

DYNAMIC SERUM CEA, CYFRA21-1, NSE, AND VEGF PROFILES AND THEIR ASSOCIATION WITH FIRST-LINE TREATMENT RESPONSE IN ADVANCED LUNG CANCER

DINAMIČKE PROMENE SERUMSKIH NIVOVA CEA, CYFRA21-1, NSE I VEGF I NJIHOVA POVEZANOST SA ODGOVOROM NA TERAPIJU PRVOG IZBORA KOD UZNAPREDOVALOG KARCINOMA PLUĆA

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Summary

Background: Carcinoembryonic antigen (CEA), cytokeratin 19 fragment antigen 21-1 (CYFRA21-1), neuron-specific enolase (NSE), and vascular endothelial growth factor (VEGF) reflect distinct aspects of lung cancer biology, but the value of longitudinal changes in these biomarkers for assessing first-line treatment response remains unclear.

Methods: This retrospective cohort included 162 patients with advanced lung cancer treated from January 2022 to June 2025. Serum CEA, CYFRA21-1, NSE, and VEGF were measured before treatment and after the first, second, and fourth cycles. Response was assessed using RECIST 1.1. Relative biomarker changes were calculated, and a decrease of at least 30% in two or more markers defined a combined biomarker response. Multivariable logistic regression evaluated associations with concurrent radiological non-response, and receiver operating characteristic analyses quantified the discrimination provided by individual baseline concentrations and T4-to-T0 relative changes.

Results: Pretreatment biomarker profiles varied by pathological type. During therapy, partial-response patients showed the greatest reductions, whereas increases were

Kratak sadržaj

Uvod: Karcinoembriionalni antigen (CEA), fragment citokeratina 19 (CYFRA21-1), neuron-specifična enolaza (NSE) i vaskularni endotelni faktor rasta (VEGF) odražavaju različite aspekte biologije karcinoma pluća, ali značaj njihovih longitudinalnih promena za procenu odgovora na terapiju prvog izbora još uvek nije dovoljno razjašnjen.

Metode: Ova retrospektivna kohortna studija je obuhvatala 162 pacijenta sa uznapredovalim karcinomom pluća koji su lečeni od januara 2022. do juna 2025. godine. Serumske koncentracije CEA, CYFRA21-1, NSE i VEGF su određivane pre početka lečenja i nakon prvog, drugog i četvrtog ciklusa terapije. Odgovor na terapiju je procenjen prema RECIST 1.1. Izračunate su relativne promene biomarkera, a smanjenje od najmanje 30% kod dva ili više markera je definisano kao kombinovani biomarkerski odgovor. Multivarijabilnom logističkom regresijom je procenjena povezanost sa istovremenim izostankom radiološkog odgovora, dok je analizom ROC krivih procenjena diskriminativna sposobnost pojedinačnih početnih koncentracija i relativnih promena od T4 do T0.

Rezultati: Profili biomarkera pre početka lečenja su razlikovali se u zavisnosti od patološkog tipa. Tokom tera-

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more common in progressive disease. Combined biomarker response rates were 68.42% in partial response, 36.23% in stable disease, and 13.89% in progressive disease ($p < 0.001$). After adjustment for pathological type, first-line treatment components, ln-transformed CRP, and ln-transformed D-dimer, insufficient decreases in CEA (OR 4.437, 95% CI 1.713–11.497; $P = 0.002$) and NSE (OR 2.623, 95% CI 1.015–6.774; $P = 0.046$), and failure to achieve a combined biomarker response (OR 8.399, 95% CI 3.501–20.151; $P < 0.001$), remained associated with non-response. Higher VEGF was associated with greater lesion burden, more metastatic organs, higher D-dimer, and pleural involvement. Dynamic biomarker changes yielded AUCs of 0.796–0.828 and showed greater discrimination than the corresponding baseline concentrations (DeLong $P < 0.004$).

Conclusions: Longitudinal changes in CEA, CYFRA21-1, NSE, and VEGF showed greater discrimination of concurrent radiological non-response than their baseline concentrations; serial multi-marker monitoring may therefore assist response assessment during first-line treatment, although prospective predictive value was not evaluated.

Keywords: advanced lung cancer, serum tumour biomarkers, longitudinal monitoring, first-line systemic therapy, treatment response

Introduction

Advanced lung cancer is biologically heterogeneous, with substantial differences in histological origin, cellular turnover, neuroendocrine differentiation, angiogenic activity, and metastatic behaviour (1). These processes may change during systemic therapy, although their changes are not necessarily synchronous and cannot be fully represented by macroscopic alterations in tumour size (2, 3). Circulating tumour-related markers originate from tumour secretion, cellular injury, structural protein degradation, and vascular activity; because they can be measured repeatedly, their longitudinal patterns may provide information on treatment-related biological changes that is not available from an isolated pretreatment measurement (4, 5).

CEA, CYFRA21-1, NSE, and VEGF reflect different aspects of lung cancer biology and are therefore unlikely to provide identical information (6–9). CEA is more commonly elevated in adenocarcinoma and is related to glandular epithelial secretion and tumour burden (10), whereas CYFRA21-1 is released during epithelial tumour cell injury and cytokeratin-19 degradation (11); NSE is mainly associated with neuroendocrine differentiation, particularly in small-cell lung cancer (12). VEGF differs from these conventional tumour markers because it is involved in angiogenesis, vascular permeability, and metastatic spread (13). Ex-

plaining their changes together may therefore show whether treatment affects these tumour-related processes in a similar or discordant manner.

Previous studies have predominantly evaluated these serum markers at a single baseline time point, usually in relation to diagnosis, staging, or prognosis, whereas their longitudinal behaviour during systemic therapy remains insufficiently characterised (14, 15). A decrease, persistent elevation, or rebound in an individual marker may reflect a different biological response, while concordant or discordant changes among markers may provide further information on tumour heterogeneity. The present study therefore characterised the dynamic serum profiles of CEA, CYFRA21-1, NSE, and VEGF during first-line systemic therapy and examined their relationships with pathological type, radiological response, tumour and metastatic burden, and coagulation-related features; the discriminatory performance of individual and combined dynamic markers was evaluated as a secondary analysis.

Zaključak: Longitudinalne promene CEA, CYFRA21-1, NSE i VEGF pokazale su veću diskriminativnu sposobnost za istovremeni izostanak radiološkog odgovora u odnosu na njihove početne koncentracije. Serijsko praćenje više biomarkera stoga može doprineti proceni odgovora tokom terapije prvog izbora, iako prospektivna prediktivna vrednost nije procenjena.

