Original Article

Monitoring early dynamic changes of plasma cell-free DNA and pretreatment pre-albumin to predict chemotherapy effectiveness and survival outcomes in advanced non-small cell lung cancer

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Background: This study aimed to determine whether plasma cell-free DNA (cfDNA) and pretreatment parameters provide useful therapeutic response and prognostic information for advanced non-small cell lung cancer (NSCLC) patients.

Methods: A total of 114 patients with advanced NSCLC who underwent systemic chemotherapy were included in this study. Detection of plasma cfDNA concentration and blood parameters before and at the sixth week after treatment was performed. The prognostic value of cfDNA dynamic changes and laboratory parameters was determined via a receiver operating characteristic (ROC) curve, and then analyzed by comparing with the therapeutic efficacy and progression-free survival (PFS). Based on the ROC curve, it revealed a pretreatment pre-albumin (PA) concentration of 21.7 mg/dL was the cut-off value. The Cox proportional hazards regression model was used to evaluate the predictive factors for treatment response and PFS via univariate and multivariate analyses.

Results: Patients with cfDNA reduction ≥20% at the sixth week after treatment reported a significantly better disease control rate (DCR) and prolonged PFS (median PFS: 10.0 *vs.* 4.0 months, P<0.001). The median PFS of low PA group (PA <21.7 mg/dL) was 6.0 months, while the median PFS of high PA group (PA ≥21.7 mg/dL) was 8.0 months. The combined assessment of cfDNA and pretreatment pre-albumin was associated with significantly better survival outcomes compared with the remaining population (P<0.001). Multivariate analysis for DCR indicated that cfDNA reduction ≥20% was an independent factor (OR =0.419, P=0.001). In addition, multivariate analysis identified 6 significant factors associated with PFS: cfDNA reduction of ≥20%, age <65 years, Eastern Cooperative Oncology Group (ECOG) score ≥2, driver gene mutation, chemotherapy combined regimen, and treatment response of complete response (CR) and partial response (PR). The nomogram could predict the 2-year PFS probability of advanced NSCLC patients after treatment, and the C-index was 0.817.

Conclusions: Monitoring cfDNA changes and pretreatment pre-albumin level in advanced NSCLC patients receiving treatment is an accurate predictor of tumor response and PFS. Combined assessment of cfDNA and pretreatment pre-albumin is helpful for predicting survival outcomes. These findings may assist in identifying high-risk patients and guiding treatment strategies.

Keywords: Advanced non-small cell lung cancer (advanced NSCLC); plasma cell-free DNA (plasma cfDNA); chemotherapy efficacy; therapeutic response

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Introduction

Lung cancer is the most frequent malignant cancer and the leading cause of cancer-related death worldwide in recent years (1). Non-small cell lung cancer (NSCLC) accounts for the majority of lung cancer cases, and most patients are diagnosed in the advanced clinical stages including IIIB, IIIC, and IV stages. Due to the limitations of treatment in the past 2 decades, clinical therapeutic efficacy and survival remain poor. The 5-year overall survival (OS) rate for patients with metastatic NSCLC is less than 5% (2). For these NSCLC patients in the advanced stage who have lost the opportunity for surgery, treatment should involve multidisciplinary therapy including chemotherapy, targeted therapy, chemoradiotherapy, and immunotherapy (2-4).

The current clinical guidelines recommend that the systemic treatment of advanced or metastatic NSCLC should be selected based on the presence of specific biomarkers (2). Molecular testing of driver mutations and expression of programmed death ligand-1 (PD-L1) should be performed for all patients with advanced or metastatic NSCLC. However, a single treatment method cannot provide substantial survival benefits to patients, and many patients still face disease progression. According to the clinical guidelines, systemic chemotherapy remains the cornerstone of treatment for advanced NSCLC, but the objective response rate (ORR) of first-line chemotherapy in NSCLC is still 30–40%. There is still a lack of effective and predictive biomarkers for prognosis after first-line chemotherapy in advanced NSCLC patients. Clinicians and researchers are devoting more efforts to determine meaningful methods or biomarkers to predict treatment efficacy.

The detection and monitoring of serum physiological parameters or indicators have always been important auxiliary methods for clinical cancer diagnosis. Therefore, it is important to identify the role of NSCLC-related hematological indicators. In the past decades, commonly used hematological indicators have included serum biomarkers, popular tumor markers, and imaging examinations, which are widely used to predict and identify therapeutic effects but with low prognostic efficacy. Based on these routine tests, multiple studies have revealed that some indicators such as prognostic nutrition index (PNI), neutrophil–lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), and serum tumor markers could be predictive markers of clinical outcome in patients with lung cancer (5-10). The synthesis of albumin and pre-albumin (PA) is inhibited by malnutrition and inflammation. As a crucial inflammatory and nutritional marker, serum pre-albumin levels have been frequently observed in cancer patients and are considered to be associated with poor survival. Currently, emerging tumor biomarkers are being used for clinical applications, such as circulating cell-free DNA (cfDNA), circulating tumor DNA (ctDNA), and circulating tumor cells (CTCs). CfDNAs are derived from dying cells and are typically short DNA fragments (average length of 120–160 bp). In individuals without cancer, most cfDNAs are derived from haemato-poietic cells. In cancer patients, a variable fraction of cfDNAs which referred as ctDNA is derived from tumors following apoptosis and/or necrosis of cancer cells. These analytes may provide information about the characteristics of the primary tumor or metastasis sites and corresponding support for clinical treatment.

Circulating cfDNA was first reported by Mandel and Metais in 1948 (11). The assessment of cfDNA is applied in the fields of early assessment of treatment responses such as chemotherapy or targeted therapy, or the characterization of mechanisms of treatment resistance to anti-cancer therapy (12-14). Moreover, dynamic monitoring and observation of cfDNA can also aid in the early prediction of treatment efficacy. Therefore, there is an urgent need to find simpler and more efficient diagnostic indicators to improve the early detection rate of NSCLC, to assist clinicians in the early judgment of patient's treatment efficacy, and improve the survival rate of patients with NSCLC.

