Oncology and Translational Medicine
DOI 10.1007/s10330-016-0130-3

April 2016, Vol. 2, No. 2, P75–P79

ORIGINAL ARTICLE

# Prognostic value of neutrophil-to-lymphocyte ratio in metastatic colorectal cancer*

**Qianqian Yu, Hong Qiu, Mingsheng Zhang, Guangyuan Hu, Liu Huang, Xianglin Yuan (✉)**

Department of Oncology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

## Abstract

**Objective** This study investigated the prognostic value of the neutrophil-to-lymphocyte ratio (NLR) in predicting clinical outcomes of patients with metastatic colorectal cancer (mCRC) undergoing irinotecan-based first-line chemotherapy.

**Methods** This study was based on a Chinese prospective multicenter trial (NCT01282658). Baseline complete blood cell counts were performed. Survival was determined using the Kaplan-Meier method. Multivariate analyses based on the Cox regression model were performed to determine the effects of independent biomarkers.

**Results** A total of 139 patients were evaluated. Values below the median NLR were associated with better progression-free survival (PFS) (9.9 vs 7.7 months, $P = 0.043$) and overall survival (OS) (21.8 vs 15.1 months, $P = 0.013$). These effects remained significant in multivariate analysis.

**Conclusion** NLR is an independent prognostic marker of mCRC treated with first-line irinotecan-based therapy in a Chinese population.

Received: 9 January 2016
Revised: 25 January 2016
Accepted: 15 February 2016

**Key words:** metastatic colorectal cancer (mCRC); outcome; neutrophil-to-lymphocyte ratio (NLR)

Representing 10% of all newly diagnosed cases annually, colorectal cancer (CRC) ranks among the top-three cancer types worldwide and is a major cause of cancer-related deaths [1]. In the last 20 years, several new active drugs, either in combination or as single agents, have broadened the spectrum of therapeutic options. Despite these management advances, the median overall survival (OS) of metastatic CRC (mCRC) patients remains less than 29 months and varies tremendously among different populations [2–3]. Therefore, there is immense interest in elucidating prognostic and predictive biomarkers that will permit classification of patients to improve clinical outcomes.

The proposed molecular biomarkers of mCRC include Kirsten ras oncogene homolog (KRAS); neuroblastoma RAS viral oncogene homolog (NRAS) [4]; B-Raf proto-oncogene, serine/threonine kinase [5]; dihydropyrimidine dehydrogenase [6]; P53; and thymidylate synthase [7]; however, only KRAS/NRAS have been generally used in clinical practice. Their limited clinical implementation may be partially due to the complexity of the testing methods and their low predictive accuracy.

The tumor microenvironment, particularly the inflammatory response, plays a crucial role in the development and progression of CRC [8]. Inflammatory response biomarkers have been incorporated into clinical outcome predictors for several malignancies [9]. The inflammatory response also features changes in the relative levels of circulating leukocytes. Increased neutrophil counts have may promote tumor growth and metastasis due to the role of these cells in immunosuppression and in the promotion of tumor angiogenesis [10]. In contrast, lymphocytes appear to be involved in the host immune response against cancer [11]. The neutrophil-to-lymphocyte ratio (NLR), a combination of circulating neutrophil and lymphocyte counts, has been associated with survival in several types of cancer, including hepatocellular carcinoma [12], pancreatic cancer [13], and CRC [14]. However, there are

✉ Correspondence to: Xianglin Yuan. Email: yxl@medmail.com.cn
* Supported by grants from the National Natural Science Foundation of China (No. 81372664 and No. 81001067), and the Natural Science Project of Hubei Province (No. 2013CFB114)

limited reports on the usefulness of NLR with respect to mCRC in Han Chinese populations.

In the present study, we investigated the association between circulating NLR and clinical outcome in a prospective series of patients with mCRC undergoing irinotecan-based first-line chemotherapy.