Ključne reči: uznapredovali karcinom pluća, serumski tumorski biomarkeri, longitudinalno praćenje, sistemska terapija prvog izbora, odgovor na terapiju

Materials and Methods

Study subjects

This single-centre retrospective cohort initially screened 274 consecutive patients with advanced

lung cancer who initiated first-line systemic therapy between January 2022 and June 2025. Exclusion criteria were applied sequentially to avoid double counting: 36 patients were excluded because complete serial measurements of CEA, CYFRA21-1, NSE, and VEGF were unavailable; 20 lacked an evaluable first radiological response assessment or had incomplete imaging data; 15 had serum samples that failed the prespecified sample-quality requirements; 16 discontinued treatment before the first response assessment for reasons unrelated to tumour progression; 13 had another active malignancy, acute infection, active autoimmune disease, or severe hepatic or renal dysfunction that could interfere with biomarker interpretation; and 12 had missing key clinical or laboratory data. Consequently, 112 patients were excluded, and 162 were included in the final complete-case analysis. Patients who underwent an earlier radiological assessment or changed treatment because of documented disease progression were retained, provided that paired T0 and T4 biomarker measurements and an evaluable RECIST 1.1 assessment were available. The study was approved by the ethics committee of Affiliated Tumour Hospital of Nantong University, and all study subjects provided written informed consent.

Inclusion and exclusion criteria

The inclusion criteria were as follows: (1) patients aged ≥ 18 years, diagnosed with LC, evaluated as having advanced disease before treatment, and receiving first-line systemic therapy; (2) patients who completed serial serum biomarker testing before and during treatment and had an evaluable first RECIST 1.1 (16) assessment paired with the T4 biomarker measurement. The exclusion criteria were as follows: (1) other active malignancies, acute infection, active autoimmune disease, or severe hepatic or renal dysfunction; (2) clinical conditions that could clearly interfere with the interpretation of serum biomarkers; (3) absence of paired T0 and T4 biomarker measurements or an evaluable first radiological response assessment; (4) missing key laboratory or imaging data.

Treatment data and response evaluation

Patients with NSCLC received platinum-based chemotherapy, immunotherapy-based combination therapy, targeted therapy, anti-angiogenic combination therapy, or other standard first-line regimens; patients with small-cell lung cancer (SCLC) mainly received platinum plus etoposide, and some patients received immunotherapy in combination. According to RECIST 1.1, treatment response was classified as complete response, partial response, stable disease, or progressive disease. Complete response

and partial response were assigned to the response group, while stable disease and progressive disease were assigned to the non-response group.

Serum sample collection

Serum CEA, CYFRA21-1, NSE, and VEGF testing covered the main observation points before and during treatment: T0 was defined as within 7 d before initiation of first-line therapy, T1 as after the end of the first treatment cycle and before the start of the second cycle, T2 as after the end of the second treatment cycle and before the start of the third cycle, and T4 was defined as the serum measurement paired with the first radiological response assessment and obtained within 7 days before or after imaging. All blood samples were fasting peripheral venous blood collected in the morning; 5 mL was collected each time into a serum separator tube (BD), left standing at 22–25 °C for approximately 30 min, and centrifuged at 1500×g for 10 min at 4 °C. CEA, CYFRA21-1, and NSE were measured within 2 h after serum separation; serum used for VEGF testing was aliquoted and stored at -80 °C, and repeated freeze-thaw cycles before testing were avoided. Samples with visible haemolysis, lipemia, jaundice, or more than one freeze-thaw cycle were not included in the VEGF analysis.

Biomarker detection

CEA, CYFRA21-1, and NSE were measured using electrochemiluminescence immunoassay (Roche cobas e 801 automated electrochemiluminescence immunoassay analyser); VEGF was measured using a human VEGF quantitative enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems), with a detection wavelength of 450 nm and a correction wavelength of 570 nm. For the VEGF ELISA, standards, controls, and serum samples were measured in duplicate, and the mean of the two acceptable readings was used; a duplicate-well CV $>15\%$ prompted repeat testing. The manufacturer-reported intra-assay CVs for serum or plasma samples were 6.7%, 4.5%, and 5.1% at low, intermediate, and high concentrations, respectively. The corresponding inter-assay CVs were 8.8%, 7.0%, and 6.2%. CEA, CYFRA21-1, and NSE were measured by electrochemiluminescence immunoassay on a Roche cobas e 801 analyser and were reported in ng/mL. VEGF was measured using a human VEGF quantitative ELISA kit from R&D Systems and was reported in pg/mL; absorbance was measured at 450 nm with wavelength correction at 570 nm. Testing personnel were unaware of the group assignments.

Definition of biomarker dynamic changes and combined detection

Treatment-related changes in CEA, CYFRA21-1, NSE, and VEGF were expressed as relative change rates, calculated as follows: $\Delta\text{marker}\% = (\text{post-treatment level} - \text{baseline level}) / \text{baseline level} \times 100\%$. Because percentage changes may be unstable when baseline concentrations are low, absolute change (T4 minus T0) and the natural-log ratio $\ln(T4/T0)$ were evaluated as supplementary change measures. Marker-specific analyses were also repeated among patients whose baseline CEA, CYFRA21-1, or NSE exceeded the operational upper limit and among patients in the upper-median VEGF subgroup. The post-treatment level was mainly based on the T4 result; in the dynamic trend analysis, T0, T1, T2, and T4 were also included. A relative decrease of $\geq 30\%$ from baseline was used as an exploratory operational definition of biochemical decline rather than as an externally validated clinical cut-off. A combined biomarker response was defined as a decrease meeting this threshold in at least two of the four biomarkers. Sensitivity analyses repeated the combined-response definition using relative-decrease thresholds of 20% and 40%. For descriptive multi-marker analyses, positivity was defined as CEA >5.0 ng/mL, CYFRA21-1 >3.3 ng/mL, or NSE >16.3 ng/mL; VEGF was divided at the cohort median of 359.5 pg/mL. The VEGF median split was used solely for internal descriptive grouping. It was not interpreted as an externally validated clinical cut-off.

Statistical analysis

Statistical analysis was performed using SPSS 26.0 (IBM). Continuous variables were first tested for normality. Normally distributed variables were expressed as mean $\pm$ standard deviation, and comparisons between groups were performed using the independent-samples t test; variables not conforming to a normal distribution were expressed as median and interquartile range, and comparisons between groups were performed using the Mann-Whitney U test or Kruskal-Wallis test. Categorical variables were expressed as numbers and percentages, and comparisons between groups were performed using the χ^2 test or Fisher's exact test. Longitudinal changes in each raw biomarker across T0, T1, T2, and T4 were evaluated using the Friedman test. To assess whether longitudinal patterns differed according to treatment exposure, natural-log-transformed concentrations were analysed using random-intercept linear mixed-effects models containing categorical time, the four treatment-component indicators, and all time-by-treatment-component interaction terms; the patient-specific random intercept accounted for within-patient correlation; logistic regression anal-