Based on the researches above, we hypothesized that dynamic monitoring of the cfDNA changes may help determine the therapeutic efficacy of patients. Therefore, we designed this retrospective study to explore whether the assessment of dynamic changes in cfDNA and pretreatment parameters over the course of the first 2 cycles (6 weeks) of chemotherapy-based treatment could predict the therapeutic effects as well as progression-free survival (PFS) in advanced NSCLC patients. We present the following article in accordance with the REMARK reporting

checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-12/rc).

Methods

Patients and blood sample collection

A total of 114 patients diagnosed with advanced NSCLC who underwent anti-tumor therapy in The Affiliated Tumor Hospital of Nantong University from Jan 2016 to Oct 2019 were retrospectively enrolled in this study. The inclusion criteria were as follows: (I) age ≥18 years; (II) cytologically or histologically confirmed as NSCLC; (III) clinical stage IIIB/IIIC/IV (according to the 8th version of the International Association for the Study of Lung Cancer TNM Staging System); (IV) patients harboring EGFR/ALK mutations had pretreatment with EGFR-TKI/ALK-TKI; (V) Eastern Cooperative Oncology Group (ECOG) Performance Status score 0–2; (VI) available blood tests within 3 weeks of chemotherapy; (VII) available chest computed tomography (CT) or fluorodeoxyglucose-positron emission tomography/CT (FDG-PET/CT) scans; (VIII) complete data collection and follow-up. The exclusion criteria were as follows: (I) patients with a second malignant tumor; (II) patients with severe comorbidities; (III) patients with acute or chronic infectious diseases; (IV) patients with psychiatric disorders who could not cooperate with the medical treatment.

All enrolled patients signed an informed consent form before participating in the study. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Affiliated Tumor Hospital of Nantong University & Nantong Tumor Hospital (No. 2019-079).

Blood samples were collected from the enrolled patients within 3 weeks prior to the first cycle of chemotherapy (baseline). After 2 cycles of therapy, blood samples were re-collected and analyzed to compare changes in plasma cfDNA concentration and other blood biomarkers.

Treatment of patients and clinical data extraction

All patients received anti-tumor therapy according to clinical guidelines, including chemotherapy, combination of chemotherapy and anti-angiogenesis drugs, or combination of chemotherapy and immune checkpoint inhibitors (ICIs) until tumor progression, development of unacceptable drug toxicity, withdrawal, or death.

The corresponding data of each patient was extracted from the hospital's computerized medical records: (I) general demographic information including name, age, gender, smoking history, alcohol history, and other comorbidities such as hypertension or diabetes; (II) clinical data, including ECOG performance status, pathological type, clinical stage, metastasis sites, treatment regimens and drugs, response evaluation, PFS, and OS. All enrolled patients received anti-tumor therapies including platinum doublet chemotherapy regimens (pemetrexed, paclitaxel, gemcitabine, or docetaxel, plus cisplatin/carboplatin), mono-chemotherapy agents (pemetrexed, paclitaxel, docetaxel, vinorelbine, or gemcitabine), and combinations with either ICIs (nivolumab, pembrolizumab, and sintilimab) or anti-angiogenesis drugs (bevacizumab).

Plasma separation and extraction of cfDNA

Each blood sample was immediately processed for plasma collection. Blood samples were collected in EDTA tubes and then centrifuged at 3,000 rpm/min for 10 minutes, then 600 μL plasma was obtained for cfDNA detection. The supernatant (serum) was collected into a tube and stored at −80 ℃ until detection. The concentration of cfDNA was measured in 20 μL plasma using the QuantiDNATM Direct cfDNA Test (DiaCarta) according to the manufacturer's instructions. QuantiDNA Direct cfDNA Test directly measures the concentration of human circulating cfDNA in plasma. It is a nucleic acid probe hybridization assay that uses branched DNA (bDNA) technology to amplify chemical signal generated in the presence of target cfDNA sequence without amplifying the cfDNA itself.

Collection of blood parameters

Complete blood count parameters including white blood cell (WBC), neutrophil, lymphocyte, monocyte, and platelet (PLT) counts and hemoglobin (Hb) concentration were retrospectively evaluated. In addition, serum albumin, pre-albumin (PA), gamma-glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), and tumor marker [carcinoembryonic antigen (CEA), neuron-specific enolase (NSE), cytokeratin-19 fragment (CYFR21-1), squamous cell carcinoma antigen (SCC), pre-gastrin-releasing peptide] levels were collected for detailed analysis. All the parameters were examined in the general clinical laboratory of The Affiliated Tumor Hospital of Nantong University. The levels of serum albumin, PA, GGT, LDH were

tested by a Hitachi 7600 automatic biochemical analyzer (Tokyo, Japan). The levels of PA were analyzed using the Immunoturbidimetry Assay.

Evaluation of treatment response

Each patient was evaluated for treatment efficacy after 2 cycles of chemotherapy (6 weeks later). According to the Response Evaluation Criteria in Solid Tumors (RECIST 1.1 criteria) (15), the treatment response was divided into 4 categories: complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). Tumor response evaluation was conducted every 2 cycles of therapy. PFS was defined as the time from enrollment to the date of PD or to the end of follow-up (Oct 31, 2020), while OS was not reached until the cut-off time. Survival data were obtained from computerized medical records and manual follow-up. None of the patients were lost to follow-up in this study. Follow-up visits were scheduled for every 3 months during the treatment until death or loss to follow up.

Statistical analysis

The cfDNA plasma levels were calculated for patients with NSCLC at baseline and at the sixth week of systemic chemotherapy, and the quantitative results of cfDNA were recorded regularly. The baseline concentration of cfDNA was recorded as cfDNA-1, while the concentration of cfDNA at the sixth week of treatment was recorded as cfDNA-2. Reduction in cfDNA was defined as the ratio of cfDNA-2/cfDNA-1. At the same time, PA, albumin, LDH, CEA, and CYFRA21-1 levels were calculated based on the blood results obtained by each patient in the time frame.