## Materials and methods

### Study patients

In this prospective longitudinal study, patients with histologically confirmed colorectal adenocarcinoma who were treated with irinotecan-based therapy were recruited consecutively at Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China) and another five cancer centers in Hubei province between November 2010 and December 2014. The selection criteria included histologically confirmed adenocarcinoma of the colon or rectum, unresectable metastases, age from 18 to 75 years, measurable disease defined according to the Response Evaluation Criteria In Solid Tumors version 1.1 (RECIST1.1) [15], no previous irinotecan exposure; no expected course of radiotherapy during first-line chemotherapy, Karnofsky index of performance status (KPS) ≥60 or Eastern Cooperative Oncology Group Performance Status Scale ≤ 2, total bilirubin ≤ 1.5 times the upper limit of normal (ULN), aspartate aminotransferase and alanine aminotransferase ≤ 2.5 times ULN (≤ 5 times ULN if liver metastases present), creatinine clearance > 50 mL/min, or serum creatinine ≤ 1.5 times ULN. Written informed consent was obtained, and blood samples were collected from each participant. This study was approved by the Ethical Committee of Huazhong University of Science and Technology under reference number NCT01282658 (registered at http://www.clinicaltrials.gov).

### Response and survival assessments

Patient responses were categorized using computed tomography or magnetic resonance imaging every 6-8 weeks according to RECIST1.1. Progression-free survival (PFS) and overall survival (OS) were chosen as the study end points. PFS was defined as the time from mCRC diagnosis to the first evidence of disease progression or to death, whichever occurred first. Data were censored if the patients were alive and progression-free at the last follow-up. OS was defined as the time from the mCRC diagnosis to death from any cause. Data were censored if the patients were alive at the last follow-up. All patients were followed up until death or for at least 36 months. Follow-up information was updated in February 2015.

### Baseline variables

The baseline clinical information collected prior to commencing chemotherapy included patient demographics, smoking status, KPS, tumor-related details, and medical history. Smoking status was coded as never, former, or current smoker, as described previously [16]. NLR was determined based on the absolute neutrophil count divided by the absolute lymphocyte count. Because peripheral hemograms are easily affected by various factors such as acute infections, post-traumatic stress reactions, cytotoxic drugs, and recombinant human granulocyte colony-stimulating factors, we performed complete blood cell counts within one month before the commencement of irinotecan-based therapy, with the exception of cases that showed clinical signs of sepsis or other inflammatory illnesses.

### Statistical analyses

All statistical analyses were performed using SPSS for Windows, version 16.0 (SPSS, Inc., USA). NLR were analyzed as categorical variables by setting the cut-off point as the median value among all study patients. Clinical outcomes, including OS and PFS, were calculated using the Kaplan-Meier method and evaluated using log-rank tests. The prognostic value of NLR on outcomes was analyzed using multivariate Cox regression models with adjustment for potential confounding covariates. Statistical significance was defined as $P < 0.05$ using a two-tailed test.

## Results

### Study population

A total of 139 NLR items were available for analysis. At the time of the most recent follow-up, 54.7% of the patients were deceased. Among those who were still alive, the mean duration of follow-up was 17 months (range, 5–50 months).

The baseline patient characteristics and tumor biological factors were shown in Table 1. The median age at the time of diagnosis was 49 years (range 18–72 years); 56.1% were males; 21.6% had a KPS of less than 80%; and 38.9% had smoked tobacco. One hundred patient cases were characterized as having a glandular histology, and 39 consisted of other subtypes, including 17 mucinous, four signet ring cell, and 12 mixed histological types, as well as six unspecified cases. Primary tumors proximal or distal to the splenic flexure were classified as right-sided ($n = 37$) or left-sided ($n = 102$), respectively, as described by Loupakis *et al* [17]. The median NLR was 2.24 (range 0.63–6.65).

