Serum tumor markers and outcomes in lung cancer patients with brain metastases: a retrospective longitudinal cohort study

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Background: Serum tumor markers (STMs) are recommended for cancer diagnosis and surveillance. However, their role in lung cancer with brain metastases (BM) is not yet clear. We aim to analyze the roles of baseline levels of STMs or ongoing STM surveillance on survival.

Methods: This retrospective longitudinal cohort study included 1,169 lung cancer patients with BM. The STM data during disease course were collected. Distinct trajectory groups were identified using the latent class growth mixed model (LCGMM). The roles of STMs on survival were further analyzed using Kaplan-Meier analysis and Cox proportional hazard models.

Results: Serum levels of cytokeratin-19 fragment (CYFRA21-1) (P<0.001), carcinoembryonic antigen (CEA) (P=0.005) and neuron-specific enolase (NSE) (P<0.001) at baseline exhibited significant correlation with overall survival (OS) of patients with BM, serving as independent prognostic factors. Further analysis indicated that baseline CYFRA21-1, CEA, NSE as well as status of key driver genes were independent prognostic factors in non-small cell lung cancer (NSCLC) patients with BM, while for small cell lung cancer (SCLC) patients with BM, baseline NSE and receiving chemotherapy show independent correlations with survival. Furthermore, we delineated the dynamic trajectories of STMs based on changes in disease course. More specifically, compared to those showing a baseline-high trend in CEA levels, the survival of patients with either persistently-rising or consistently normal levels seemed to be more promising. For CYFRA21-1, both early-rising and later-rising trends were observed, indicating a prognosis inferior to that of individuals with normal-level trajectory. Likewise, for NSE, patients with persistently-rising or persistently-descending trends showed no significantly survival difference. However, in comparison with the status of driver genes, receiving radiotherapy and targeted therapy, the dynamic changes in STM levels lacked independent prognostic significance. Further analysis indicated that among BM patients lacking key driver genes, NSE trajectory (P<0.05), CYFRA21-1 trajectory (P<0.05) and receiving chemotherapy (P<0.001) were independent prognostic factors.

Conclusions: Baseline levels of serum CYFRA21-1, CEA and NSE, as well as status of key driver genes are recommended for evaluating BM patients' outcome. Dynamic changes of STMs during disease course were not significantly associated with the final outcome of BM patients.

Introduction

Lung cancer is the primary cause of cancer-related mortality globally (1-3). Over 50% of lung cancers are diagnosed as unresectable or at an advanced stage, and 15–20% of lung cancer patients have been found to have brain metastases (BM) at the time of diagnosis (4,5). With the development of treatment options, different approaches such as tyrosine kinase inhibitors (TKIs) and immune checkpoint therapy have emerged alongside traditional cytotoxic chemotherapy, improving the long-term survival rate of patients with BM, highlighting the importance of ongoing disease surveillance. In terms of disease monitoring in clinical practice, serum tumor markers (STMs) are widely used in lung cancer (6,7), with carcinoembryonic antigen (CEA), cytokeratin-19 fragment (CYFRA21-1), neuron-specific enolase (NSE), squamous cell carcinoma antigen (SCC-Ag), cancer antigen 125 (CA125) and carbohydrate antigen 199 (CA199), being most sensitive. However, their role in BM remains unclear, lacking clinical evidence.

Previous studies have also emphasized the utilization of STMs alone or in combination to monitor treatment effectiveness and prognosis (8,9). Typically, both CEA

and other STMs have been reported as predictive factors at baseline or prognostic factors for disease recurrence, progression-free survival (PFS), or overall survival (OS) (6,10). Moreover, a few studies have suggested that the trajectory of STMs in colorectal cancer can reflect dynamic patterns of changes during the disease progression, providing more information on the relationship between STMs and outcomes (11,12). However, due to the limited number of measurements, the relationship between STMs and the prognosis of patients with BM is still uncertain, and the longitudinal trajectory of STMs is often overlooked. Specifically, there is a lack of understanding regarding the trajectory of STMs in lung cancer patients with BM, and its association with the OS of these patients.

Therefore, we employed a latent class growth mixture model using a large-sample retrospective longitudinal cohort to define the dynamic pattern of STMs, and simultaneously analyzed the role of baseline levels and dynamic changes in STMs in predicting prognosis of lung cancer patients with BM. We present this article in accordance with the STROBE reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-404/rc).