ysis used concurrent radiological non-response as the dependent variable, coded as 1 for stable or progressive disease and 0 for partial response; no complete responses were observed. Candidate variables included age ≥ 65 years, sex, smoking history, ECOG score ≥ 2 , stage IV disease, involvement of at least two metastatic organs, pleural involvement or pleural effusion, any liver, brain, or bone metastasis, pathological type, the four non-mutually exclusive treatment components, LDH elevation, In-transformed CRP, In-transformed D-dimer, baseline biomarker elevation, upper-median VEGF, the four individual dynamic-marker indicators, and failure to achieve the combined biomarker response. Model A included pathological type, the four treatment components, In-transformed CRP, In-transformed D-dimer, and the four individual indicators of a relative decrease of $<30\%$; Model B included the same adjustment variables and failure to achieve the combined biomarker response instead of the four individual dynamic-marker indicators. Pathological type was entered using indicator variables with adenocarcinoma as the reference category, and the four treatment components were entered as separate binary variables. Model A included pathological type, the four treatment components, In-transformed CRP, In-transformed D-dimer, and the four individual indicators of a relative decrease of $<30\%$; Model B included the same adjustment variables and failure to achieve the combined biomarker response instead of the four individual dynamic-marker indicators. Receiver operating characteristic (ROC) curves were used to quantify the ability of each baseline biomarker concentration and its corresponding T4-to-T0 relative change to discriminate concurrent radiological non-response. The AUC, 95% CI, optimal cut-off, sensitivity, specificity, and Youden index were calculated, and paired baseline and dynamic AUCs for the same marker were compared using the DeLong test. All tests were two-sided, and $P < 0.05$ was considered statistically significant.

Results

Baseline characteristics of patients

There were no significant differences in age, sex, smoking history, Eastern Cooperative Oncology Group (ECOG) score, pathological type, and other variables between the two groups ($P > 0.05$), but pretreatment C-reactive protein (CRP) and D-dimer levels were higher in the non-response group than in the response group ($P < 0.001$, $P = 0.014$), *Table 1*.

Table I Baseline clinical and laboratory characteristics according to treatment response.

	Overall n=162	Response group n=57	Non-response group n=105	Statistic	P value
Age (years)	64.26±9.95	62.89±9.60	65.00±10.10	t=-1.309	0.193
Sum of target lesion diameters (mm)	74.67±38.02	68.85±34.98	77.83±39.38	t=-1.492	0.138
ALB (g/L)	38.42±5.24	39.20±4.89	38.00±5.39	t=1.430	0.155
LDH (U/L)	257.00 (191.00, 348.00)	235.00 (174.00, 324.00)	267.00 (193.00, 351.00)	Z=-1.445	0.149
CRP (mg/L)	15.85 (7.90, 37.00)	11.50 (4.30, 22.50)	19.30 (9.50, 51.20)	Z=-3.399	<0.001
D-dimer (mg/L)	1.13 (0.58, 2.46)	0.89 (0.46, 1.91)	1.44 (0.68, 2.55)	Z=-2.452	0.014
Male	101 (62.35)	33 (57.89)	68 (64.76)	$\chi^2=0.742$	0.389
Smoking history	96 (59.26)	31 (54.39)	65 (61.90)	$\chi^2=0.865$	0.352
ECOG score ≥ 2	50 (30.86)	14 (24.56)	36 (34.29)	$\chi^2=1.637$	0.201
Overall distribution of pathological type	-	-	-	$\chi^2=3.199$	0.362
Adenocarcinoma	86 (53.09)	35 (61.40)	51 (48.57)	-	-
SCC	47 (29.01)	15 (26.32)	32 (30.48)	-	-
SCLC	23 (14.20)	5 (8.77)	18 (17.14)	-	-
Others	6 (3.70)	2 (3.51)	4 (3.81)	-	-
Stage IV	123 (75.93)	40 (70.18)	83 (79.05)	$\chi^2=1.591$	0.207
Metastatic organs ≥ 2	77 (47.53)	23 (40.35)	54 (51.43)	$\chi^2=1.818$	0.178
Pleural involvement/pleural effusion	64 (39.51)	17 (29.82)	47 (44.76)	$\chi^2=3.449$	0.063
Any liver/brain/bone metastasis	61 (37.65)	17 (29.82)	44 (41.90)	$\chi^2=2.297$	0.13
First-line treatment components	-	-	-	-	-
Platinum-based chemotherapy	44 (27.16)	13 (22.81)	31 (29.52)	$\chi^2=0.842$	0.359
Immunotherapy-based combination therapy	56 (34.57)	21 (36.84)	35 (33.33)	$\chi^2=0.201$	0.654
Targeted therapy	36 (22.22)	16 (28.07)	20 (19.05)	$\chi^2=1.740$	0.187
Anti-angiogenic combination therapy	26 (16.05)	7 (12.28)	19 (18.10)	$\chi^2=0.927$	0.336

Note: Treatment components were coded separately and were not mutually exclusive; 41 patients had more than one listed component, and 49 had none of the four listed components.