All statistical analyses were performed using SPSS 25.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 8.0 (GraphPad Inc., San Diego, CA, USA). Receiver operating characteristic (ROC) curves were constructed to determine the superior predictive markers out of cfDNA-1, cfDNA-2, cfDNA reduction, PA, albumin, and LDH, and to define the cut-off values for the indicators that yielded maximum sensitivity and specificity. The area under the ROC curve (AUC) was also calculated. A chi-square test was performed to compare baseline clinical characteristics. The Kruskal-Wallis rank-sum test was used to compare the groups. The Cox proportional hazards regression model was used to evaluate the predictive factors for PFS via univariate and multivariate analyses. Results of PFS were determined by the Kaplan-Meier method using log-rank analysis. All statistical tests were two-sided, and a P value <0.05 was considered to be statistically significant. PFS was defined as the time from the first day of the chemotherapy protocol to the day of disease progression or death, whichever occurred first. Based on the results of multivariable analysis, the nomogram was formulated by R 3.6.3 (http://www.r-project.org) with the survival and rms package. The final model was conducted using a backward step-down process, which used the Akaike information criterion as a stopping rule.

Results

Patient characteristics

From Jan 2016 to Oct 2019 a total of 114 patients were included in the study. The baseline clinical characteristics of the patients are summarized in *Table 1*. The median age was 67 years (range, 44–80 years). The majority of patients were male (73.70%) and current/former smokers (44.7%), with current/former alcoholic history (43.00%). Most patients had an ECOG score <2 (67.50%), and according to the clinical stage, IIIB + IIIC patients comprised 21.90%, IVA comprised 21.10%, and IVB comprised 57.00%. According to the distant metastatic sites (108 sites in stage IVA/IVB patients), bone was the most common metastatic site (39.80%), followed by brain/meninges (16.70%), kidney, adrenal gland, intra-abdominal metastasis (13.00%), bilateral lungs (14.80%), and subcutaneous metastasis (4.60%). As for the histological type, adenocarcinoma accounted for 63.20%, squamous cell carcinoma accounted for 28.90%, and NSCLC-not otherwise specified (NOS) accounted for 7.90%.

All patients received the following systemic chemotherapies of various regimens based on their pathological subtype and ECOG performance scores: platinum-doublet chemotherapy (67.50%), third-generation chemotherapy agents alone (8.80%), chemotherapy combined with anti-angiogenesis drugs (14.9%), and chemotherapy combined with immunotherapy (8.80%). Among them, 76.30% of patients underwent chemotherapy while the rest (23.70%) received combined regimens. PR and SD were observed in 44.70% and 21.1% of patients, respectively, and 34.2% had PD. CR of the patients was not observed in this study. Most patients had first-line therapy (62.3%), while the others (37.7%) had more than first-line treatment. The median follow-up duration was 16.5 months (range, 1.5–42.3 months) while the median

Table 1 Baseline characteristics of patients

Characteristics	Total (n=114)	%
Median age (years, range)	67 [44–80]	
Sex		
Male	84	73.70
Female	30	26.30
Smoking history		
Never/unknown	63	55.30
Current/former	51	44.70
Alcoholic history		
Never/unknown	65	57.00
Current/former	49	43.00
Comorbidity		
Hypertension	39	34.20
Diabetes	12	10.60
Other	5	4.40
Absent	58	50.80
ECOG		
0–1	77	67.50
≥2	37	32.50
Stage subgroup		
IIIB + IIIC	25	21.90
IVA	24	21.10
IVB	65	57.00
Histology		
Adenocarcinoma	72	63.20
Squamous cell carcinoma	33	28.90
NSCLC-NOS	9	7.90
Distant metastatic sites[‡] (108 sites in stage IVA/IVB patients)		
Liver	12	11.10
Brain/meninges	18	16.70
Bone	43	39.80
Kidney, adrenal gland, intra-abdominal metastasis	14	13.00
Bilateral lungs	16	14.80
Subcutaneous metastasis	5	4.60
Metastatic sites[§]		
≥3 organs/sites	42	36.80
<3 organs/sites	72	63.20
Driver mutation		
EGFR	24	21.10
ALK	3	2.70
Negative	87	76.20
EGFR mutation type		
19del	6	25.00
21L858R	17	70.80
Other rare mutation	1	4.20
Line of therapy		
<2	71	62.30
≥2	43	37.70
Chemotherapy regimen		
Platinum-doublets	77	67.50
Third-generation chemotherapy agents alone	10	8.80
Chemotherapy combined with anti-angiogenesis drugs	17	14.90
Chemotherapy combined with immunotherapy	10	8.80
Chemotherapy regimen		
Chemotherapy	87	76.30
Combination regimen	27	23.70
Best response		
CR	0	0
PR	51	44.70
SD	24	21.10
PD	39	34.20

[‡], distant metastatic sites represented 108 sites in 89 stage IVA/IVB patients, the number of distant metastases sites is not consistent with the total number of patients [114]; [§], metastatic sites included both lymph nodes and distant metastatic sites. ECOG, Eastern Cooperative Oncology Group; NSCLC-NOS, non-small cell lung cancer, not otherwise specified; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; third-generation chemotherapy agents, docetaxel/gemcitabine/paclitaxel/pemetrexed; platinum-doublets, third-generation chemotherapy agents plus cisplatin/carboplatin; CR, complete response; PR, partial response; SD, stable disease, PD, progressive disease.

