/6	12/9	1.000	13/3	8/12	0.019
CA199（<27 u/mL/≥27 u/mL）	13/2	8/13	0.006	12/4	9/11	0.096
CEA（<5.2 ng/mL/≥5.2 ng/mL）	12/3	8/13	0.019	10/6	10/10	0.515

表 2　晚期胃癌患者 PFS 的单、多因素 COX 分析
Tab. 2　Univariate and multivariate COX analysis of PFS of advanced gastric cancer patients

项目	例数	单因素分析			多因素分析		
		HR	95%*CI*	*P* 值	*HR*	95%*CI*	*P* 值
性别							
女	8	0.988	0.357～2.736	0.982	—	—	—
男	28						
年龄（岁）							
<60	17	0.974	0.935～1.015	0.205	—	—	
≥60	19						
分化程度							
中、高分化	6	0.200	0.027～1.500	0.118	—	—	—
低分化	30						
淋巴结转移							
N1	15	4.545	1.447～14.278	0.010	1.294	0.31～5.397	0.723
N2～N3	21						
脉管侵犯							
无	15	4.482	1.583～12.691	0.005	2.729	0.878～8.482	0.083
有	21						
治疗方案							
化疗	21	1.767	0.732～4.262	0.205	—	—	—
免疫联合化疗	15						
CTC							
≤15	15	9.976	2.257～44.099	0.002	7.441	1.048～52.847	0.045
>15	21						
NLR							
≤2.32	16	6.915	2.261～21.146	0.001	4.690	1.426～15.426	0.011
>2.32	20						

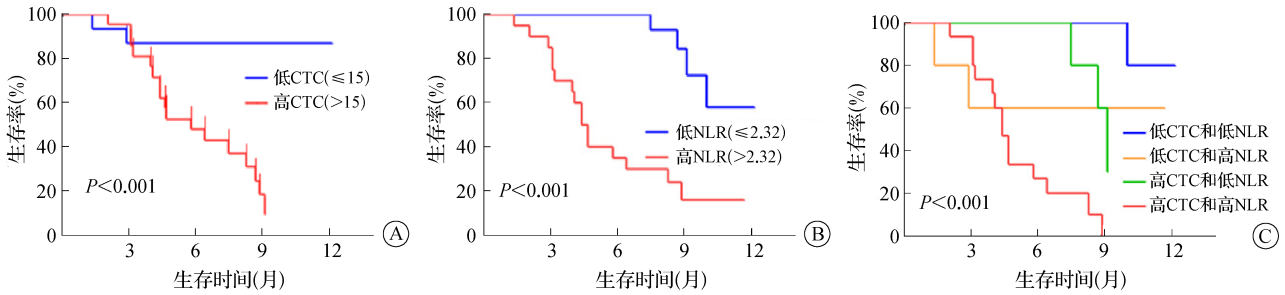

图 2　不同 CTC 组 NLR 组晚期胃癌患者的 PFS 生存曲线

Fig. 2　PFS survival curves of advanced gastric cancer patients in different CTC groups and NLR groups

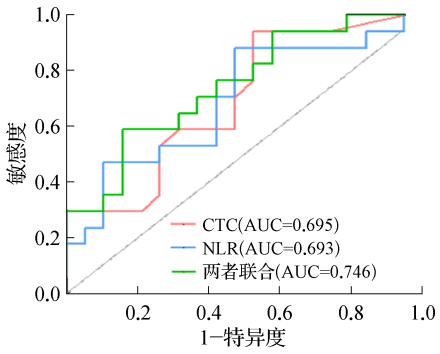

图 3　CTC,NLR 和两者联合的 ROC 曲线

Fig. 3　The ROC curves of CTC, NLR and their combination

3　讨　论

我国是胃癌高发国家,患者早期诊断率低,许多患者初诊即为晚期[17]。近年来,新兴研发的免疫检查点抑制剂如纳武利尤单抗,帕博利珠单抗已逐渐被纳入晚期胃癌的一线治疗方案,但晚期胃癌患者治疗后复发转移率仍然较高,生存结果仍不乐观[18-19]。因此寻找新的外周血标志物在帮助及时评估晚期胃癌的治疗疗效,指导临床后续治疗中起到重要作用。