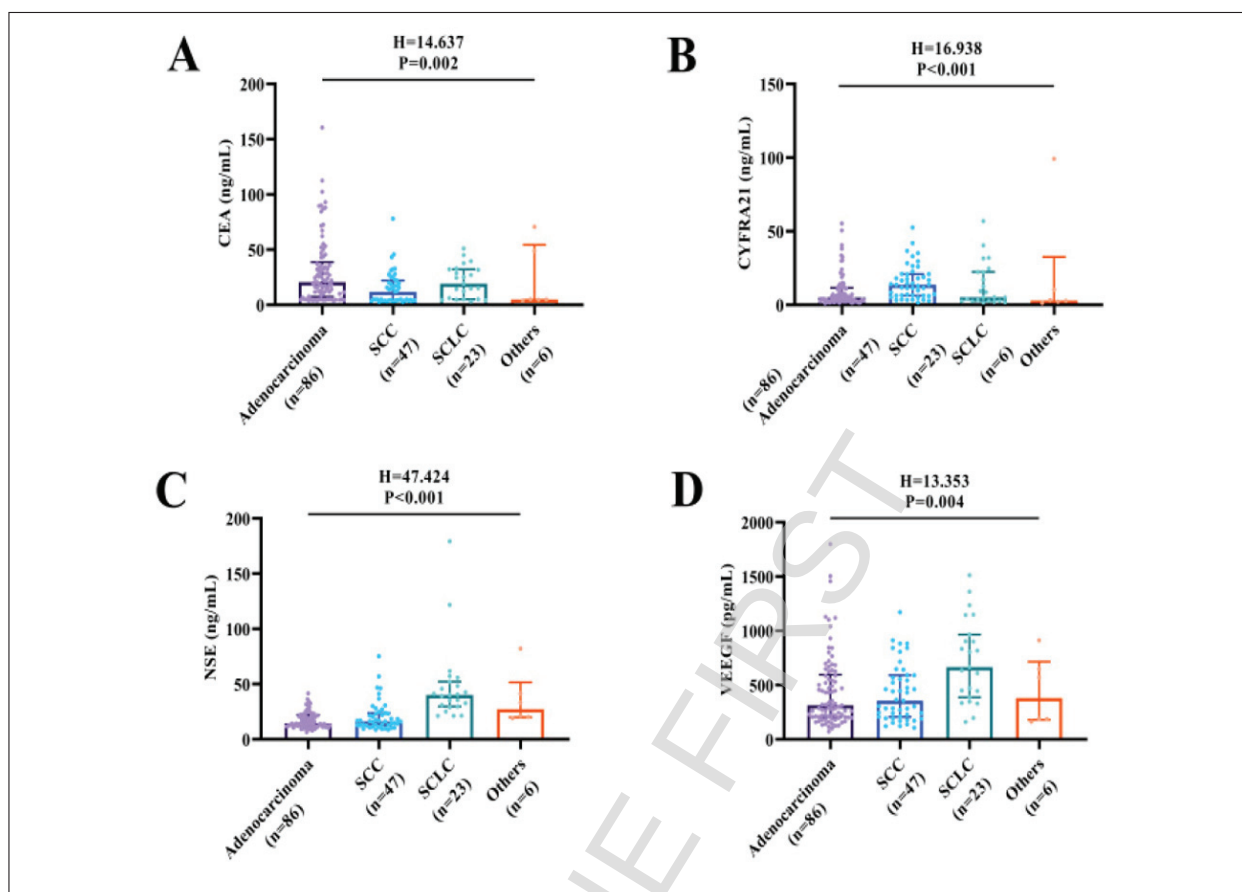


Figure 1 Pretreatment serum levels of CEA, CYFRA21-1, NSE, and VEGF according to pathological type.

(A) Pretreatment serum CEA level according to pathological type; (B) pretreatment serum CYFRA21-1 level according to pathological type; (C) pretreatment serum NSE level according to pathological type; (D) pretreatment serum VEGF level according to pathological type.

Pretreatment serum levels of CEA, CYFRA21-1, NSE, and VEGF

Before treatment, CEA was relatively higher in patients with adenocarcinoma ($P=0.002$), CYFRA21-1 was higher in patients with squamous cell carcinoma (SCC) ($P<0.001$), and NSE was most prominently elevated in patients with SCLC ($P<0.001$). VEGF levels also differed according to pathological type, with a higher median level in patients with SCLC ($P=0.004$), Figure 1.

Pretreatment multi-marker expression patterns

In terms of positive detection, the pretreatment positive rates of CEA, CYFRA21-1, and NSE were 67.28%, 71.60%, and 54.32%, respectively, and patients with high VEGF level accounted for 50.00%. Combined detection improved the identification rate of biomarker abnormalities: 91.98% of patients were positive for any biomarker, 79.63% were positive for ≥ 2 biomarkers, 51.85% were posi-

tive for ≥ 3 biomarkers, and 19.75% were positive for all 4 biomarkers.

Dynamic serum marker profiles during first-line therapy

During first-line systemic therapy, CEA, CYFRA21-1, NSE, and VEGF changed significantly across T0, T1, T2, and T4 (all Friedman $P<0.001$). The recalculated four-marker standardised score was 0.00 ± 0.59 at T0, -0.06 ± 0.65 at T1, -0.14 ± 0.73 at T2, and -0.26 ± 0.86 at T4, with a significant overall difference across the four time points (Friedman $\chi^2=52.319$, $P<0.001$), Figure 2.

Response-associated gradients in serum marker changes

No complete response was observed at the first radiological assessment; therefore, the response group consisted of 57 patients with partial response,

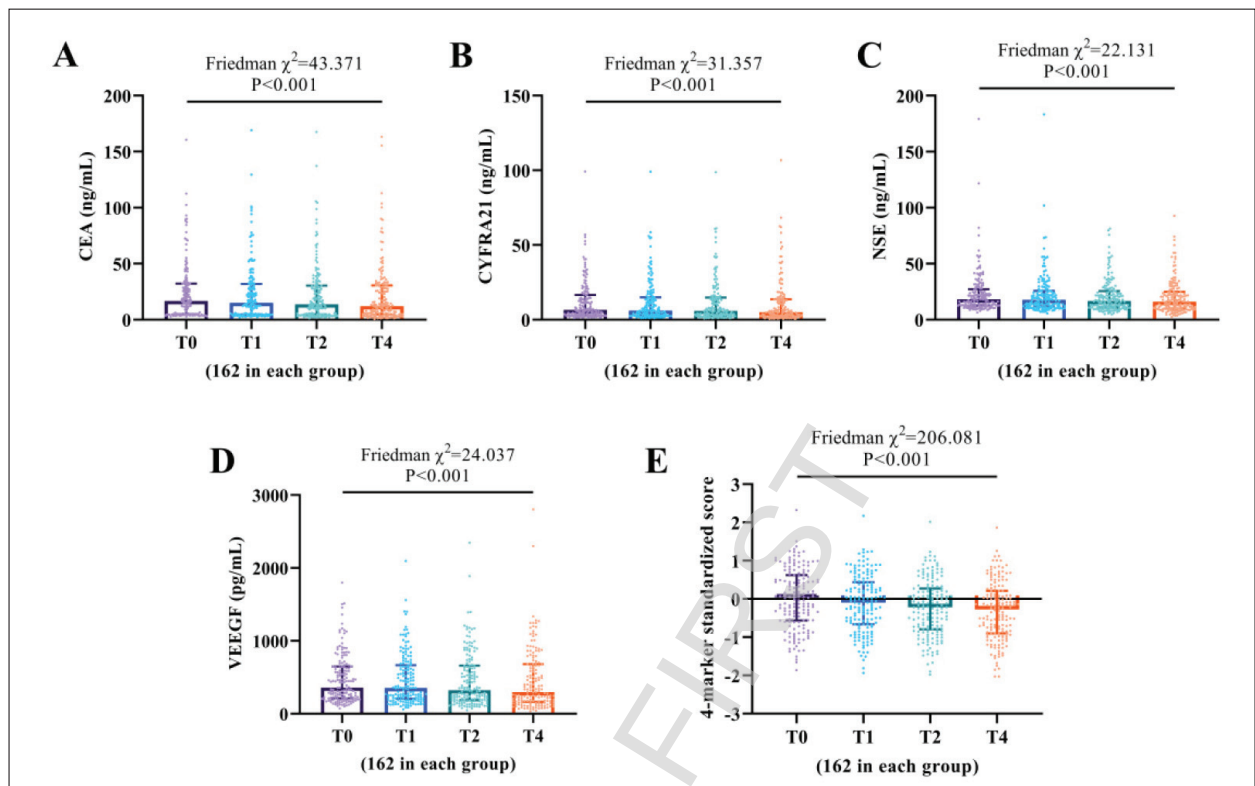


Figure 2 Dynamic changes in serum biomarkers and the 4-marker standardised score during first-line systemic therapy.

