Original Article

Monocytes-to-lymphocytes ratio increases the prognostic value of circulating tumor cells in non-small cell lung cancer: a prospective study

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Background: Circulating tumor cells (CTCs) has shown important prognostic value in non-small cell lung cancer (NSCLC). However, the present low sensitivity of CTC capture technology restricts their clinical application. This study aims to explore the feasibility of combining the peripheral blood cell (PBC)-derived inflammation-based score with CTCs to increase the prognostic value of CTCs in NSCLC.

Methods: Sixty volunteers diagnosed with NSCLC were recruited. CTC count and six inflammation-based scores were examined and the association with progression-free survival (PFS) and overall survival (OS) was explored. The changes in the CTC counts before and after the immunotherapy were observed.

Results: Multivariate analysis showed that CTCs >7 [hazard ratio (HR) =9.07; 95% confidence interval (CI): 3.68–22.37, P<0.001] and monocytes-to-lymphocytes ratio (MLR) > 0.2 (HR =3.07; 95% CI: 1.21–7.84; P=0.01) were associated with shorter OS and PFS in patients with NSCLC. Patients with CTCs >7 and MLR >0.2 had 12.30 times increased risk of death (P<0.001) and 6.10 times increased risk of disease progression (P=0.002) compared with those with CTCs ≤7 and MLR ≤0.2. Decreased CTC counts after immunotherapy were closely related to disease control (r=0.535, P=0.01).

Conclusions: CTCs and MLR are both independent risk factors for prognosis in patients with NSCLC. The combination of CTCs with MLR significantly increased the prognostic value of CTCs, which would contribute to stratification of NSCLC patients and providing precise treatment. Dynamic monitoring of CTCs efficiently shows the immunotherapy response in NSCLC.

Keywords: Circulating tumor cells (CTCs); monocyte-to-lymphocyte ratio (MLR); non-small cell lung cancer (NSCLC); survival; immunotherapy

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Introduction

Lung cancer (LC) is the most common malignancy worldwide. According to the "Global Cancer Statistics 2020", LC is still the most malignant tumor with the highest mortality globally (1). About 85% of LC belongs to non-small cell lung cancer (NSCLC). Despite the breakthroughs in treatment strategies for NSCLC in the previous decade, the overall survival (OS) of NSCLC is still unfavorable. The main factors leading to the death of patients with LC are late diagnosis and metastasis (2). Therefore, it is urgent

to find simple, noninvasive, reliable biomarkers for the prediction of prognosis in NSCLC patients and carry out effective treatment timely.

Circulating tumor cells (CTCs) are malignant cells originating from either primary tumors or metastases that migrate into the bloodstream (3). The detection of CTCs provides a noninvasive approach that allows for the retrieval of multiple samples with low risk (4). A meta-analysis revealed that pretreatment CTC count was significantly associated with worse OS and shorter progression-free survival (PFS), indicating that CTC count can be an effective tool to predict the disease prognosis in patients with NSCLC (5). However, in the bloodstream, CTCs are very rare; the present low sensitivity of CTC capture technology restricts their clinical application.

Recently, many studies have focused on inflammation, which impacts each step of tumor genesis (6). Inflammation cells induced changes within the cancer microenvironment that favor cancer progression (7). It has been recently shown some alterations in the peripheral blood cell (PBC)-derived inflammation-based scores are linked to different cancers, including NSCLC (8-10). However, the optimal indicator for NSCLC patients is uncertain.

Inflammation is strictly linked with cancer, and CTCs survive in the blood microenvironment by interacting with PBCs, including neutrophils, platelets, and macrophages (11). It has been reported that the formation of heterotypic cell clusters between CTCs and white blood cells (WBCs) predicts poor survival in patients with NSCLC (12). Herein, we hypothesized that the combination of CTCs and inflammation-based scores could improve the prognostic value of CTCs for NSCLC, and CTCs could be a predictive biomarker for immunotherapy response. To address this hypothesis, we measured CTCs as well as inflammation-based markers in the peripheral blood to evaluate OS and PFS in patients with NSCLC, and compared the change in CTC counts before and after immune checkpoint inhibitor (ICI) therapy, aiming to explore the potential of the combination of CTCs with inflammation-based scores as a predictive biomarker for prognosis of NSCLC, and predictive value of CTC monitoring for the efficiency of immunotherapy in NSCLC. We present this article in accordance with the REMARK reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-10/rc).