CTC 多年前便已经应用于以血行转移为主的实体肿瘤中,并且已被美国 FDA 批准作为监测乳腺癌、肺癌、结直肠癌患者预后的生物标志物[20-21]。但作为转移方式主要为淋巴结转移和腹盆腔播散转移,而非血行转移的恶性肿瘤,胃癌细胞进入体循环的数量相比其他血行转移为主的癌种要少,检测结果经常出现假阴性率高的问题。而随着 CTC 检测技术的发展,胃癌患者外周血中 CTC 的检出率明显提高。尤其是在一项 2023 年最新的研究中,晚期胃癌患者 CTC 阳性率可超 80%[22]。本研究以每 7.5 mL 中测得超过 5 个 CTC 作为阳性标准,晚期胃癌患者外周血中测得的基线 CTC 阳性率高达 83.3%(30/36),进一步支持了 CTC 作为一种潜在标志物在胃癌中应用的可能。基线 CTC 阳性或计数较高通常预示着不佳疗效或较差预后,本文中以 15 作为 CTC 的最佳截断值,高 CTC 是接受一线治疗患者无进展生存期的独立影响因素,CTC 计数更高的患者的 PFS 更短,疗效更差。

除了探究 CTC 这一单一指标在晚期胃癌疗效预测中的作用外,本文还探究了炎症指标在疗效预测中的作用。NLR 作为一项经典的外周血炎症指标,只需进行血常规检查便可得到,简单易得。Sun 等的研究表明 NLR 在胃癌中与患者的预后相关,NLR 正常的胃癌患者比高 NLR 的患者总生存期更长[23]。在 Wu 等的研究中,NLR 还与治疗反应相关,高 NLR 预示着较差的疗效总生存期。CTC 可与免疫炎症细胞相互作用,促进肿瘤的转移,加速患者死亡[25]。在乳腺癌患者中,CTC 与中性粒细胞形成特殊的循环肿瘤细胞-中性粒细胞簇,通过调控细胞周期帮助肿瘤细胞转移[26]。因此,目前许多研究也聚焦于将炎症指标与 CTC 计数相结合,辅助预测肿瘤疗效及预后。本文结果显示,NLR 与 CTC 一样,也可以作为预测晚期胃癌 PFS 的独立预后因子。生存分析提示同时具有高 CTC 和高 NLR 的患者 PFS 最短,治疗疗效最差。ROC 曲线表示 CTC 联合 NLR 用于预测晚期胃癌患者疗效较任一指标单独应用,准确度明显提高,提示 CTC 联合 NLR 有助于更好地提前预测疗效,指导后续治疗。

NLR 在接受免疫治疗的恶性肿瘤中有一定的预测作用,在行免疫治疗的结直肠癌患者中,基线低 NLR 及治疗后 NLR 的下降均提示着预后好,总生存期明显延长[27]。近年来,一些大型多中心三期临床研究证明了免疫联合化疗作为一线治疗在晚期胃癌患者治疗中的优势[28]。本文也纳入了部分接受免疫联合化疗作为一线治疗的患者,在所有患者中,高、低

NLR 组间的治疗方法间存在差异,而高、低 CTC 组间治疗方法无差别。可惜的是,本文中接受免疫治疗的患者样本量较小,还不足以进行相关亚组分析,未来有望纳入更多接受免疫治疗的患者,进一步探索 CTC、NLR 与晚期胃癌免疫治疗疗效的关系。

综上,CTC 联合 NLR 可作为预测晚期胃癌患者一线治疗疗效的潜在预测指标,在临床工作中能有一定的参考价值。但本研究进行随访的时间较短,尚不能对患者的总生存期进行分析,后期需长期随访探究 CTC 联合 NLR 在总生存期中的预测价值。同时本文作为一个样本量较小的回顾性分析,仍需要扩大样本量进一步验证。

利益冲突　无

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