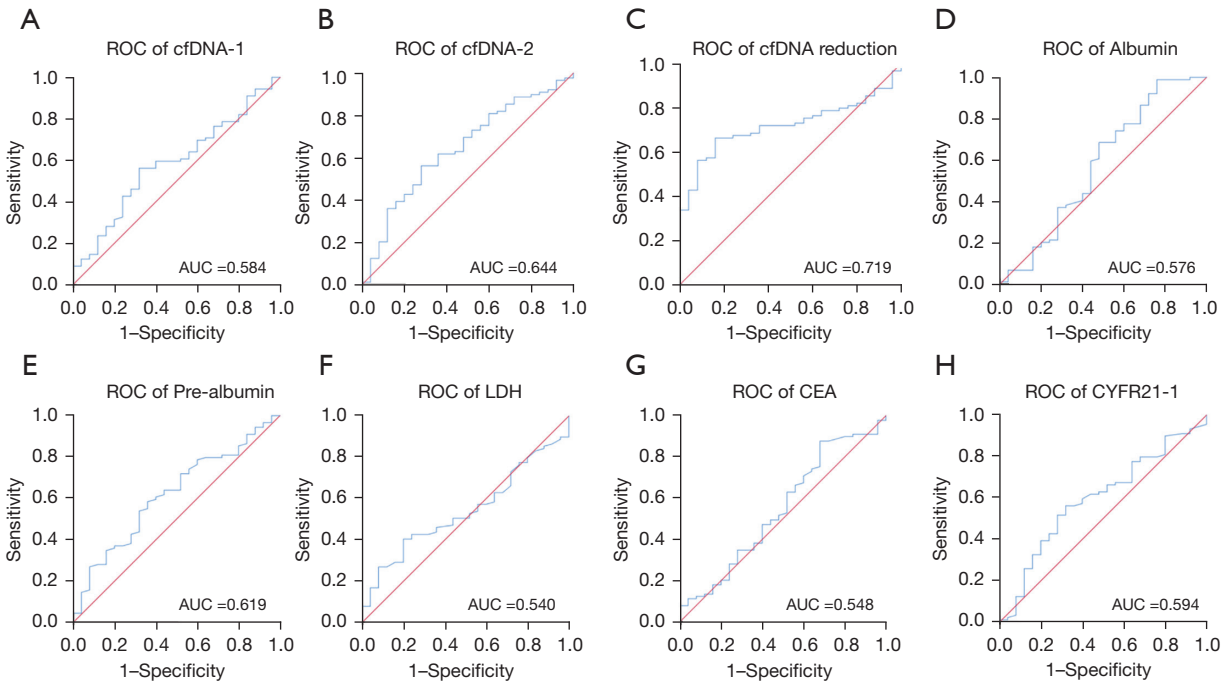

Figure 1 ROC curves of cfDNA and clinical biomarkers. (A) ROC curve of cfDNA-1. (B) ROC curve of cfDNA-2. (C) ROC curve of cfDNA reduction. (D) ROC curve of albumin. (E) ROC curve of pre-albumin. (F) ROC curve of LDH. (G) ROC curve of CEA. (H) ROC curve of CYFR21-1. ROC, receiver operating characteristic; cfDNA, cell-free DNA; LDH, lactate dehydrogenase; CEA, carcinoembryonic antigen.

PFS was 7.0 months (95% CI, 6.0–8.0 months) in the overall study group.

ROC curves of cfDNA and clinical biomarkers

ROC curves were constructed to determine which of cfDNA-1, cfDNA-2, cfDNA reduction (defined as cfDNA-2/cfDNA-1), albumin, PA, LDH, CEA, and CYFR21-1 might be potential predictive markers (*Figure 1*). Considering the individual differences in cfDNA concentration, and through comprehensive evaluation and comparison of cfDNA-1 (*Figure 1A*), cfDNA-2 (*Figure 1B*), and cfDNA reduction (*Figure 1C*), it was determined that the objective ratio of cfDNA reduction could be used as a predictive marker. Based on the results of ROC curve analysis, 0.80 was accepted as the cut-off value of cfDNA reduction for PFS, with an AUC of 0.719, a sensitivity of 76.0%, and a specificity of 67.4% (*Figure 1D*). In addition, 21.7 mg/dL was determined as the cut-off value of PA for PFS, with an AUC of 0.619 (*Figure 1E*), a sensitivity of 58.4%, and a specificity of 62.0%.

Pretreatment PA concentration and PFS

At the time of survival analysis, disease progression occurred in 89 patients, while 25 patients did not reach disease progression at the time of cut-off. The median DFS was 7.0 months (95% CI, 6.0–8.0 months) in the overall study group. To further explore the association between pretreatment PA level and PFS in advanced NSCLC patients, Kaplan-Meier curves were calculated. As shown in *Figure 2A*, the median PFS of the low PA group (PA <21.7 mg/dL) was 6.0 months (95% CI, 4.7–7.3 months), while the median PFS of the high PA group (PA ≥21.7 mg/dL) was 8.0 months (95% CI, 6.7–9.3 months). The PFS (P=0.035) was significantly improved in the high PA group compared with the low PA group as determined by the log-rank test.

Dynamic changes in cfDNA and PFS

As mentioned above, the objective ratio of cfDNA reduction was determined as a predictive marker of PFS in advanced NSCLC patients, and Kaplan-Meier curves were calculated.