Highlight box

Key findings

- The combination of circulating tumor cells (CTCs) with monocyte-to-lymphocyte ratio (MLR) could aid in risk stratification of patients with non-small cell lung cancer (NSCLC). Dynamic monitoring of CTCs efficiently shows the immunotherapy response in NSCLC.

What is known and what is new?

- CTCs play an essential role in initiating metastasis, but they are very rare in the bloodstream, which means that the value of CTC counts in assessing the prognosis of NSCLC is limited. Inflammation cells induced changes within the cancer microenvironment that favor cancer progression.
- This study found that CTCs and MLR are independent prognostic factors for survival in patients with NSCLC. The combination of CTCs with MLR significantly increased the prognostic value of CTCs.

What is the implication, and what should change now?

- In the present study, we identified for the first time that the combination of CTCs and MLR may further improve the predictive value of prognosis for NSCLC, which might have the potential to provide valuable information when deciding on the best treatment approach. Future research should focus on personalizing treatment strategies, and improving CTC capture device to increase the detection rate.

Methods

Patients' selection

This study utilized a prospective design. A total of 60 patients who were diagnosed with NSCLC at Henan Provincial People's Hospital from May 2020 to May 2021 were enrolled [31 males and 29 females, median age: 62 (range, 35–80) years]. Disease stages were based on the eighth edition of the International Association for the Study of LC on the tumor-node-metastasis (TNM) classification of LC (13). Patients who did not receive any treatment before the blood samples were obtained and those who had pathologically confirmed NSCLC were included. Patients who also had end-stage liver disease or kidney disease and other malignant tumors in the past 5 years were excluded. All patients were diagnosed by histopathology examination. Seventeen patients with advanced epidermal growth factor receptor (EGFR)/anaplastic lymphoma kinase (ALK) negative NSCLC received pembrolizumab or atezolizumab treatment irrespective of the number of previous therapies or programmed death-ligand 1 (PD-L1) expression levels and continued until they were either confirmed disease progression or experienced a serious adverse event. The