Among 139 patients, 125 received the irinotecan with fluorouracil and folinic acid regimen, eight received the modified combination regimen of capecitabine and irinotecan, and six received only irinotecan. Response evaluations were available for 136 patients; three achieved com-

**Table 1** Demographic and clinical characteristics of the patients

| Characteristic | *n* | % |
|---|---|---|
| Age (years) | | |
| ≤ 49 | 75 | 54.0 |
| > 49 | 64 | 46.0 |
| Gender | | |
| Male | 78 | 56.1 |
| Female | 61 | 43.9 |
| KPS | | |
| ≥ 80% | 109 | 78.4 |
| < 80% | 30 | 21.6 |
| Smoking status | | |
| Never | 85 | 61.2 |
| Former | 19 | 13.7 |
| Current | 35 | 25.2 |
| Histology | | |
| Glandular | 100 | 71.9 |
| Others | 39 | 28.1 |
| Primary tumor | | |
| Right-sided | 37 | 26.6 |
| Left-sided | 102 | 73.4 |

plete responses (2.2%), 41 had partial responses (29.5%), 62 were stabilized (44.6.0%), and 30 had disease progression (21.6%). In three patient cases, the response could not be evaluated because of early cessation of chemotherapy (fewer than three cycles) due to insufferable toxicity or the interference of another anti-cancer therapy with the therapeutic effect.

### Patient- and tumor-related characteristics and outcomes

Potential associations between patient- and tumor-related characteristics and survival were tested using univariate analyses. As shown in Table 2, patients with a KPS of less than 80% had a higher risk of death ($P$ = 0.025), indicating that KPS was associated with OS. Left-sided tumors tended to be predictors for longer OS compared with right-sided tumors, but the correlation was not significant ($P$ = 0.052). No significant associations with PFS or OS were observed with other patient- or tumor-related characteristics.

### NLR and outcomes

We assessed the potential associations of NLR with PFS and OS using univariate Kaplan-Meier and multivariate Cox models (Table 3). The distributions of PFS and OS as a function of NLR (as a quantitative variable) were shown in Fig. 1a and Fig. 1b, respectively. An inverse correlation between survival and NLR was observed. For PFS, the regression coefficient was -1.636 ± 0.516, and the correlation coefficient was -0.238 ($P$ = 0.005). The OS regression coefficient was -2.933 ± 0.792, while the correlation coefficient was -0.284 ($P$ = 0.001). Age, gender, KPS, smoking status, and primary tumor site did not differ significantly between the two groups classified by NLR median value. In contrast, glandular cancer histology was associated with higher NLR values ($P$ = 0.016).

In the 139 patients, there were 108 (77.7%) and 76 (54.7%) OS and PFS events, respectively. NLRs lower than the median value were associated with better PFS in univariate (median PFS: 9.9 vs 7.7 months, $P$ = 0.043) and multivariate (HR, 1.559; 95% CI, 1.035-2.348; $P$ = 0.033) analyses. NLRs were also a predictor of OS: patients with lower NLRs showed a median OS of 21.8 months compared with 15.1 months for patients with higher NLRs ($P$ = 0.013). In multivariate analysis, lower NLRs remained significantly associated with increased OS (HR, 1.764; 95% CI, 1.089-2.856; $P$ = 0.021). Fig. 1c and 1d showed the PFS and OS curves associated with NLR, respectively.

## Discussion

In this prospective series of patients with mCRC, NLR could stratify the patient groups based on the observed 2.2-month increase in PFS and a 6.7-month longer OS

**Table 2** Univariate analyses of patient- and tumor-related characteristics predictive of survival

| Variables | PFS | | | OS | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | *P* | HR | 95% CI | *P* |
| Age (years) | | | | | | |
| ≤ 49 vs > 49 | 0.901 | 0.614–1.322 | 0.593 | 0.979 | 0.623–1.538 | 0.925 |
| Gender | | | | | | |
| Male vs female | 1.155 | 0.791–1.689 | 0.456 | 1.329 | 0.845–2.090 | 0.219 |
| KPS | | | | | | |
| ≥ 80% vs < 80% | 1.385 | 0.886–2.165 | 0.153 | 1.784 | 1.075–2.961 | 0.025 |
| Smoking status | 0.865 | 0.583–1.283 | 0.471 | 0.760 | 0.468–1.234 | 0.267 |
| Histology | | | | | | |
| Glandular vs others | 0.911 | 0.600–1.384 | 0.662 | 0.800 | 0.480–1.334 | 0.393 |
| Primary tumor | | | | | | |
| Right- vs left-sided | 1.155 | 0.751–1.775 | 0.512 | 0.623 | 0.387–1.004 | 0.052 |