Methods

Ethics

This retrospective study was approved by the Ethics Committee of West China Hospital (No. 2022127). The study was conducted according to the guidelines of the Declaration of Helsinki (as revised in 2013). Individual consent for this analysis was waived due to retrospective nature.

Patients

Referring to our previous literature (13), we obtained the clinicopathological and follow-up data of lung cancer patients with BM from the linked electronic medical care records at West China Hospital during the period from

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Highlight box

Key findings
• Baseline serum tumor markers (STMs) can predict the prognosis of lung cancer patients with brain metastases (BM).

What is known and what is new?
• Baseline STMs are recommended for cancer diagnosis and surveillance.
• The relationship between baseline levels and dynamic changes of STMs in lung cancer patients with BM and prognosis for the first time was reported.

What is the implication, and what should change now?
• Baseline cytokeratin-19 fragment, carcinoembryonic antigen and neuron-specific enolase levels, as well as status of key driver genes are recommended to predict the prognosis of BM patients. Dynamic changes of STMs during disease course were not significantly associated with the final outcome of BM patients.

December 1, 2013 to September 30, 2020. Lung cancer patients with BM were verified by pathology reports and brain imaging. The exclusion criteria for this study included: (I) patients aged below 18 years or above 80 years, (II) incomplete STMs determinations, (III) lack of survival information, and (IV) less than 3 visits. BM patients with STMs records from the first admission and follow-up were included for STMs trajectory analysis. Participants who met the inclusion criteria for all STMs trajectory analyses simultaneously were subjected to joint analysis. The treatment regimen (radiotherapy, chemotherapy, immunotherapy, and targeted therapy) for all our included patients was used after diagnosis of BM. Targeted therapy includes anti-angiogenesis treatment and small molecule targeted therapy.

STMs measurement

Serum CYFRA21-1, CEA and NSE were selected in this study. A total of 3 mL of peripheral venous blood was withdrawn, serum was separated at 4,000 r/min and stored at –80 ℃ for measurement. An electrochemiluminescence immunoassay (Roche Cobas e601) was used to evaluate the three STMs. Normal reference values of CEA, CYFRA21-1 and NSE are <3.4, <3.0, and <15.0 ng/mL, respectively. Each patient underwent at least three follow-up measurements before being included in the analysis.

Surveillance outcome

The primary outcome was to evaluate the prognostic roles of STMs in the OS of lung cancer patients with BM. Kaplan-Meier analysis, Cox proportional hazards model, and log-rank test were performed.

Statistical analysis

The relationship between the baseline levels of CEA, CYFRA21-1, as well as NSE and BM outcomes was evaluated by Kaplan-Meier analysis and Cox proportional hazard models, which included age, gender, smoking, status of key driver genes, Graded Prognostic Assessment (GPA) score of BM and treatment regimen (radiotherapy, chemotherapy, immunotherapy, and targeted therapy). Kaplan-Meier analysis and cox proportional hazard models were performed via the "survival" package in R (version 4.2.1).

We utilized longitudinal mode to analyze the patterns and trajectories of STMs in lung cancer patients with BM.

The logarithms of CEA, CYFRA21-1, and NSE values were then used for trajectory fitting due to their non-normal distribution. A latent class growth mixed model (LCGMM) was utilized to identify different trajectory patterns, considering the heterogeneity of the population. This model included longitudinal measurements as linear or nonlinear functions of time, with 2–5 potential groups considered. The optimal number of groups and the best fitting shape were determined based on the Bayesian information criterion, ensuring a representative proportion in each group. LCGMM analysis was carried out using the "lcmm" package in R (version 4.2.1). The relationship between trajectory groups and BM outcomes was evaluated by Kaplan-Meier analysis and Cox proportional hazard models with age, sex, and baseline levels adjusted.