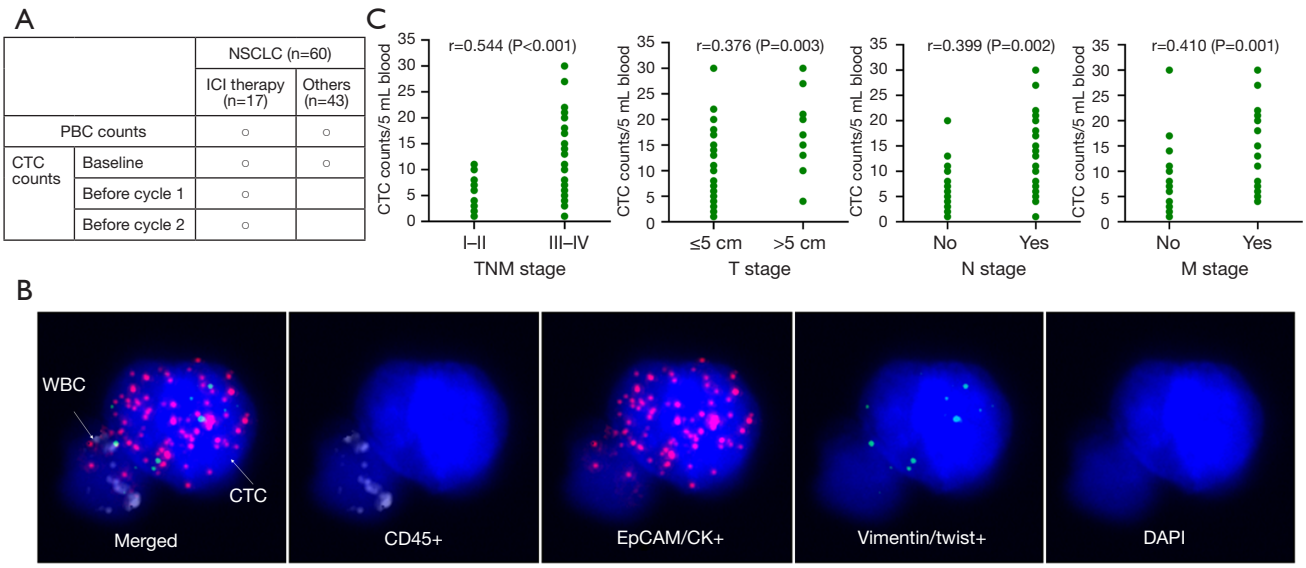

Figure 1 Study design (blood sample collection), CTC identification, and the relationship between CTC count and clinical characteristics of patients with NSCLC. (A) Complete blood samples were collected for inflammation-based score and CTC counts. (B) CTCs were identified as EpCAM/CK+, vimentin/twist+, and CD45− cells using immunofluorescent staining and formed a heterotypic cluster with CD45+ leukocyte. Images were shown as 400 magnifications. (C) The significant relationship existed between CTC counts and TNM stage, T stage, N stage and M stage. PBC, peripheral blood cell; CTCs, circulating tumor cells; NSCLC, non-small cell lung cancer; ICI, immune checkpoint inhibitor; TNM, tumor-node-metastasis; WBC, white blood cells; DAPI, 4',6-diamidino-2-phenylindole; EpCAM, epithelial cell adhesion molecule; CK, cytokeratin.

follow-up of patients with NSCLC started after treatments, and then repeated at every 3 months until July 2023. In the analysis of risk, we included the following clinical information: CTC, tumor size, lymph node metastasis, distant metastasis and six inflammation-based scores, and the sample size was determined accordingly. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Henan Medical College (Zhengzhou, China) (No. HNYZLLWYH-2021-013) and informed consent was obtained from all individual participants.

Enrichment of CTCs

The study design about blood sample collection is shown in *Figure 1A*. Peripheral venous blood samples (5.0 mL) were collected from all patients before treatment and from 17 patients with advanced NSCLC before ICI treatment cycle 1 and 2. CTCs were enriched using the CanPatrol™ CTC technique (SurExam, Guangzhou, China). First, erythrocyte lysis buffer was added to the red blood cells; the remaining cells were resuspended in phosphate-buffered saline containing 4% formaldehyde (Sigma-Aldrich, St. Louis, USA) and allowed to suspend for 5 minutes. Subsequently, the cell suspension was transferred to the CTCs filtration device as described previously (14). CTCs were identified using antibodies against epithelial biomarkers cytokeratin (CK)8/18/19 and epithelial cell adhesion molecule (EpCAM), mesenchymal biomarkers vimentin and twist, and leukocyte biomarker CD45 by the RNA *in situ* hybridization. 4',6-diamidino-2-phenylindole (DAPI) stained nuclei. As shown in *Figure 1B*, CTCs were detected and formed a heterotypic cluster with CD45+ leukocyte.

Analysis of inflammation-based score

Complete blood counts were collected from all patients with NSCLC before treatment. Inflammation-based scores included neutrophil-to-lymphocyte ratio (NLR); derived NLR (dNLR) [absolute neutrophil count /(WBC count – absolute neutrophil count)]; platelet-to-lymphocyte ratio (PLR); monocytes-to-lymphocytes ratio (MLR); systemic-inflammation index (SII) (absolute neutrophil count ×

Table 1 Comparison of CTC count in different clinical feature groups of NSCLC patients (n=60)

Characteristics	Number of case	CTC counts, median [range]	P value
Age (years)			0.33
≤62	33	7 [1–30]	
>62	27	8 [1–30]	
Sex			0.78
Male	31	7 [1–30]	
Female	29	7 [1–30]	
Smoking			0.20
No	37	7 [1–30]	
Yes	33	8 [1–22]	
Pathological type			0.15
ADC	35	6 [1–30]	
SCC	25	8 [1–27]	
TNM stage			<0.001
I–II	26	4 [1–11]	
III–IV	34	10.5 [1–30]	
Tumor size (cm)			0.004
≤5	50	7 [1–30]	
>5	10	16 [4–30]	
Lymph node metastasis			0.002
No	22	4 [1–20]	
Yes	38	8 [1–30]	
Distant metastasis			0.002
No	36	5 [1–30]	
Yes	24	11 [4–30]	