(A) Dynamic change in serum CEA level across T0, T1, T2, and T4; (B) dynamic change in serum CYFRA21-1 level across T0, T1, T2, and T4; (C) dynamic change in serum NSE level across T0, T1, T2, and T4; (D) dynamic change in serum VEGF level across T0, T1, T2, and T4; (E) four-marker standardised score calculated as the arithmetic mean of the T0-referenced z-scores of natural-log-transformed CEA, CYFRA21-1, NSE, and VEGF.

Table II Changes in serum biomarkers according to radiological treatment response.

	PR n=57	SD n=69	PD n=36	Statistic	P value
ΔCEA (%)	-45.20 (-54.70, -20.00)	-6.50 (-35.80, 8.00)	15.55 (3.83, 33.50)	H=67.851	<0.001
ΔCYFRA21-1 (%)	-46.20 (-58.60, -24.30)	-9.00 (-37.50, 5.90)	24.80 (5.40, 53.58)	H=61.616	<0.001
ΔNSE (%)	-29.10 (-51.20, -13.20)	-2.80 (-24.40, 6.40)	16.50 (-2.52, 27.62)	H=57.599	<0.001
ΔVEGF (%)	-34.20 (-50.00, -15.50)	-13.30 (-31.40, 8.10)	29.40 (6.97, 54.67)	H=64.485	<0.001
≥2 biomarkers decreased by ≥30%	39 (68.42)	25 (36.23)	5 (13.89)	χ²=28.824	<0.001
No decrease in all 4 biomarkers	6 (10.53)	22 (31.88)	23 (63.89)	χ²=29.137	<0.001

whereas the non-response group comprised 69 patients with stable disease and 36 patients with progressive disease. After stratification according to RECIST response, serum biomarker changes showed a relatively clear correspondence with radiological response. Patients with partial response (PR) had larger decreases in CEA, CYFRA21-1, NSE, and VEGF;

patients with stable disease (SD) mostly showed mild decreases or small fluctuations, whereas patients with progressive disease (PD) more often showed increases ($P<0.001$). Meanwhile, the proportion of patients with ≥ 2 biomarkers decreased by $\geq 30\%$ was 68.42% in the PR group, higher than 36.23% in the SD group and 13.89% in the PD group ($P<0.001$).

Supplementary Table S1 Sensitivity analyses of the combined biomarker response using 20%, 30%, and 40% relative-decrease thresholds.

Relative-decrease threshold	PR, n/N (%)	SD, n/N (%)	PD, n/N (%)	Overall, n/N (%)	Adjusted OR for failure to achieve a combined biomarker response (95%CI)	P value
20%	49/57 (85.96)	31/69 (44.93)	5/36 (13.89)	85/162 (52.47)	15.861 (5.894–42.684)	<0.001
30%	39/57 (68.42)	25/69 (36.23)	5/36 (13.89)	69/162 (42.59)	8.399 (3.501–20.151)	<0.001
40%	33/57 (57.89)	11/69 (15.94)	1/36 (2.78)	45/162 (27.78)	19.536 (6.973–54.729)	<0.001

In contrast, patients with no decrease in all 4 biomarkers accounted for the highest proportion in the PD group, at 63.89%, which was markedly higher than in the PR and SD groups ($P<0.001$), *Table II*. At the 20% threshold, combined biomarker response was observed in 49 of 57 patients with PR (85.96%), 31 of 69 with SD (44.93%), and 5 of 36 with PD (13.89%); failure to achieve this response was associated with non-response after adjustment (OR 15.861, 95% CI 5.894–42.684; $P<0.001$). At the primary 30% threshold, the corresponding response rates were 68.42%, 36.23%, and 13.89%, and the adjusted OR was 8.399 (95% CI 3.501–20.151; $P<0.001$). At the 40% threshold, the response rates were 57.89%, 15.94%, and 2.78%, and the adjusted OR was 19.536 (95% CI 6.973–54.729; $P<0.001$; *Supplementary Table S1*).

Associations of serum VEGF with tumour, metastatic, and coagulation-related features

Patients were divided at the cohort median pretreatment VEGF concentration of 359.5 pg/mL, yielding 81 patients in the lower-median group and 81 in the upper-median group. These labels describe an internal cohort split and do not represent clinically validated VEGF categories. Compared with the VEGF-low group, the VEGF-high group had a larger sum of target lesion diameters, more metastatic organs, and higher D-dimer level ($P<0.05$), whereas lactate dehydrogenase (LDH) level did not differ significantly between the two groups ($P=0.184$). Further analysis of clinically aggressive features showed that the proportion of pleural involvement or pleural effusion was higher in the VEGF-high group than in the VEGF-low group (48.15% vs 30.86%, $P=0.024$; *Table III*).

Table III Clinical features according to the cohort-median pretreatment VEGF split.

	Lower-median VEGF, n=81	Upper-median VEGF, n=81	Statistic	P value
VEGF (pg/mL)	211.00 (171.00, 272.00)	647.00 (502.00, 882.00)	$Z=-10.989$	<0.001
Sum of target lesion diameters (mm)	68.23±36.64	81.11±38.52	$t=-2.181$	0.031
Number of metastatic organs	1.00 (1.00, 2.00)	2.00 (1.00, 3.00)	$Z=-2.711$	0.007
LDH (U/L)	234.00 (174.00, 327.00)	273.00 (201.00, 352.00)	$Z=-1.330$	0.184
D-dimer (mg/L)	0.99 (0.53, 1.78)	1.55 (0.82, 2.97)	$Z=-2.755$	0.006
Metastatic organs ≥2	33 (40.74)	44 (54.32)	$\chi^2=2.995$	0.084
Pleural involvement/pleural effusion	25 (30.86)	39 (48.15)	$\chi^2=5.062$	0.024
Any liver/brain/bone metastasis	26 (32.10)	35 (43.21)	$\chi^2=2.130$	0.144
Disease control rate (DCR)	68 (83.95)	58 (71.60)	$\chi^2=3.571$	0.059
Early progression	13 (16.05)	23 (28.40)	$\chi^2=3.571$	0.059

Table IVa Univariable logistic regression analyses.