Results

Participant characteristics

During the period from December 2013 and September 2020, a total of 1,169 lung cancer patients with BM were included in this study. Of these, 702 were males and 467 were females. The age distribution was as follows: 323 patients were under 50 years old, 419 were aged 50–60 years old, and 427 were over 60 years old. Among the participants, 591 were never-smokers, 180 were current smokers, and 398 were former smokers. According to the World Health Organization/International Association for the Study of Lung Cancer (WHO/IASLC) classification criteria for lung tumors, SCC accounted for 10.9%, adenocarcinoma (AD) for 66.9%, small cell lung cancer (SCLC) for 14.5%, and not otherwise specified (NOS) for the remaining 7.4%. In terms of treatment, 485 cases (41.5%) underwent targeted therapy, 39 cases (3.3%) underwent immunotherapy, 627 cases (53.6%) underwent radiotherapy and 954 cases (81.6%) underwent chemotherapy. The baseline characteristics of patients are shown in *Table 1*.

Association of single STMs at baseline with BM survival

In order to investigate the clinical significance of individual STMs (CEA, CYFRA21-1, and NSE) baseline levels, we collected baseline STM data and utilized the median as the cutoff value to classify patients into high- and low-level groups. The baseline characteristics of patients were further analyzed in detail (*Table 1*). In the high NSE levels group (≥15.5 ng/mL), there was a higher proportion of male

Table 1 Baseline characteristics of lung cancer brain metastases participants

Features	Level	Total	CYFRA21-1, ng/mL			NSE, ng/mL			CEA, ng/mL		
			≥4.66	<4.66	P	≥15.5	<15.5	P	≥9.35	<9.35	P
n		1,169	585	584		585	584		585	584	
Histology	AD	782 (66.9)	407 (69.6)	375 (64.2)	<0.001	351 (60.0)	431 (73.8)	<0.001	449 (76.8)	333 (57.0)	<0.001
	AD_SCC	3 (0.3)	2 (0.3)	1 (0.2)		1 (0.2)	2 (0.3)		2 (0.3)	1 (0.2)	
	SAR	1 (0.1)	1 (0.2)	0 (0.0)		0 (0.0)	1 (0.2)		0 (0.0)	1 (0.2)	
	SCC	127 (10.9)	81 (13.8)	46 (7.9)		61 (10.4)	66 (11.3)		39 (6.7)	88 (15.1)	
	SCLC	169 (14.5)	45 (7.7)	124 (21.2)		121 (20.7)	48 (8.2)		54 (9.2)	115 (19.7)	
	NOS	87 (7.3)	49 (8.4)	38 (6.5)		51 (8.7)	36 (6.2)		41 (7.0)	46 (7.9)	
Age (years)	<50	323 (27.6)	144 (24.6)	179 (30.7)	0.03	149 (25.5)	174 (29.8)	0.25	154 (26.3)	169 (28.9)	0.047
	>60	427 (36.6)	232 (39.7)	195 (33.4)		222 (37.9)	205 (35.1)		234 (40.0)	193 (33.0)	
	50–60	419 (35.8)	209 (35.7)	210 (36.0)		214 (36.6)	205 (35.1)		197 (33.7)	222 (38.0)	
Gender	Male	702 (60.1)	370 (63.2)	332 (56.8)	0.03	371 (63.4)	331 (56.7)	0.02	330 (56.4)	372 (63.7)	0.01
	Female	467 (39.9)	215 (36.8)	252 (43.2)		214 (36.6)	253 (43.3)		255 (43.6)	212 (36.3)	
Smoking	No	591 (76.7)	280 (73.5)	311 (79.7)	0.049	269 (72.9)	322 (80.1)	0.02	322 (78.2)	269 (74.9)	0.33
	Yes	180 (23.3)	101 (26.5)	79 (20.3)		100 (27.1)	80 (19.9)		90 (21.8)	90 (25.1)	
KPS	<70	78 (6.7)	37 (6.3)	41 (7.0)	0.76	39 (6.7)	39 (6.7)	0.67	31 (5.3)	47 (8.0)	0.17
	>90	33 (2.8)	15 (2.6)	18 (3.1)		14 (2.4)	19 (3.3)		17 (2.9)	16 (2.7)	
	70–90	1,058 (90.5)	533 (91.1)	525 (89.9)		532 (90.9)	526 (90.1)		537 (91.8)	521 (89.2)	
EM	No	212 (18.1)	75 (12.8)	137 (23.5)	<0.001	79 (13.5)	133 (22.8)	<0.001	66 (11.3)	146 (25.0)	<0.001
	Yes	957 (81.9)	510 (87.2)	447 (76.5)		506 (86.5)	451 (77.2)		519 (88.7)	438 (75.0)	
MT	>3	560 (47.9)	297 (50.8)	263 (45.0)	0.02	301 (51.5)	259 (44.3)	0.052	308 (52.6)	252 (43.2)	0.004
	1	441 (37.7)	197 (33.7)	244 (41.8)		205 (35.0)	236 (40.4)		197 (33.7)	244 (41.8)	
	2–3	168 (14.4)	91 (15.6)	77 (13.2)		79 (13.5)	89 (15.2)		80 (13.7)	88 (15.1)	
BMI (kg/m^2)		22.32 [20.34, 24.45]	22.03 [20.20, 24.06]	22.66 [20.70, 24.77]	<0.001	22.04 [20.05, 24.24]	22.60 [20.62, 24.58]	0.01	22.31 [20.42, 24.43]	22.40 [20.31, 24.47]	0.65
EGFR	Negative	238 (20.4)	126 (21.5)	112 (19.2)	0.40	109 (18.6)	129 (22.1)	0.002	119 (20.3)	119 (20.4)	0.01
	Positive	265 (22.6)	137 (23.4)	128 (21.9)		113 (19.3)	152 (26.0)		153 (26.2)	112 (19.2)	
	Unknown	666 (57.0)	322 (55.0)	344 (58.9)		363 (62.1)	303 (51.9)		313 (53.5)	353 (60.4)	
ALK	Negative	369 (31.6)	199 (34.0)	170 (29.1)	0.11	169 (28.9)	200 (34.2)	0.04	186 (31.8)	183 (31.3)	0.99
	Positive	60 (5.1)	25 (4.3)	35 (6.0)		25 (4.3)	35 (6.0)		30 (5.1)	30 (5.1)	
	Unknown	740 (63.3)	361 (61.7)	379 (64.9)		391 (66.8)	349 (59.8)		369 (63.1)	371 (63.5)	
ROS	Negative	311 (26.6)	165 (28.2)	146 (25.0)	0.42	143 (24.4)	168 (28.8)	0.12	149 (25.5)	162 (27.7)	0.61
	Positive	14 (1.2)	6 (1.0)	8 (1.4)		5 (0.9)	9 (1.5)		8 (1.4)	6 (1.0)	
	Unknown	844 (72.2)	414 (70.8)	430 (73.6)		437 (74.7)	407 (69.7)		428 (73.2)	416 (71.2)	
Immunotherapy	No	1,130 (96.7)	562 (96.1)	568 (97.3)	0.33	565 (96.6)	565 (96.7)	>0.99	575 (98.3)	555 (95.0)	0.003
	Yes	39 (3.3)	23 (3.9)	16 (2.7)		20 (3.4)	19 (3.3)		10 (1.7)	29 (5.0)	
Targeted therapy	No	684 (58.5)	325 (55.6)	359 (61.5)	0.046	363 (62.1)	321 (55.0)	0.02	304 (52.0)	380 (65.1)	<0.001
	Yes	485 (41.5)	260 (44.4)	225 (38.5)		222 (37.9)	263 (45.0)		281 (48.0)	204 (34.9)	