CTC, circulating tumor cell; NSCLC, non-small cell lung cancer; ADC, adenocarcinoma; SCC, squamous cell carcinoma; TNM, tumor-node-metastasis.

absolute platelet count/absolute lymphocyte count), systemic-inflammatory-response index (SIRI) (absolute neutrophil count × absolute monocyte count/absolute lymphocyte count).

The evaluation criterion for treatment

The immunotherapy efficacy before cycle 4 was evaluated according to the Response Evaluation Criteria in Solid Tumours (RECIST) (version 1.1) (15), with responses categorized as complete response (CR), partial response (PR), progressive disease (PD), and stable disease (SD). We described disease control (DC) as CR + PR + SD.

Statistical analysis

SPSS 20.0 was used to analyze all data. The comparative analysis of continuous variables between groups was performed using the Mann-Whitney *U* test. The comparison of categorical data was made using Pearson's Chi-square or Fisher's exact test, and the contingency coefficient represented the correlation. The cut-off values for CTCs, NLR, dNLR, MLR, PLR, SII, and SIRI were calculated based on X-tile bioinformatics software version 3.6.1. Survival curves were analyzed by the Kaplan-Meier method, and the differences in survival were assessed using the log-rank test. Multivariable analyses of survival were performed using a Cox proportional hazards model. A two-tailed P value of <0.05 was considered statistically significant.

The study endpoints were PFS and OS. PFS was defined as the interval (in months) from the first treatment to disease progression or death. OS was measured from the first treatment to death or the last follow-up.

Results

Association between CTC count and clinical features of patients with NSCLC

CTCs were detected in all patients. The comparison of the CTC count in different clinical feature groups of NSCLC patients is shown in *Table 1*. There were 12 cases with stage I (median 3; range, 1–8), 14 with stage II (median 4; range, 1–11), 10 with stage III (median 10; range, 1–30), and 24 cases with stage IV (median 11; range, 4–30). The CTC count in patients with III–IV stage NSCLC was significantly more than that in those with I–II stage NSCLC, with a statistically significant difference (P<0.001). The CTC count in patients with ≤5 cm tumor size was also significantly lower than that in those with >5 cm tumor size (P=0.004). There was a significant difference in the CTC count between patients with NSCLC with and without lymph node metastasis (P=0.002) and those with and without distant metastasis (P=0.002). The CTC count was positively associated with TNM stage (r=0.544, P<0.001), tumor invasion depth (r=0.376, P=0.003), lymph node metastasis (r=0.399, P=0.002), and distant metastasis (r=0.410,