Abbreviations: HR, hazard ratio; CI, confidence interval

**Table 3** Associations between NLR and survival in the univariate and multivariate models

| Variables | *n* | Univariate analysis | | | Multivariate analysisa | |
|---|---|---|---|---|---|---|
| | | No. of events | Median survival (95% CI) | *P* | HR (95% CI) | *P* |
| PFS | | | | | | |
| NLR ≤ Median | 70 | 55 | 9.9 (7.3–12.5) | 0.043 | 1.000 | 0.033 |
| NLR > Median | 69 | 53 | 7.7 (5.6–9.8) | | 1.559 (1.035–2.348) | |
| OS | | | | | | |
| NLR ≤ Median | 70 | 35 | 21.8 (17.6–26.0) | 0.013 | 1.000 | 0.021 |
| NLR > Median | 69 | 41 | 15.1 (9.1–21.0) | | 1.764 (1.089–2.856) | |

in the patient group with NLRs below the median value. This degree of difference in PFS and OS stratified by simple blood test findings is clinically significant.

These results are consistent with previous observations on the association of NLR with a variety of cancers [18]. More than three decades ago, lymphocytes were reported to play a role in defense mechanisms against solid tumors in murine models [19]. One decade later, an increase in the number of mature polymorphonuclear cells was found to be associated with poor prognosis in patients with several non-hematological malignancies [20]. Numerous functional studies have demonstrated that neutrophils affect the tumor microenvironment to promote tumor progression and metastasis via their proangiogenic activity [21] and ability to modulate the biology of tumor cells [22]. Conversely, lymphocytes, immunological cells derived from the lymphoid lineage, are more likely to defend the host against abnormal cells through the immune response [11].

Numerous predictive and prognostic biomarkers for CRC have emerged, but none have been adopted as a predictor for targeting irinotecan-based therapy. Common variants of uridine diphosphate glucuronosyltransferase 1A have been associated with toxicity outcomes in patients treated with irinotecan; however, their prognostic value in predicting clinical outcomes remains controversial [23–24]. Compared with aspiration and biopsy, which are not routinely performed for the diagnosis of recurrent disease, evaluation of biomarkers in the peripheral blood will be much easier and more convenient in clinical practice.

Emerging evidence suggests that clinical outcomes are strongly affected by host response, particularly through systemic inflammation. A growing body of evidence in recent years has supported use of immune-based cancer therapy, including nonspecific immune stimulation, antitumor monoclonal antibodies, cancer vaccines, and the adoptive transfer of ex vivo-activated T and natural killer cells [25]. After further validation, the marker identified as a predictor for the risk of disease progression and death in the current study may be useful in selecting candidates