Table 1 (*continued*)

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Table 1 *(continued)*

Features	Level	Total	CYFRA21-1, ng/mL			NSE, ng/mL			CEA, ng/mL		
			≥4.66	<4.66	P	≥15.5	<15.5	P	≥9.35	<9.35	P
Radiotherapy	No	542 (46.4)	295 (50.4)	247 (42.3)	0.006	284 (48.5)	258 (44.2)	0.15	300 (51.3)	242 (41.4)	0.001
	Yes	627 (53.6)	290 (49.6)	337 (57.7)		301 (51.5)	326 (55.8)		285 (48.7)	342 (58.6)	
Chemotherapy	No	215 (18.4)	122 (20.9)	93 (15.9)	0.04	115 (19.7)	100 (17.1)	0.30	125 (21.4)	90 (15.4)	0.01
	Yes	954 (81.6)	463 (79.1)	491 (84.1)		470 (80.3)	484 (82.9)		460 (78.6)	494 (84.6)	
GPA	0–1.0	413 (35.3)	235 (40.2)	178 (30.5)	<0.001	238 (40.7)	175 (30.0)	<0.001	242 (41.4)	171 (29.3)	<0.001
	1.5–2.0	518 (44.3)	263 (45.0)	255 (43.7)		249 (42.6)	269 (46.0)		249 (42.6)	269 (46.1)	
	2.5–3.0	217 (18.6)	83 (14.2)	134 (22.9)		92 (15.7)	125 (21.4)		90 (15.4)	127 (21.7)	
	3.5–4.0	21 (1.8)	4 (0.6)	17 (2.9)		6 (1.0)	15 (2.6)		4 (0.6)	17 (2.9)	