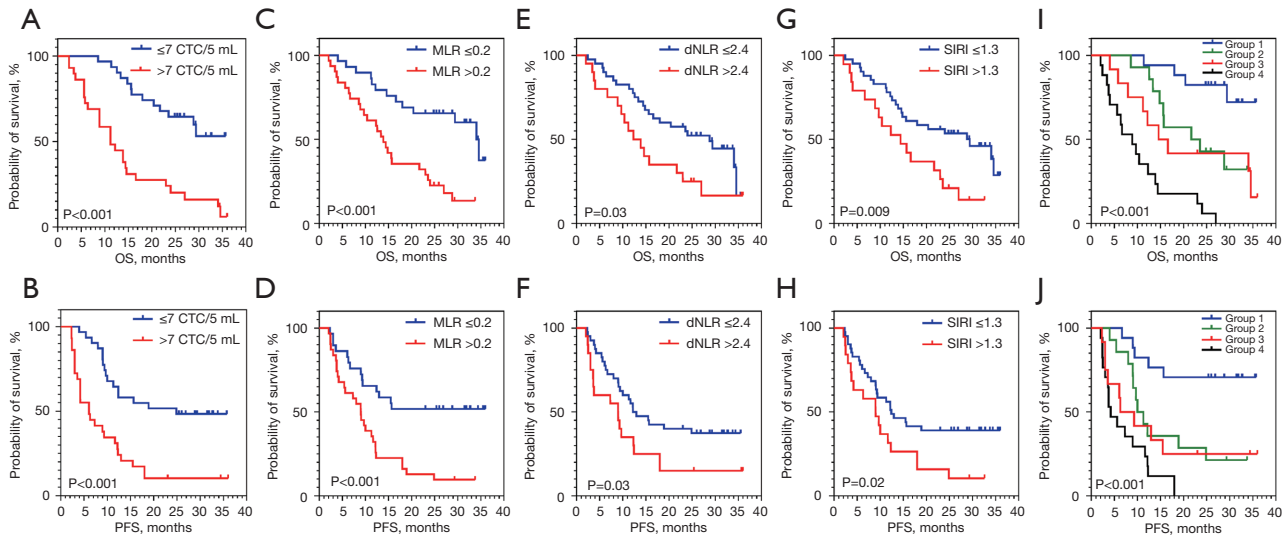

Figure 2 OS and PFS analysis based on CTCs and inflammation-based scores. (A,B) CTCs; (C,D) MLR; (E,F) dNLR; (G,H) SIRI; (I,J) the combination of CTCs and MLR. Group 1: CTCs ≤7 and MLR ≤0.2; Group 2: CTCs ≤7 and MLR >0.2; Group 3: CTCs >7 and MLR ≤0.2; Group 4: CTCs >7 and MLR >0.2. CTCs, circulating tumor cells; OS, overall survival; MLR, monocytes-to-lymphocytes ratio; dNLR, derived neutrophil-to-lymphocyte ratio; SIRI, systemic-inflammatory-response index; PFS, progression-free survival.

P=0.001). The CTC counts were not significantly associated with age, sex, smoking, and histopathology (*Figure 1C*).

Relationship between CTC count and clinical characteristics of patients with NSCLC

The relationship between CTCs and clinical characteristics in 60 patients with NSCLC is shown in *Table 1*. The CTC count in patients with III–IV stage NSCLC was significantly more than that in those with I–II stage NSCLC, with a statistically significant difference (P<0.001). The CTC count in patients with ≤5 cm tumor size was also significantly lower than that in those with >5 cm tumor size (P=0.004). There was a significant difference in the CTC count between patients with NSCLC with and without lymph node metastasis (P=0.002) and those with and without distant metastasis (P=0.002).

The prognostic value of CTCs and inflammation-based scores in NSCLC

All patients were followed up. The median follow-up was 32 months, with a range of 2–36 months. The Kaplan-Meier's survival curves revealed that patients with CTCs >7 had a significantly poorer median OS (11.2 months *vs.* not reached) and PFS (6.1 *vs.* 24.9 months) than those with CTCs ≤7 (*Figure 2A,2B*). CTCs >7 was associated with 3.99 times increased risk of disease mortality [95% confidence interval (CI): 2.03–7.85, P<0.001] and 3.44 times increased risk of disease progression (95% CI: 1.82–6.51, P<0.001). Patients with higher values of MLR had a significantly poorer median OS (13.9 *vs.* 34.6 months; *Figure 2C*) and PFS (9.0 months *vs.* not reached; *Figure 2D*). Patients with higher values of dNLR had a poorer prognosis (OS: 13.1 *vs.* 28.8 months, PFS: 9.0 *vs.* 12.6 months; *Figure 2E,2F*). Patients with higher values of SIRI had a worse survival rate (OS: 14.4 *vs.* 29.4 months, PFS: 9.0 *vs.* 12.4 months; *Figure 2G,2H*) The values of NLR, SII, and PLR did not aid in distinguishing patients with survival risk (OS and PFS) from the total population (Figure S1). In multivariate analysis, CTC count and MLR were both significant factors for OS [hazard ratio (HR) =9.07, 95% CI: 3.68–22.37 for CTC; HR =3.07, 95% CI: 1.21–7.84 for MLR] and PFS (HR =3.59, 95% CI: 1.72–7.52 for CTC; HR =2.97, 95% CI: 1.24–7.14 for MLR). Among clinical variables, lymph node metastasis and distant metastasis were also independently associated with OS and PFS (*Table 2*).