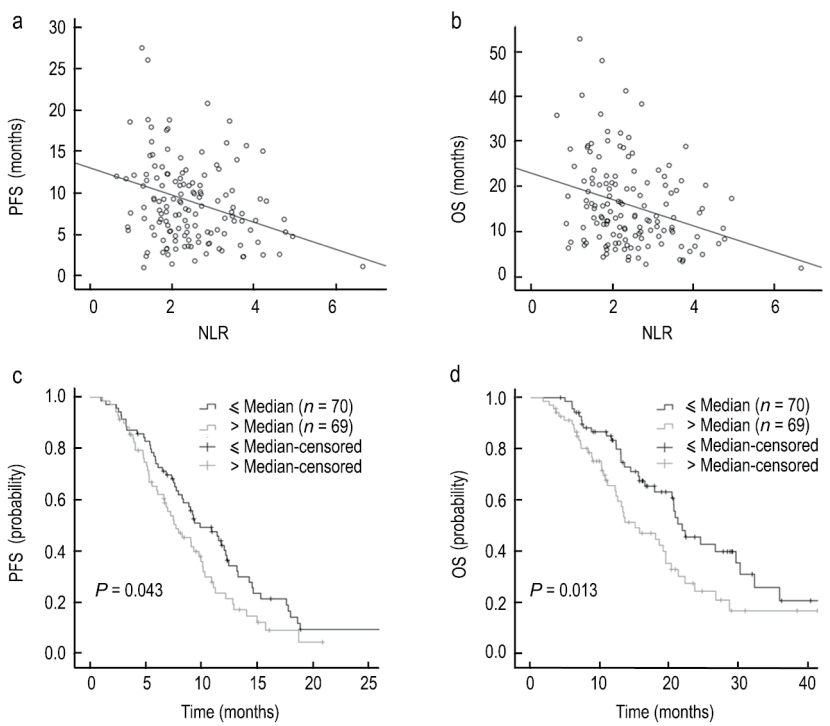


Fig. 1 Prognostic value of the NLR in metastatic colorectal cancer. Survival as a function of NLR (as a quantitative variable) in the patients ($n$ = 139). A linear regression line was shown for (a) PFS and NLR (correlation coefficient $r$ = -0.238, $P$ = 0.005) and for (b) OS and NLR (correlation coefficient $r$ = -0.284, $P$ = 0.001). Kaplan-Meier curves of the estimated survival classified by the median NLR value for PFS (c) and OS (d). The $P$ values denote comparisons of two groups by the log-rank test

for additional immunotherapeutic interventions.

Although our hypothesis was confirmed by the present results, the current study has several limitations. Only 139 patient cases were available for the NLR analyses. Because of the limited number of samples, we were unable to perform further subgroup analysis of associations. Thus, the results of the current study require further validation in future, large-scale prospective studies.

In conclusion, we confirmed the prognostic value of NLR in a Chinese prospective multicenter longitudinal study of irinotecan-based chemotherapy. With validation, NLR could be used as a predictor for survival outcome in Chinese patients with mCRC.

### Conflicts of interest

The authors indicated no potential conflicts of interest.