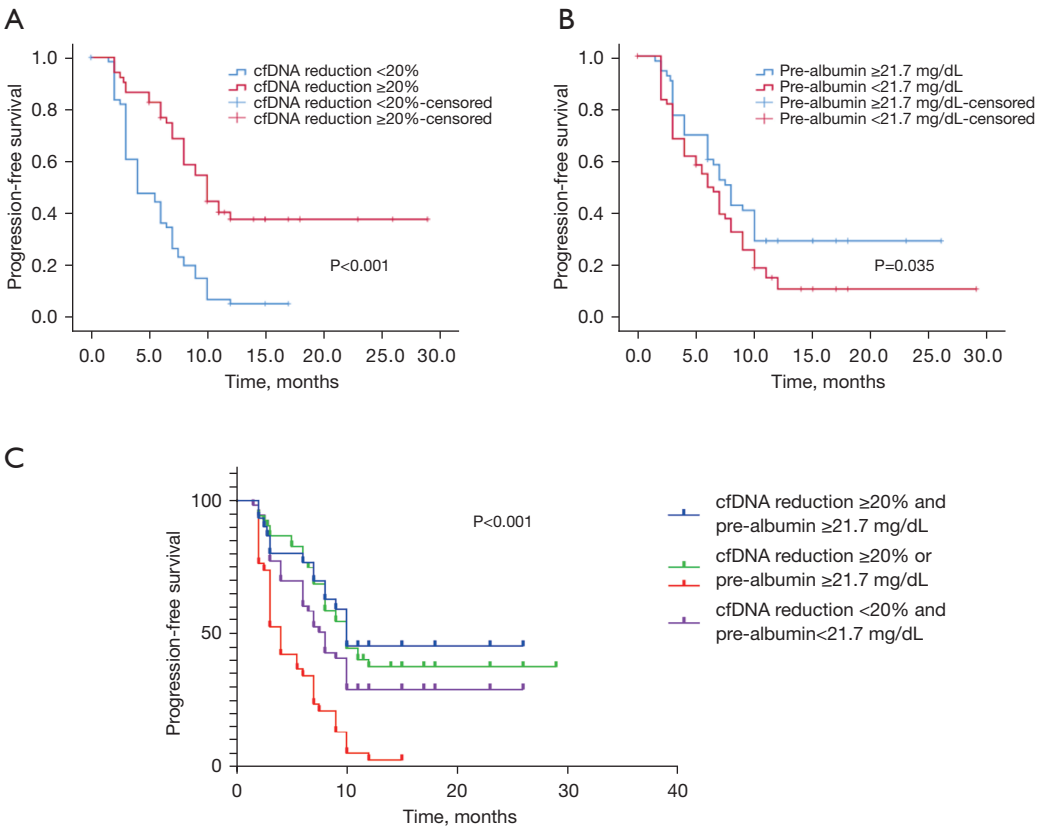

Figure 2 KM curves of PFS in advanced NSCLC patients. (A) KM curve of PFS stratified by the pretreatment pre-albumin concentration. (B) KM curve of PFS stratified by cfDNA reduction. (C) KM curve of PFS according to the pretreatment pre-albumin and cfDNA reduction. cfDNA, cell-free DNA; KM, Kaplan-Meier; PFS, progression-free survival; NSCLC, non-small cell lung cancer.

In *Figure 2B*, it was revealed that the median PFS of the low cfDNA reduction group (reduction <20%) was 4.0 months (95% CI, 2.1–5.9 months), while the high cfDNA reduction group (reduction ≥20%) showed a median PFS of 10.0 months (95% CI, 8.1–11.9 months). A significant difference in PFS was also observed between the 2 cfDNA reduction groups (P<0.001).

Combination of cfDNA dynamic changes with pretreatment PA and patient survival

The combination of both cfDNA reduction and pretreatment PA between baseline and the sixth week of therapy was associated with significantly better survival outcomes compared with patients with neither such parameters (median PFS: 10.5 versus 7.5 months, P<0.001; *Figure 2C*).

Univariate and multivariate analyses for therapeutic response

Therapeutic response was evaluated by the disease control rate (DCR) and calculated by univariate and multivariate analyses. As shown in *Table 2*, in the multivariate analysis, cfDNA reduction ≥20% (HR =0.419, P=0.001) and positive driver mutation (OR =0.496, P=0.009) were associated with a better DCR.

Univariate and multivariate analyses for survival outcomes

The factors associated with PFS were assessed by univariate and multivariate analyses. The univariate Cox proportional regression analysis showed that cfDNA reduction ≥20% (HR =0.336, P<0.001), pretreatment PA ≥21.7 mg/dL (HR =1.529,

Table 2 Univariate and multivariate analysis for DCR

Variable	Univariate analysis			Multivariate analysis		
	P value	OR	95% CI	P value	OR	95% CI
cfDNA reduction (≥20% *vs.* <20%)	0.005	0.485	0.295–0.800	0.001	0.419	0.251–0.702
Pre-albumin (≥21.7 *vs.* <21.7 mg/dL)	0.328	1.260	0.793–2.003			
Age (<65 *vs.* ≥65 years)	0.150	0.700	0.430–1.138			
Sex (male *vs.* female)	0.207	1.447	0.815–2.568			
Smoking history (current/former *vs.* never/unknown)	0.856	1.044	0.659–1.654			
Alcoholic history (current/former *vs.* never/unknown)	0.576	1.140	0.720–1.803			
Comorbidity (yes *vs.* no)	0.509	1.167	0.738–1.844			
ECOG (≥2 *vs.* <2)	0.607	1.193	0.609–2.337			
Stage subgroup (IIIB + IIIC *vs.* IVA + IVB)	0.748	1.098	0.621–1.944			
Histological type (adenocarcinoma *vs.* squamous cell carcinoma)	0.444	1.065	0.907–1.251			
Metastatic sites (≥3 *vs.* <3)	0.401	1.225	0.763–1.965			
Driver mutation (yes *vs.* no)	0.039	0.581	0.347–0.972	0.009	0.496	0.293–0.839
Line of therapy (<2 *vs.* ≥2)	0.267	1.314	0.811–2.126			
Chemotherapy regimen (combined regimen *vs.* chemotherapy)	0.413	0.817	0.503–1.327			

OR, odds ratio; CI, confidence intervals; DCR, disease control rate; cfDNA, cell-free DNA; ECOG, Eastern Cooperative Oncology Group.

P=0.049), age <65 years (HR =0.553, P=0.011), an ECOG performance score of ≥2 (HR =3.148, P<0.001), positive driver mutation (HR =0.378, P=0.001), chemotherapy combined regimen (HR =0.418, P=0.002), and treatment response of CR and PR (HR =0.231, P<0.001) were associated with a better PFS (*Table 3*).