Variable	β	SE	OR (95% CI)	P value
Clinical and disease characteristics				
Age ≥ 65 years	0.316	0.33	1.371 (0.718–2.618)	0.339
Male	0.29	0.337	1.337 (0.690–2.588)	0.39
Smoking history	0.31	0.333	1.363 (0.709–2.619)	0.353
ECOG score ≥ 2	0.472	0.37	1.602 (0.776–3.310)	0.203
Stage IV disease	0.472	0.376	1.603 (0.767–3.350)	0.209
Metastatic organs ≥ 2	0.448	0.333	1.565 (0.815–3.007)	0.179
Pleural involvement or pleural effusion	0.645	0.35	1.907 (0.961–3.785)	0.065
Any liver, brain, or bone metastasis	0.529	0.351	1.697 (0.854–3.374)	0.131
Pathological type				
Squamous cell carcinoma vs. adenocarcinoma	0.205	0.368	1.227 (0.597–2.525)	0.578
Small-cell lung cancer vs. adenocarcinoma	0.766	0.535	2.152 (0.754–6.141)	0.152
Other pathological types vs. adenocarcinoma	0.085	0.882	1.089 (0.193–6.137)	0.923
First-line treatment components				
Platinum-containing chemotherapy	0.349	0.381	1.418 (0.671–2.994)	0.36
Immunotherapy-based combination therapy	-0.154	0.344	0.857 (0.437–1.682)	0.654
Targeted therapy	-0.506	0.386	0.603 (0.283–1.284)	0.189
Anti-angiogenic combination therapy	0.456	0.477	1.578 (0.620–4.016)	0.338
Laboratory variables				
ln(CRP)	0.498	0.152	1.646 (1.222–2.218)	0.001
ln(D-dimer)	0.497	0.187	1.643 (1.140–2.368)	0.008
Baseline CEA elevation	0.406	0.346	1.500 (0.761–2.957)	0.241
Baseline CYFRA21-1 elevation	0.496	0.358	1.642 (0.814–3.313)	0.166
Baseline NSE elevation	0.323	0.33	1.381 (0.723–2.638)	0.328
Upper-median VEGF	0.601	0.334	1.824 (0.948–3.509)	0.072
Dynamic biomarker variables				
CEA decrease $<30\%$	1.777	0.364	5.913 (2.898–12.066)	<0.001
CYFRA21-1 decrease $<30\%$	1.381	0.347	3.977 (2.013–7.858)	<0.001
NSE decrease $<30\%$	1.609	0.375	4.998 (2.398–10.417)	<0.001
VEGF decrease $<30\%$	1.623	0.364	5.067 (2.483–10.342)	<0.001
Failure to achieve a combined biomarker response	1.689	0.358	5.417 (2.688–10.917)	<0.001

Table IVb Multivariable logistic regression analyses.

Model	Variable	β	SE	Adjusted OR (95% CI)	P value
Model A	Intercept	-3.353	0.817	0.035 (0.007–0.173)	<0.001
	Squamous cell carcinoma vs adenocarcinoma	0.312	0.507	1.366 (0.506–3.689)	0.538
	Small-cell lung cancer vs adenocarcinoma	-0.017	0.756	0.983 (0.224–4.327)	0.982
	Other pathological types vs adenocarcinoma	0.937	1.184	2.551 (0.251–25.956)	0.429
	Platinum-containing chemotherapy	0.858	0.501	2.358 (0.883–6.297)	0.087
	Immunotherapy-based combination therapy	-0.213	0.458	0.808 (0.329–1.985)	0.642
	Targeted therapy	-0.247	0.505	0.781 (0.290–2.104)	0.625
	Anti-angiogenic combination therapy	0.306	0.586	1.358 (0.431–4.281)	0.601
	ln(CRP)	0.43	0.194	1.537 (1.051–2.246)	0.027
	ln(D-dimer)	0.676	0.246	1.965 (1.213–3.185)	0.006
	CEA decrease <30%	1.49	0.486	4.437 (1.713–11.497)	0.002
	CYFRA21-1 decrease <30%	0.681	0.48	1.975 (0.771–5.059)	0.156
	NSE decrease <30%	0.964	0.484	2.623 (1.015–6.774)	0.046
	VEGF decrease <30%	0.756	0.494	2.130 (0.808–5.612)	0.126
Model B	Intercept	-2.087	0.646	0.124 (0.035–0.440)	0.001
	Squamous cell carcinoma vs adenocarcinoma	0.405	0.473	1.499 (0.593–3.787)	0.392
	Small-cell lung cancer vs adenocarcinoma	0.117	0.708	1.124 (0.281–4.500)	0.868
	Other pathological types vs. adenocarcinoma	0.721	1.071	2.057 (0.252–16.798)	0.501
	Platinum-containing chemotherapy	0.705	0.451	2.024 (0.836–4.899)	0.118
	Immunotherapy-based combination therapy	-0.186	0.43	0.830 (0.358–1.928)	0.665
	Targeted therapy	-0.168	0.486	0.845 (0.326–2.189)	0.729
	Anti-angiogenic combination therapy	0.654	0.567	1.923 (0.633–5.842)	0.248
	ln(CRP)	0.44	0.179	1.552 (1.093–2.205)	0.014
	ln(D-dimer)	0.713	0.232	2.040 (1.294–3.218)	0.002
	Failure to achieve a combined biomarker response	2.128	0.447	8.399 (3.501–20.151)	<0.001

Notes: The dependent variable was concurrent radiological non-response, coded as 1 for stable or progressive disease and 0 for partial response; no complete responses were observed. Panel A presents separate univariable logistic regression models. Model A included pathological type, the four first-line treatment components, ln-transformed CRP, ln-transformed D-dimer, and the four individual dynamic biomarker indicators. Model B included the same pathological type, treatment, CRP, and D-dimer variables but replaced the four individual dynamic indicators with failure to achieve the combined biomarker response. Both multivariable models included 162 patients and 105 non-response events.

Association of dynamic serum marker changes with radiological response

In the univariable analyses, ln-transformed CRP, ln-transformed D-dimer, insufficient decreases in each of the four biomarkers, and failure to achieve the combined biomarker response were associated with concurrent radiological non-response. After adjustment for pathological type, the four non-mutually exclusive first-line treatment components, ln-transformed CRP, and ln-transformed D-dimer, insufficient decreases in CEA and NSE remained associated with non-response, with adjusted ORs of 4.437 (95% CI 1.713–11.497; $P=0.002$) and 2.623 (95% CI 1.015–6.774; $P=0.046$), respectively; the corresponding associations for CYFRA21-1 and VEGF were attenuated. In Model B, failure to achieve the combined biomarker response remained associated with non-response (adjusted OR 8.399, 95% CI 3.501–20.151; $P<0.001$; Table IV).

Pathological type was indicator coded, with adenocarcinoma as the reference category. The four treatment components were entered as separate, non-mutually exclusive binary variables, coded as 1 for recorded exposure and 0 for no recorded exposure. Baseline biomarker elevation was defined as CEA >5.0 ng/mL, CYFRA21-1 >3.3 ng/mL, or NSE >16.3 ng/mL; upper-median VEGF was defined as >359.5 pg/mL. Insufficient dynamic decline was coded as 1 when the T4 concentration decreased by $<30\%$ from T0 and 0 when it decreased by $\geq 30\%$. Failure to achieve a combined biomarker response was coded as 1 when fewer than two of the four biomarkers decreased by $\geq 30\%$ and 0 when at least two biomarkers met this criterion.