Data are presented as n (%) or median [IQR]. CYFRA21-1, cytokeratin-19 fragment; NSE, neuron-specific enolase; CEA, carcinoembryonic antigen; AD, adenocarcinoma; SCC, squamous cell carcinoma; SAR, sarcomatoid; SCLC, small cell lung cancer; NOS, not otherwise specified; KPS, Karnofsky Performance Status; EM, extracranial metastases; MT, brain metastasis; BMI, body mass index; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; ROS, reactive oxygen species; GPA, Graded Prognostic Assessment; IQR, interquartile range.

individuals (63.4% *vs.* 56.7%), a lower body mass index (BMI), a higher prevalence of SCLC pathology type (20.7% *vs.* 8.2%), a higher prevalence of smoking habits (27.1% *vs.* 19.9%), and a lower GPA score (40.7% *vs.* 30%). Similarly, in the high CYFRA21-1 levels group (≥4.66 ng/mL), there was a higher prevalence of male individuals (63.2% *vs.* 56.8%), a lower BMI, or a higher prevalence of SCC pathology types (13.8% *vs.* 7.9%), had more number of brain metastatic lesions (50.8% *vs.* 45%), and a lower GPA score (40.2% *vs.* 30.5%). Conversely, the high CEA levels group (≥9.35 ng/mL) shows a tendency towards female gender (43.6% *vs.* 36.3%), age over 60 years (40% *vs.* 33%), presence of AD pathology (76.8% *vs.* 57%), more number of brain metastatic lesions (52.6% *vs.* 43.2%), a higher prevalence of positive epidermal growth factor receptor (EGFR) (26.2% *vs.* 19.2%), and a lower GPA score (41.4% *vs.* 29.3%). Furthermore, we observed that a significant proportion of BM patients with AD exhibit elevated levels of CEA (76.8%), CYFRA21-1 (69.6%) or NSE (60%).

In terms of survival, Kaplan-Meier survival analysis revealed that all STMs (CEA, CYFRA21-1, and NSE) were negatively correlated with survival outcomes (*Figure 1A-1C*). Patients with low serum NSE levels (<15.5 ng/mL) exhibited a significantly extended median survival time (MST) (P<0.001) compared to those with high NSE levels (≥15.5 ng/mL) [29 months (95% CI: 27–31) *vs.* 19 months (95% CI: 18–21)], indicating a survival benefit (P<0.001) for patients with low baseline serum NSE levels. Similarly, patients

with low baseline serum CYFRA21-1 levels (<4.66 ng/mL) had a longer MST of 27 months (95% CI: 24–29) in contrast to patients with high CYFRA21-1 levels (≥4.66 ng/mL) with an MST of 21 months (95% CI: 19–23). Additionally, patients with low baseline serum CEA levels (<9.35 ng/mL) displayed a prolonged MST (P=0.01) of 24 months (95% CI: 22–27) compared to patients with high CEA levels (≥9.35 ng/mL), who had an MST of 23 months (95% CI: 21–25).

Multivariate Cox regression analysis to identify poor prognostic factors at baseline levels