Increased prognostic value of CTCs in combination with MLR in NSCLC

According to the MLR value and CTC counts, we divided

Table 2 Cox proportional hazard regression analysis

Variables	OS			PFS		
	HR	95% CI	P	HR	95% CI	P
CTCs >7 (n=29)	9.07	3.68–22.37	<0.001	3.59	1.72–7.52	0.001
dNLR >2.4 (n=20)	1.26	0.57–2.80	0.57	1.26	0.56–2.85	0.57
MLR >0.2 (n=31)	3.07	1.21–7.84	0.01	2.97	1.24–7.14	0.01
SIRI >1.3 (n=19)	0.51	0.18–1.46	0.20	0.36	0.13–1.03	0.056
Tumor size >5 cm (n=10)	1.76	0.67–4.63	0.25	2.67	0.99–7.16	0.052
Lymph node metastasis (n=38)	2.64	1.07–6.50	0.03	3.67	1.51–8.96	0.004
Distant metastasis (n=24)	8.86	3.40–23.11	<0.001	3.27	1.35–7.93	0.009

CTCs, circulating tumor cells; dNLR, derived neutrophil-to-lymphocyte ratio; MLR, monocytes-to-lymphocytes ratio; SIRI, systemic-inflammatory-response index; OS, overall survival; PFS, progression-free survival; HR, hazard ratio; CI, confidence interval.

the patients into the following four subgroups: Group 1 (n=17), CTCs ≤7 and MLR ≤0.2; Group 2 (n=14), CTCs ≤7 and MLR >0.2; Group 3 (n=12), CTCs >7 and MLR ≤0.2; and Group 4 (n=17), CTCs >7 and MLR >0.2. Patients in Group 4, Group 3 or Group 2 had a significantly poorer median OS (8.87, 14.63, 21.70 months *vs.* not reached) and PFS (4.23, 6.33, 10.03 months *vs.* not reached) compared to those in Group 1 (*Figure 2I,2J*). Univariate analysis showed the combination of CTCs with MLR significantly increased the prognostic value of CTCs for NSCLC (P<0.001). With the Group 1 as a reference, the risks of adverse prognosis in the Group 2, 3 and 4 gradually increased, with HRs of 4.02, 4.37 and 15.61 for OS and 3.87, 4.78 and 10.02 for PFS, respectively. Multivariate analysis confirmed the prognostic value of the combination of CTCs and MLR as an independent risk factor for OS and PFS. In comparison to those with both low CTC count and MLR, patients with both high CTC count and MLR had 12.30 times increased risk of death (95% CI: 3.71–40.79; P<0.001) and 6.10 times increased risk of disease progression (95% CI: 1.95–19.05; P=0.002); and those with high CTC count and low MLR had 3.69 times increased risk of death (95% CI: 1.06–12.87; P=0.004) and 3.44 times risk of disease progression (95% CI: 1.11–10.61; P=0.03). Among the tested clinical variables, lymph node metastasis and distant metastasis also significantly increased the risk of disease death and progression (*Table 3*).

Dynamic changes of CTCs in patients with NSCLC

The CTC counts were determined in 17 patients with advanced NSCLC before ICI treatment cycles 1 and 2. We defined the CTC counts before cycle 1 as CTC_0 and before cycle 2 as CTC_1. The RECIST version 1.1 was used to evaluate immunotherapy response before cycle 4. Patients with increased CTCs at cycle 2 were more likely to have PD and those with reduced CTCs to have DC (P=0.03). The changes in the CTC counts before and after the treatment were closely related to PD and DC (r=0.535, P=0.01) (*Table 4*).