Discriminatory performance of baseline and dynamic serum marker profiles

ROC analysis showed that the AUCs of baseline CEA, CYFRA21-1, NSE, and VEGF were 0.626,

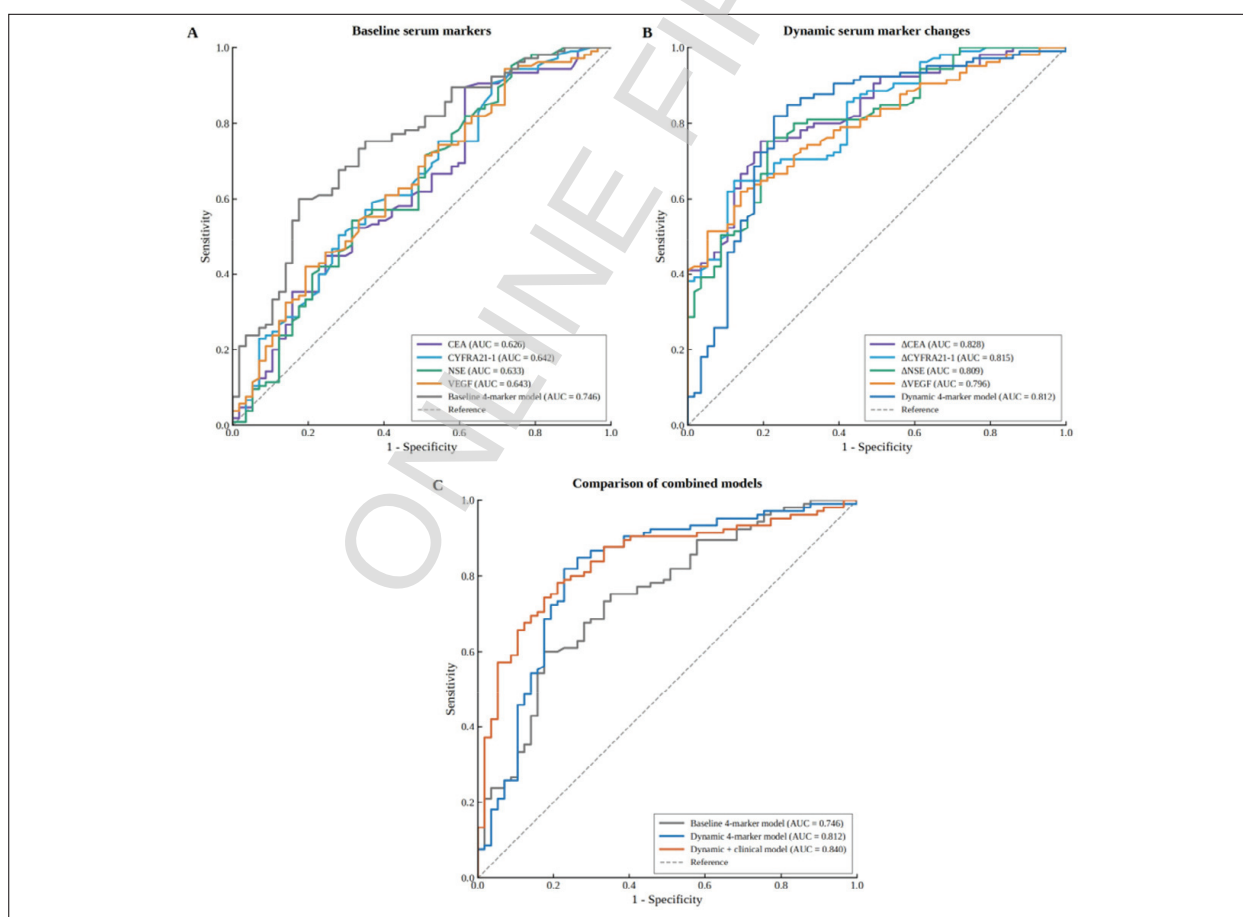


Figure 3 Receiver operating characteristic curves of baseline biomarkers, dynamic biomarker changes, and combined models for treatment non-response

(A) ROC curves of baseline serum CEA, CYFRA21-1, NSE, VEGF, and the 4-marker baseline combined model; (B) ROC curves of Δ CEA, Δ CYFRA21-1, Δ NSE, Δ VEGF, and the 4-marker dynamic combined model; (C) comparison of the 4-marker baseline combined model, 4-marker dynamic combined model, and 4-marker dynamic model combined with clinical variables. The four-marker dynamic model did not significantly outperform Δ CEA (DeLong $P=0.682$), and the dynamic-clinical model did not significantly outperform either Δ CEA ($P=0.787$) or the four-marker dynamic model ($P=0.573$).

Table V Discriminatory performance of individual baseline biomarkers and dynamic biomarker changes for concurrent radiological non-response.

Predictor	AUC	95%CI	Optimal cut-off value	Sensitivity (%)	Specificity (%)	Youden index
Baseline CEA level	0.626	0.534–0.719	4.8	89.524	38.596	0.281
Baseline CYFRA21-1 level	0.642	0.551–0.733	8.4	50.476	71.93	0.224
Baseline NSE level	0.633	0.540–0.727	19	54.286	68.421	0.227
Baseline VEGF level	0.643	0.554–0.733	556	41.905	80.702	0.226
D CEA%	0.828	0.766–0.891	-18.2	75.238	80.702	0.559
D CYFRA21-1%	0.815	0.750–0.880	-11.5	64.762	87.719	0.525
D NSE%	0.809	0.742–0.876	-11.6	75.238	78.947	0.542
D VEGF%	0.796	0.729–0.863	-5.1	61.905	85.965	0.479
4-marker baseline combined model	0.746	0.667–0.825	0.549	60	82.456	0.425
4-marker dynamic combined model	0.812	0.736–0.887	0.443	81.905	77.193	0.591
4-marker dynamic model + clinical variables	0.84	0.778–0.903	0.461	78.095	78.947	0.57

Supplementary Table S2 Bootstrap assessment and calibration of the exploratory combined models.