## References


1. Torre LA, Bray F, Siegel RL, *et al.* Global cancer statistics, 2012. CA Cancer J Clin, 2015, 65: 87–108.
2. Grothey A, Van Cutsem E, Sobrero A, *et al*. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. Lancet, 2013, 381: 303–312.
3. Li J, Qin S, Xu R, *et al.* Regorafenib plus best supportive care versus placebo plus best supportive care in Asian patients with previously treated metastatic colorectal cancer (CONCUR): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol, 2015, 16: 619–629.
4. Douillard JY, Oliner KS, Siena S, *et al*. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. N Engl J Med, 2013, 369: 1023–1034.
5. Maughan TS, Adams RA, Smith CG, *et al*. Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of advanced colorectal cancer: results of the randomised phase 3 MRC COIN trial. Lancet, 2011, 377: 2103–2114.
6. Koopman M, Venderbosch S, van Tinteren H, *et al*. Predictive and prognostic markers for the outcome of chemotherapy in advanced colorectal cancer, a retrospective analysis of the phase III randomised CAIRO study. Eur J Cancer, 2009, 45: 1999–2006.
7. Paradiso A, Simone G, Petroni S, *et al.* Thymidilate synthase and p53 primary tumour expression as predictive factors for advanced colorectal cancer patients. Br J Cancer, 2000, 82: 560–567.
8. Coussens LM, Werb Z. Inflammation and cancer. Nature, 2002, 420:860-867.
9. Abu-Remaileh M, Bender S, Raddatz G, *et al.* Chronic inflammation induces a novel epigenetic program that is conserved in intestinal adenomas and in colorectal cancer. Cancer Res, 2015, 75: 2120–2130.
10. Dumitru CA, Lang S, Brandau S. Modulation of neutrophil granulocytes in the tumor microenvironment: mechanisms and consequences for tumor progression. Semin Cancer Biol, 2013, 23: 141–148.
11. Robbins PF, Morgan RA, Feldman SA, *et al.* Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. J Clin Oncol, 2011, 29: 917–924.
12. Gao F, Li X, Geng M, *et al*. Pretreatment neutrophil-lymphocyte ratio: an independent predictor of survival in patients with hepatocellular carcinoma. Medicine (Baltimore), 2015, 94: e639.
13. Stotz M, Gerger A, Eisner F, *et al*. Increased neutrophil-lymphocyte ratio is a poor prognostic factor in patients with primary operable and inoperable pancreatic cancer. Br J Cancer, 2013, 109: 416–421.
14. Chua W, Charles KA, Baracos VE, *et al.* Neutrophil/lymphocyte ratio predicts chemotherapy outcomes in patients with advanced colorectal cancer. Br J Cancer, 2011, 104: 1288–1295.
15. Eisenhauer EA, Therasse P, Bogaerts J, *et al*. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer, 2009, 45: 228–247.
16. Li Q, Wu H, Chen B, *et al.* SNPs in the TGF-beta signaling pathway are associated with increased risk of brain metastasis in patients with non-small-cell lung cancer. PLoS One, 2012, 7: e51713.
17. Loupakis F, Yang D, Yau L, *et al*. Primary tumor location as a prognostic factor in metastatic colorectal cancer. J Natl Cancer Inst, 2015, 107: dju427.
18. Templeton AJ, McNamara MG, Seruga B, *et al.* Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. J Natl Cancer Inst, 2014, 106: dju124.
19. Hanna N, Burton RC. Definitive evidence that natural killer (NK) cells inhibit experimental tumor metastases in vivo. J Immunol, 1981, 127: 1754–1758.
20. Shoenfeld Y, Tal A, Berliner S, *et al*. Leukocytosis in non hematological malignancies--a possible tumor-associated marker. J Cancer Res Clin Oncol, 1986, 111: 54–58.
21. Bekes EM, Schweighofer B, Kupriyanova TA, *et al*. Tumor-recruited neutrophils and neutrophil TIMP-free MMP-9 regulate coordinately the levels of tumor angiogenesis and efficiency of malignant cell intravasation. Am J Pathol, 2011, 179: 1455–1470.
22. Wu Y, Zhao Q, Peng C, *et al*. Neutrophils promote motility of cancer cells via a hyaluronan-mediated TLR4/PI3K activation loop. J Pathol, 2011, 225: 438–447.
23. Cecchin E, Innocenti F, D'Andrea M, *et al*. Predictive role of the UGT1A1, UGT1A7, and UGT1A9 genetic variants and their haplotypes on the outcome of metastatic colorectal cancer patients treated with fluorouracil, leucovorin, and irinotecan. J Clin Oncol, 2009, 27: 2457–2465.
24. Liu X, Cheng D, Kuang Q, *et al*. Association between UGT1A1*28 polymorphisms and clinical outcomes of irinotecan-based chemotherapies in colorectal cancer: a meta-analysis in Caucasians. PLoS One, 2013, 8: e58489.
25. Fournie JJ, Sicard H, Poupot M, *et al.* What lessons can be learned from gammadelta T cell-based cancer immunotherapy trials? Cell Mol Immunol, 2013, 10: 35–41.


**DOI 10.1007/s10330-016-0130-3**
**Cite this article as:** Yu QQ, Qiu H, Zhang MS, *et al*. Prognostic value of neutrophil-to-lymphocyte ratio in metastatic colorectal cancer. Oncol Transl Med, 2016, 2: 75–79.