Multivariate analysis identified that cfDNA reduction ≥20% (HR =0.361, P<0.001), age <65 years (HR =0.608, P=0.027), an ECOG performance score of ≥2 (HR =2.282, P=0.001), driver gene mutation (HR =0.401, P=0.005), chemotherapy combined regimen (HR =0.465, P=0.009), and treatment response of CR and PR (HR =0.500, P=0.013) were independent factors associated with better PFS (*Table 3*).

Kaplan-Meier curves of other independent indicators

Based on the results of the multivariate analysis, we further calculated the Kaplan-Meier curves to evaluate the association between other important independent indicators and PFS. PFS was significantly related to combined regimen (P=0.001), ECOG score (P<0.001), driver gene mutation (P<0.001), age (P=0.026), and therapeutic response (P<0.001, *Figure 3*).

Prognostic nomograms for PFS of advanced NSCLC

Significant independent factors based on the multivariate analysis, such as cfDNA reduction, PA, age, ECOG score, driver mutation, combination chemotherapy, and treatment efficacy were eventually included in the nomogram to predict the 2-year PFS probability of advanced NSCLC patients after treatment. The C-index of the nomogram was 0.817 (Figure S1).

Discussion

Our study presented a retrospective analysis of systemic chemotherapy in patients with advanced NSCLC and showed 3 significant findings. First, the results of this work

Table 3 Univariate and multivariate analysis for progression-free survival

Variable	Univariate analysis			Multivariate analysis		
	P value	HR	95% CI	P value	HR	95% CI
cfDNA reduction (≥20% *vs.* <20%)	<0.001	0.336	0.215–0.526	<0.001	0.361	0.216–0.603
Pre-albumin (≥21.7 *vs.* <21.7 mg/dL)	0.049	1.529	1.002–2.334			
Age (<65 *vs.* ≥65 years)	0.038	0.638	0.418–0.976	0.027	0.608	0.392–0.946
Sex (male *vs.* female)	0.915	0.974	0.601–1.579			
Smoking history (current/former *vs.* never/unknown)	0.952	0.987	0.649–1.501			
Alcoholic history (current/former *vs.* never/unknown)	0.699	0.92	0.604–1.401			
Comorbidity (yes *vs.* no)	0.673	0.914	0.602–1.387			
ECOG (≥2 *vs.* <2)	<0.001	3.148	2.025–4.894	0.001	2.282	1.378–3.778
Stage subgroup (IIIB + IIIC *vs.* IVA + IVB)	0.973	1.009	0.601–1.693			
Histological type (adenocarcinoma *vs.* squamous cell carcinoma)	0.789	1.019	0.886–1.173			
Metastatic sites (≥3 *vs.* <3)	0.604	0.892	0.580–1.373			
Driver mutation (yes *vs.* no)	0.001	0.378	0.209–0.685	0.005	0.401	0.212–0.756
Line of therapy (<2 *vs.* ≥2)	0.143	1.376	0.897–2.111			
Chemotherapy regimen (combined regimen *vs.* chemotherapy)	0.002	0.418	0.243–0.722	0.009	0.465	0.261–0.828
Objective response (CR + PR *vs.* SD + PD)	<0.001	0.231	0.146–0.366	0.013	0.500	0.290–0.865

HR, hazard ratio; CI, confidence intervals; cfDNA, cell-free DNA; ECOG, Eastern Cooperative Oncology Group; CR, complete response; PR, partial response; SD, stable disease, PD, progressive disease.

demonstrated that an early dynamic change of plasma cfDNA is a potential predictive biomarker in advanced NSCLC patients treated with chemotherapy, regardless of whether they are newly diagnosed or previously treated. Our study is consistent with some previous research, while there are some differences on certain issues. Hyun *et al.* (16) enrolled 177 NSCLC patients of different clinical stages and found that a high cfDNA concentration was an independent negative prognostic factor for PFS and OS, suggesting that the serum cfDNA concentration is associated with the prognosis of patients with NSCLC. In our research focused on advanced NSCLC patients (IIIB/IIIC/IV stage) who underwent systemic chemotherapy, the objective ratio of cfDNA reduction ≥20% in the course of the first 6 weeks of chemotherapy was associated with better clinical response and prolonged PFS, while a reduction of <20% indicated a poor outcome. Thus, the dynamic change of cfDNA might serve as a potential predictive marker in the real-time monitoring of chemotherapy response and survival outcome.

Second, we revealed a PA concentration of 21.7 mg/dL was the cut-off value based on the ROC curve, which suggested that a low level of pretreatment PA is associated with the poor prognosis of advanced NSCLC. Besides, monitoring cfDNA changes and pretreatment PA level in advanced NSCLC patients receiving treatment could help to predict treatment effectiveness and survival outcomes. These data are in line with the results of other studies, as the presence of low PA was shown to be an independent predictor of poor survival outcome in malignant tumor patients (17-19). This provides insights into the significance of the combined use of various indicators for prediction, as the application of a single marker for prediction may not meet clinical needs.

Third, multivariate analysis showed that cfDNA reduction ≥20% is an independent prognostic factor for clinical efficacy and PFS in advanced NSCLC patients.