Model	AUC	Bootstrap 95% CI	Calibration intercept	Calibration slope	Brier score
Four-marker baseline combined model	0.746	0.664–0.824	0.736	1.04	0.211
Four-marker dynamic combined model	0.812	0.733–0.886	0.851	1.405	0.192
Four-marker dynamic model combined with clinical variables	0.84	0.775–0.901	0.87	1.554	0.183

0.642, 0.633, and 0.643, respectively; after combining the four baseline indicators, the AUC of the 4-marker baseline combined model increased to 0.746. The predictive performance of dynamic change indicators was superior to that of baseline levels ($P < 0.05$). In addition, the AUCs of Δ CEA%, Δ CYFRA21-1%, Δ NSE%, and Δ VEGF% were 0.828, 0.815, 0.809, and 0.796, respectively, and the AUC of the 4-marker dynamic combined model was 0.812; after clinical variables were further added, the model AUC increased to 0.840 ($P < 0.05$). *Figure 3* and *Table V*. The AUCs of Δ CEA, Δ CYFRA21-1, Δ NSE, and Δ VEGF were 0.828, 0.815, 0.809, and 0.796, respectively, whereas the four-marker dynamic combined model had an AUC of 0.812 and the dynamic-clinical model had an AUC of 0.840. The four-marker dynamic model did not outperform

Δ CEA, with an AUC difference of -0.016 (95% CI -0.095 to 0.062; DeLong $P = 0.682$). The dynamic-clinical model was also not significantly superior to Δ CEA (difference 0.012, 95% CI -0.076 to 0.100; $P = 0.787$) or to the four-marker dynamic model (difference 0.029, 95% CI -0.071 to 0.128; $P = 0.573$). In the bootstrap assessment, the 95% CIs of the AUCs were 0.664–0.824 for the baseline combined model, 0.733–0.886 for the dynamic combined model, and 0.775–0.901 for the dynamic-clinical model. Calibration was imperfect, particularly for the two dynamic combined models, as detailed in *Supplementary Table S2*.

Discussion

The present study characterised the longitudinal serum behaviour of CEA, CYFRA21-1, NSE, and VEGF during first-line systemic therapy in advanced lung cancer. Pretreatment concentrations differed according to pathological type, consistent with the distinct biological origins of these analytes, while their treatment-related changes showed clear gradients across partial response, stable disease, and progressive disease. Serum VEGF was additionally associated with lesion size, metastatic burden, D-dimer levels, and pleural involvement, distinguishing it from conventional tumour markers that mainly reflect tumour lineage, cellular injury, or neuroendocrine activity. Taken together, these findings indicate that serial measurements contain information on several tumour-related processes rather than merely reproducing the results of a single baseline assay.

CEA was relatively higher in adenocarcinoma, which is consistent with its association with glandular epithelial differentiation, secretory activity, and adenocarcinoma-related tumour burden (17); CYFRA21-1 was more prominently elevated in squamous cell carcinoma, in keeping with increased cytokeratin-19 turnover and release during epithelial tumour cell injury (18). NSE showed its highest concentrations in small-cell lung cancer, reflecting neuroendocrine differentiation and the high cellular activity characteristic of this pathological phenotype (19). These differences indicate that serum marker concentrations are conditioned by tumour lineage and cellular biology and should not be interpreted as uniform surrogates of total tumour burden. The broad multi-marker expression pattern observed before treatment therefore reflects the heterogeneous biological composition of advanced lung cancer. It provides a basis for interpreting subsequent longitudinal changes.

During therapy, the overall decline in the four serum markers was accompanied by distinct response-related gradients: patients with partial response generally showed the greatest reductions, those with stable disease showed limited decreases or fluctuations, whereas increasing concentrations occurred more frequently in patients with progressive disease. This pattern suggests that the direction and magnitude of marker change are associated with treatment-related differences in tumour burden, epithelial cell turnover, neuroendocrine phenotype, and angiogenesis-related activity; however, serum measurements alone cannot establish corresponding biological changes within tumour tissue. CEA and NSE retained independent associations with radiological response after multivariable adjustment, while the associations of CYFRA21-1 and VEGF were attenuated, indicating that the contribution of each analyte was not equivalent. The

combined biomarker response should therefore be interpreted as a summary of several tumour-associated processes rather than as evidence that all four markers provide identical or interchangeable information.

VEGF differed conceptually from CEA, CYFRA21-1, and NSE because it is associated with angiogenic and vascular processes rather than primarily with tumour lineage or structural-protein release; nevertheless, serum VEGF cannot be interpreted as a direct quantitative measure of tissue angiogenesis (20, 21). Higher serum VEGF was associated with a larger sum of target-lesion diameters, more metastatic organs, higher D-dimer levels, and more frequent pleural involvement or pleural effusion (22); these associations are biologically compatible with increased angiogenic signalling, vascular permeability, metastatic activity, and tumour-associated coagulation activation (23). Serum VEGF should not, however, be regarded as a direct quantitative measurement of tissue angiogenesis, since its concentration may also be affected by platelet release and preanalytical conditions. In the present study, it is therefore more appropriately interpreted as an angiogenesis-related serum signal associated with tumour, metastatic, pleural, and coagulation-related burden.

The ROC analyses provided a secondary assessment of concurrent discrimination by baseline and longitudinal serum measurements rather than an evaluation of prospective prediction; changes in individual markers consistently showed greater discrimination than their isolated pretreatment concentrations, supporting the greater information content of serial measurements. The four-marker dynamic model did not exceed every single dynamic marker, indicating that its main value lies in combining biologically different signals rather than automatically producing the highest AUC; after clinical variables were added, the increase in discrimination suggested that serum marker changes and conventional clinical characteristics contributed complementary information. These models should therefore be regarded as analytical summaries of longitudinal marker behaviour rather than stand-alone instruments for determining treatment decisions, and the cut-offs were derived and evaluated in the same cohort; therefore, they require prospective external validation before they can be considered for clinical application.

This was a single-centre retrospective analysis, and patient-level dates for treatment cycles, T4 blood collection, and imaging were not retained in the analytical dataset; consequently, the exact treatment exposure before the first assessment could not be reconstructed, while heterogeneity among treatment components may also have affected the interpretation of biomarker changes. Second, CRP

and D-dimer already differed between groups at baseline, suggesting that the non-response group may have had a higher inflammatory or coagulation burden; future studies need to control further confounding through stricter stratification, propensity score methods, or multicentre validation. Third, VEGF testing is affected by sample type, platelet release, and preanalytical conditions. Although the testing process was standardised as much as possible in this study, the findings still need further validation under standardised testing conditions. In addition, this study mainly evaluated the first treatment response, and long-term outcomes such as progression-free survival and overall survival were not sufficiently included.

Conclusion

CEA, CYFRA21-1, NSE, and VEGF displayed distinct pathology-associated distributions and longitudinal serum patterns during first-line treatment of advanced lung cancer. Their changes were associated with concurrent radiological response, and each dynamic measure showed greater discrimination of non-response than its corresponding baseline concentration; higher serum VEGF was also associated with lesion burden, metastatic extent, pleural involvement, and D-dimer. Serial measurements

may therefore assist response assessment, but they should not be interpreted as direct measurements of tumour-cell burden, neuroendocrine activity, or tissue angiogenesis. These findings require external validation in cohorts with standardised treatment timing, pre-analytical procedures, and survival follow-up.

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Data availability statement

The datasets used and/or analysed during the current retrospective cohort study are available from the corresponding author on reasonable request.

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

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