Discussion

The TNM classification for LC has proven to be predictive of OS. However, some patients have the same pathological stage but different outcomes; nevertheless, repeated imaging examination increases the patients' risk of exposure of radiation, invasive test also increases the patient's physical pain. With the development of precision medicine, the study of LC has gone to the molecular level. Liquid biopsy has a broad prospect. It can be used for prognosis assessment, monitoring response to therapeutic regimens (16). Currently, CTCs have been approved for clinical use by the Food and Drug Administration (FDA) (17).

In the present study, we found that CTC counts were closely associated with TNM stage, tumor size, lymph node metastasis, and distant metastasis, indicating that CTC counts can evaluate the stage and metastasis of tumor and then non-invasively predict prognosis of patients with NSCLC. We followed up 60 patients with NSCLC and found CTCs >7 was independently risk factors for OS and PFS. CTC count could be considered as a significantly predictive biomarker for prognosis of NSCLC, consistent with previous studies (18,19). However, CTC risk stratification was too simple to discriminate among lower or

Table 3 Univariate and multivariate analysis for the association between the combination of CTCs with MLR and OS or PFS

Variables	Univariate analysis				Multivariate analysis			
	OS		PFS		OS		PFS	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
CTCs ≤7, MLR ≤0.2	–	<0.001	–	<0.001	–	<0.001	–	0.003
CTCs ≤7, MLR >0.2	4.02 (1.22–13.24)	0.02	3.87 (1.34–11.21)	0.01	1.01 (0.27–3.78)	0.99	1.99 (0.62–6.35)	0.24
CTCs >7, MLR ≤0.2	4.37 (1.32–14.44)	0.01	4.78 (1.59–14.38)	0.005	3.69 (1.06–12.87)	0.004	3.44 (1.11–10.61)	0.03
CTCs >7, MLR >0.2	15.61 (5.04–48.31)	<0.001	10.02 (3.59–27.97)	<0.001	12.30 (3.71–40.79)	<0.001	6.10 (1.95–19.05)	0.002
Tumor size >5 cm	4.32 (2.02–9.21)	<0.001	5.48 (2.51–11.97)	<0.001	1.40 (0.60–3.25)	0.43	1.71 (0.74–3.96)	0.21
Lymph node metastasis	4.99 (2.18–11.47)	<0.001	5.36 (2.43–11.80)	<0.001	3.46 (1.41–8.48)	0.007	3.61 (1.51–8.67)	0.004
Distant metastasis	7.52 (3.54–15.99)	<0.001	7.24 (3.28–16.00)	<0.001	8.42 (3.36–21.07)	<0.001	2.96 (1.24–7.10)	0.01

CTCs, circulating tumor cells; MLR, monocytes-to-lymphocytes ratio; OS, overall survival; PFS, progression-free survival; HR, hazard ratio; CI, confidence interval.

Table 4 Correction between the change of CTCs and efficacy of therapy

CTCs variations	Evaluation of therapeutic response		P value (for Fisher's exact test)	r	P value (for correlation)
	DC	PD			
$CTC_1 - CTC_0 \leq 0$	9	2	0.03	0.535	0.01
$CTC_1 - CTC_0 > 0$	1	5			

CTCs, circulating tumor cells; DC, disease control; PD, progressive disease.

higher-risk patients in the era of novel therapies.