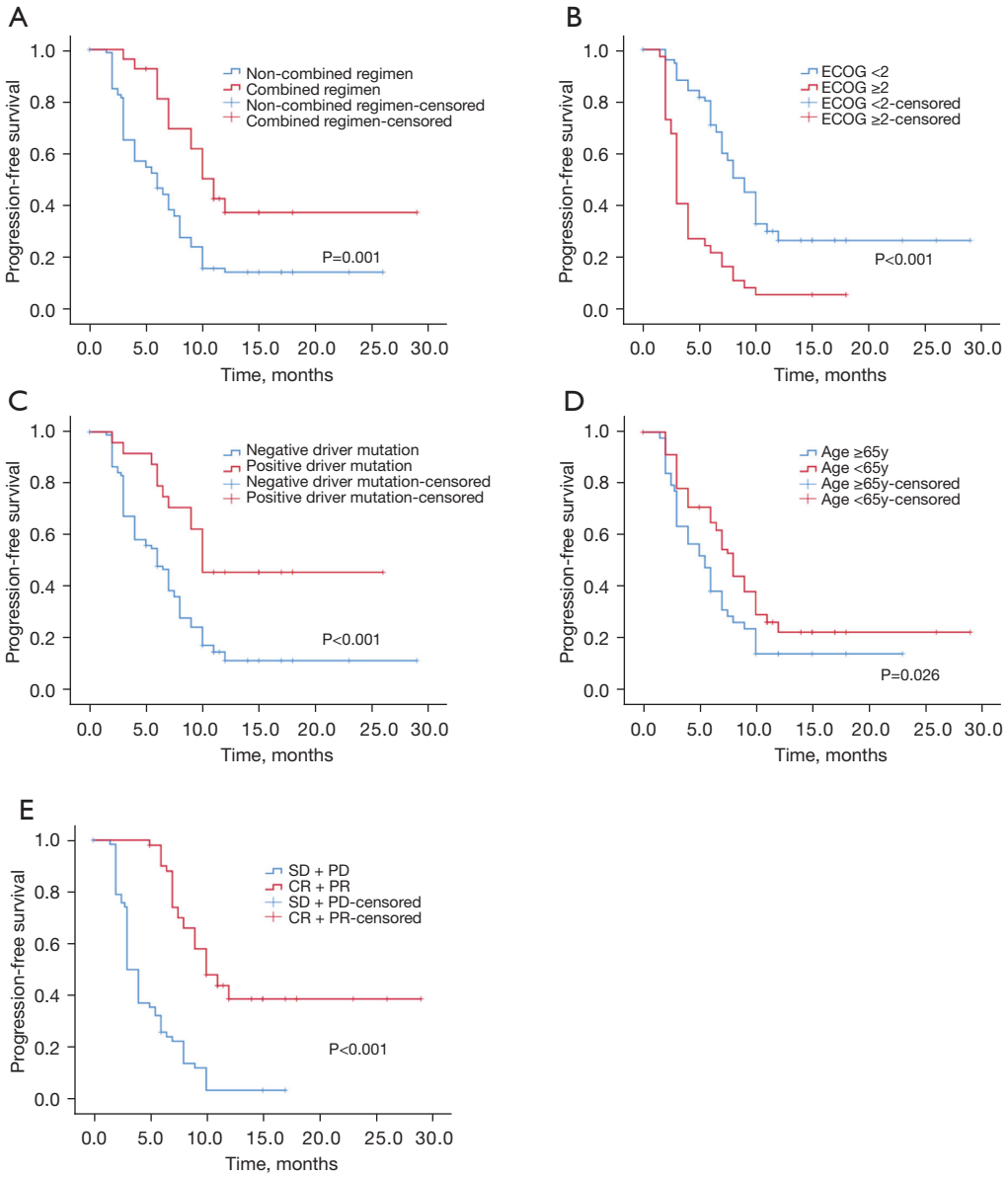

Figure 3 KM curves of PFS in advanced NSCLC patients. (A) KM curve of PFS stratified by regimen. (B) KM curve of PFS stratified by ECOG. (C) KM curve of PFS stratified by driver mutation. (D) KM curve of PFS stratified by age. (E) KM curve of PFS stratified by therapeutic response. ECOG, Eastern Cooperative Oncology Group; SD, stable disease, PD, progressive disease; CR, complete response; PR, partial response; KM, Kaplan-Meier; PFS, progression-free survival; NSCLC, non-small cell lung cancer.

Meanwhile, along with the above, there were also several independent prognostic factors such as age, ECOG performance, combination chemotherapy regimen, positive driver gene mutation, and treatment response. Further analysis confirmed the prognostic value of all these indicators, which were stratified by Kaplan-Meier curves. These findings are consistent with previous research in other tumors (20-24). One study (24) demonstrated the significance of the longitudinal assessment of ctDNA to predict response to anti-PD1 antibodies in metastatic melanoma, and the importance of persistent elevation of ctDNA on monitoring whole disease management. Therefore, all this evidence reveals the importance for every clinician to comprehensively identify independent

prognostic factors and individually evaluate the prognosis of patients.

Currently, the most studied biomarkers in the field of NSCLC are focused on liquid biopsy (25), including cfDNA, ctDNA, and CTCs. As a footprint in the blood left by the tumor (26), cfDNA can be analyzed to identify numerous routine DNAbased alterations observed in tumors, including mutations, gene fusions, copy number variations, and DNA methylation changes. Using NSCLC as an example, for newly diagnosed patients who are medically unfit to undergo invasive tissue sampling, or there is insufficient material for molecular testing if an oncogenic driver is not identified, cfDNA or ctDNA can be considered in specific clinical circumstances (27). Besides, cfDNA analysis could also help to determine comprehensive genomic biomarkers in patients with newly diagnosed metastatic NSCLC, proving that cfDNA is a clinically feasible option to guide the first-line treatment selection for these patients with advanced NSCLC (28). Moreover, the clinical applications of cfDNA are rapidly developing. The use of digital polymerase chain reaction for plasma genotyping is clinically effective for selecting patients who have progressed during first-line treatment with osimertinib (29).

In addition, our research suggested that despite the cfDNA reduction, there were other independent indicators such as chemotherapy combined with anti-angiogenesis drugs (bevacizumab) or ICIs (nivolumab, pembrolizumab, and sintilimab). The Kaplan-Meier curve of PFS in advanced NSCLC patients stratified by treatment regimen showed that the combination group had significantly longer PFS than the chemotherapy group. In clinical settings, high clinical evidence-based clinical trials such as KEYNOTE-189 have laid solid foundation for the combination use of checkpoint inhibitors with chemotherapy (30,31), which indicated that adding pembrolizumab with chemotherapy to the first-line treatment of advanced NSCLC patients could prolong PFS and OS. In the next step, our team is planning to further explore the relationship between serum biomarkers and treatment efficacy or survival outcomes for patients undergoing immunotherapy or anti-angiogenesis therapy. However, there are still some limitations within our investigation. As a retrospective analysis at a single-center, the sample size is limited and some confounding factors are inevitable. In the future, our research group intends to further expand the sample size and conduct studies in conjunction with multiple clinical centers in the local district.