We conducted a multivariate Cox regression analysis and observed a significant correlation between OS and CYFRA21-1 (P<0.001), CEA (P=0.005) as well as NSE (P<0.001), serving as independent prognostic factors (*Figure 1D-1F*). When all three were included in the Cox model as continuous variables, only CYFRA21-1 (P<0.001) as well as NSE (P=0.03) remained statistically different (Figure S1). In either scenario, the mutation status of anaplastic lymphoma kinase (ALK) (P<0.05) and EGFR (P<0.05) as well as receiving radiotherapy and targeted therapy were independent prognostic factors for patients with BM (*Figure 1D-1F*). Further analysis indicated that baseline CYFRA21-1, CEA, NSE as well as status of key driver genes were independent prognostic factors in non-small cell lung cancer (NSCLC) patients with BM, while for SCLC patients with BM, baseline NSE and receiving
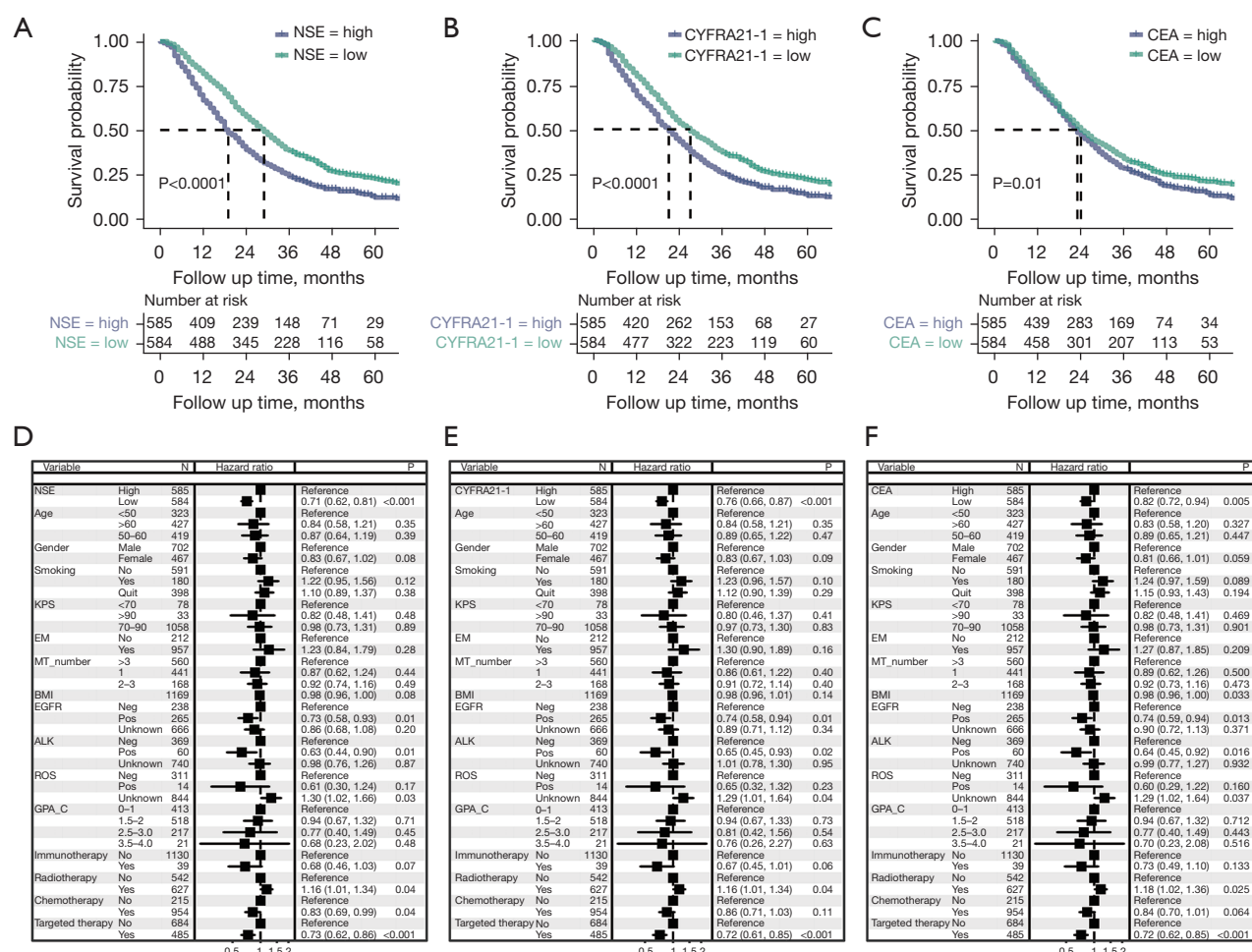
Figure 1 Kaplan-Meier curves and multivariate Cox's forest plots of overall survival in lung cancer patients with brain metastases based on baseline levels of different serum tumor markers. (A) Kaplan-Meier curves of patients stratified by baseline serum NSE levels. (B) Kaplan-Meier curves of patients stratified by baseline serum CYFRA21-1 levels. (C) Kaplan-Meier curves of patients stratified by baseline serum CEA levels. (D) Multivariable Cox regression analysis of patients stratified by baseline serum NSE levels. (E) Multivariable Cox regression analysis of patients stratified by baseline serum CYFRA21-1 levels. (F) Multivariable Cox regression analysis of patients stratified by baseline serum CEA levels. NSE, neuron-specific enolase; CYFRA21-1, cytokeratin-19 fragment; CEA, carcinoembryonic antigen; KPS, Karnofsky Performance Status; EM, extracranial metastases; MT_number, brain metastasis number; BMI, body mass index; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; ROS, reactive oxygen species; GPA_C, Graded Prognostic Assessment score.