Systemic inflammation is associated with the immune resistance in cancer. It can promote tumor growth by changing the turnover rate of stromal cell and polarizing the immunosuppressive ability of immune cells (20). Monocytes appear to be recruited throughout tumor progression (21). In this study, the MLR displayed the best predictive performance for prognosis in patients with NSCLC among six inflammation-based scores. MLR >0.2 was a significantly risk factor for OS and PFS, which was also confirmed in advanced LC patients by Mandaliya and colleagues using a cut-off of 0.25 (22), including for breast cancer (7) and uterine cancer (23). Monocytes could differentiate into tumor-associated macrophages (TAMs) during cancer. Single-cell RNA sequencing demonstrates TAMs in primary lung tumors and distant metastases mainly propagated from monocyte-derived macrophages that are ontologically different from tissue-resident macrophages, along with T-cell exhaustion (24). TAMs play an important role in furthering tumor genesis by promoting immune suppression, remodeling extracellular matrix, regulating angiogenesis, and helping intravasation of tumor cells. It provides a new idea for immunotherapy to shift the balance toward monocyte fates that aid in antitumor immunity (25). Real-time monitoring of MLR is a convenient and efficient approach for evaluating the prognosis of patients with NSCLC and adjusting the treatment strategy in time. Interestingly, NLR and PLR are the most studied inflammatory-based markers and some published data have confirmed the association of elevated PLR and NLR with poorer PFS and OS (26,27), but did not demonstrate prognostic significance in this study. With similar results, Song *et al.* investigated on 16 inflammation/nutrition-based indicators and validated all but PLR were independent predictors of OS in a cohort of 1,772 LC patients (10). Several studies demonstrated that high SIRI or dNLR resulted in increased hazard for shorter OS and PFS in NSCLC (28,29), which were not confirmed by multivariate analysis in this study. The differences in patients selected maybe the main reason. Patients we recruited were in stage I–IV, but those in most of studies were in stage III–IV. No unified standard for selecting the optimal cutoff value, and

sample sizes could also be the reasons for the inconsistent research results. Although different studies choose different cutoff value, the prognostic value of inflammation-based scores in patients with NSCLC has been confirmed to some extent.

In our study, the clusters between CTCs and WBCs were found, which results in enhanced CTCs survival and induces proliferation of CTCs by epigenetically reprograming the attached neighbor CTCs (30). In addition, leucocytes induce monocyte-macrophage differentiation and promote the release and invasion of CTCs through TAMs (31). In papers by Gast and colleagues, the authors pointed out the fusion of CTCs and macrophages contributed to tumor heterogeneity, resulted in a higher efficiency in metastasis behavior (32). We tried to explore the value of combining CTCs and MLR and found the combination of the two provided a more detailed risk assessment of prognosis in patients with NSCLC. A strong association was shown between adverse outcome and elevated MLR in CTCs >7 patients. Similar results were reported in patients with primary breast cancer by De Giorgi *et al.* (7). It will contribute to stratifying patients and providing precise treatment for NSCLC patients to prevent metastatic progression.

ICIs are among the most notable advances in cancer immunotherapy; however, some patients with low programmed death-ligand 1 (PD-L1) expression respond to ICIs, which means that a single biomarker may not be indicative for patient selection. Tamminga *et al.* demonstrated CTC detection was an independent predictive factor for shorter PFS and OS at baseline and on-treatment (33). Spiliotaki *et al.* analyzed CTC surface markers and found monitoring PD-L1-positive CTCs of NSCLC patients was predictive for ICI efficacy (34). The present study found that the change in CTC counts before and after ICI therapy was closely associated with the efficacy of immunotherapy. Reduced CTC counts at cycle 2 (compared with those at cycle 1) meant the immunotherapy benefit. Therefore, CTC monitoring may be predictive for the efficiency of ICI therapy. Moreover, monitoring treatment efficacy using CTC allows for a timely change of treatment in order to minimize financial costs and potential toxicities.

There are several limitations in this study. We only included 60 NSCLC patients from our institution from May 2020 to May 2021. The sample size was relatively small and the patients were from a single center which could potentially influence our research conclusions. For future studies, randomized controlled multi-center clinical trials are needed to obtain more favorable results for the prognosis evaluation of CTCs and MLR and the predictive value of CTC monitoring for the efficiency of immunotherapy in NSCLC. Additionally, a bias could have been introduced owing to ICI selection, possibly because of the individual financial circumstance.

Conclusions

In the present study, we identified for the first time that the combination of CTCs and MLR may further improve the predictive value of prognosis for NSCLC, which might have the potential to provide valuable information when deciding on the best treatment approach. Our results confirmed that CTCs could be used for prognostic prediction, immunotherapy response monitoring. Compared with other inflammatory indicators, the MLR showed the best performance in predicting the prognosis of patients with NSCLC that needs further investigation.

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-10/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-10/dss

Peer Review File: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-10/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-10/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are

appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Henan Medical College (Zhengzhou, China) (No. HNYZLLWYH-2021-013) and informed consent was obtained from all individual participants.

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