In this rapidly developing field, liquid biopsy has brought great advances and gained increasing attention as an alternative and complementary method compared to traditional tumor biopsy (32). The promise of liquid biopsies including cfDNA/ctDNA detection is undeniable since they offer many advantages in addressing issues related to conventional biopsies. Potential advantages include identifying treatment failures, especially for patients without driven mutations, and broader detection of genomic changes obtained during treatment, especially in later lines of treatment and high-risk patients. Based on the results of this research, we suggest that dynamic monitoring of cfDNA at the sixth week from treatment is a sensitive and reliable biomarker in advanced NSCLC. Early response data may allow for the early initiation of combination therapy, for example, the addition of drugs such as ICIs or anti-angiogenesis agents to the backbone of chemotherapy. Furthermore, the process of evaluating the use of dynamic monitoring of cfDNA changes in the criteria for treatment decisions will help to confirm the role of cfDNA in routine clinical practice. Finally, despite the huge number of predictive biomarkers, we should still be cautious in choosing the most suitable and individualized markers for patients to predict the treatment responses and survival outcomes, or to identify high-risk patients to guide treatment strategies by favoring the use of combined therapy or the most effective therapy in a frontline setting.

Conclusions

Monitoring cfDNA changes and pretreatment PA levels in advanced NSCLC patients receiving treatment is an accurate predictor of tumor response and PFS. Combined assessment of cfDNA and pretreatment PA is helpful for predicting survival outcomes. These findings may assist in identifying high-risk patients and guiding treatment strategies.

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://atm.

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-12/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 The Affiliated Tumor Hospital of Nantong University & Nantong Tumor Hospital (No. 2019-079). All enrolled patients signed an informed consent form before participating in the study.

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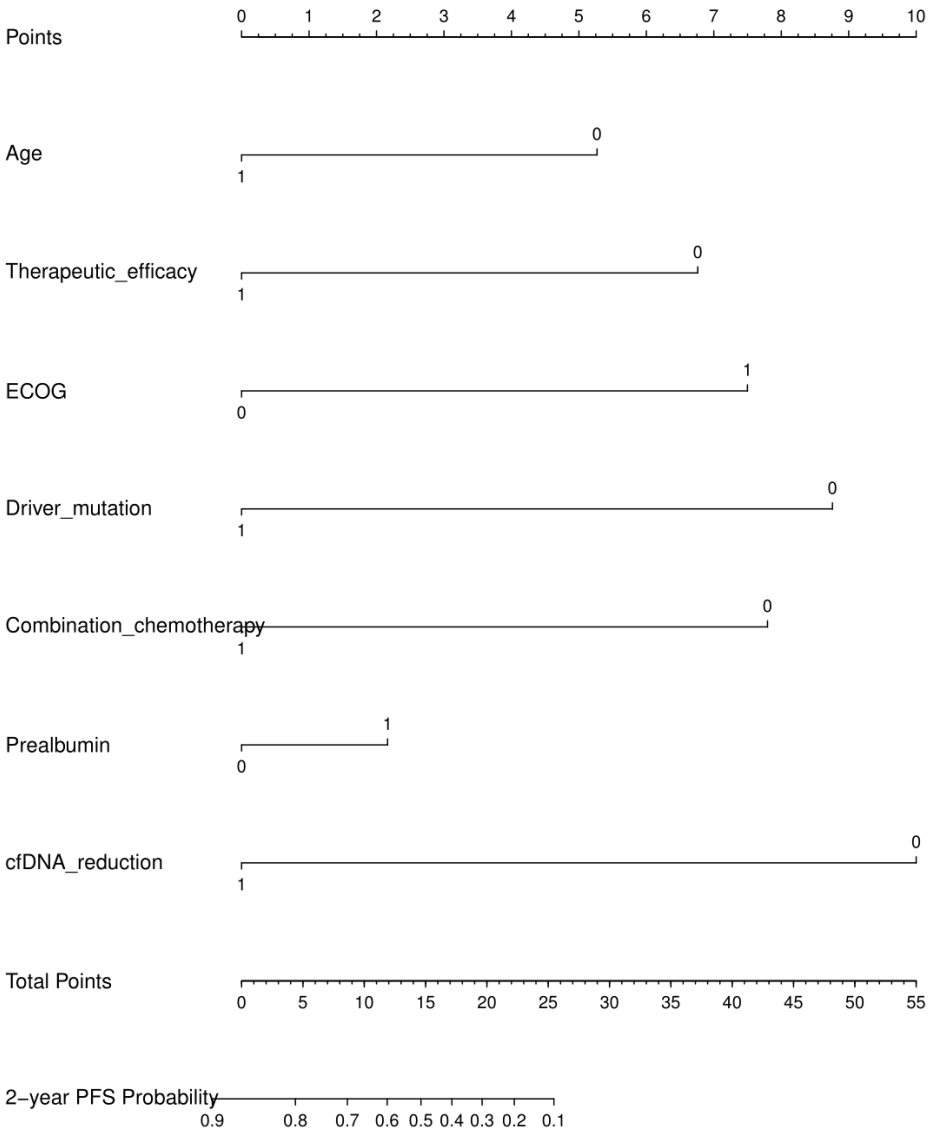

Figure S1 Prognostic nomograms for PFS of advanced NSCLC. ECOG, Eastern Cooperative Oncology Group; cfDNA, cell-free DNA; PFS, progression-free survival; NSCLC, non-small cell lung cancer.

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