chemotherapy show independent correlations with survival (Figures S2-S4).

Net reclassification index (NRI) for individual STMs baseline levels

When each STM was individually integrated into the NRI clinical model, as compared to traditional GPA scores, each STM demonstrated an enhancement in discriminatory ability (*Table 2*). Among these, NSE exhibited the most substantial enhancement in NRI. The inclusion of NSE [NRI (categorical) 0.11, 95% CI: 0.04–0.2; NRI (continuous) 0.27, 95% CI: 0.1–0.38], CYFRA21-1 [NRI (categorical) 0.10, 95% CI: 0.03–0.2; NRI (continuous) 0.25, 95% CI: 0.09–0.38], or CEA [NRI (categorical) 0.08, 95% CI: 0.018–0.18; NRI (continuous) 0.21, 95% CI: 0.07–0.33] led to an increase in the accurate reclassification rate for both positive and negative outcomes.

Table 2 NRI of adding STMs into a traditional GPA risk prediction model

Basic model	NRI (categorical) (95% CI)	NRI (continuous) (95% CI)
+NSE	0.11109563 (0.04212546–0.2003059)	0.27211232 (0.09736432–0.3777031)
+CYFRA21-1	0.09746602 (0.030884354–0.1973936)	0.24931891 (0.09230719–0.3756895)
+CEA	0.07822513 (0.01807649–0.18462074)	0.20980775 (0.06645839–0.3290698)

NRI, net reclassification improvement; STMs, serum tumor markers; GPA, Graded Prognostic Assessment; CI, confidence interval; NSE, neuron-specific enolase; CYFRA21-1, cytokeratin-19 fragment; CEA, carcinoembryonic antigen.

Clinical value of STMs trajectory modeling

We conducted a longitudinal analysis to analyze the trajectories of STMs and understand how they evolve over time in the progression and treatment process of BM patients. *Table 3* shows the baseline characteristics of participants in the trajectory groups of STMs. Based on LCGMM, the trajectories of STMs were classified by three classes. The distribution of the 1,169 patients with NSE across the three classes was 48 (class 1, persistently-rising trajectory), 1,014 (class 2, normal-level trajectory), and 107 (class 3, persistently-descending); the distribution of CYFRA21-1 was 933 (class 1, normal-level trajectory), 62 (class 2, early-rising trajectory), and 174 (class 3, later-rising trajectory); and the distribution of CEA was 914 (class 1, normal-level trajectory), 67 (class 2, baseline-high trajectory) and 188 (class 3, persistently-rising trajectory) (*Figure 2A-2C*).

Relationship between BM survival and STM trajectories

Kaplan-Meier survival analyses showed that dynamic changes in all STMs (CEA, CYFRA21-1 and NSE) correlated with prognosis. In terms of NSE, class 1 (persistently-rising trajectory) had the worst prognosis, with a median time of 11 months (95% CI: 10–19). In contrast, the class 2 (normal-level trajectory) had the best prognosis, with an MST of 25 months (95% CI: 23–27), and the class 3 (persistently-descending) had a more intermediate median time of 18 months (95% CI: 15–23). In the dynamic analysis of serum CYFRA21-1 levels, the class 1 (normal-level trajectory) group had the best prognosis (P<0.001), with an MST of 25 months (95% CI: 23–27), and the class 2 (early-rising trajectory) and 3 (later-rising trajectory) groups all had a worse prognosis, with an MST of 17 months (95% CI: 14–23) and 19.5 months (95% CI: 17–23), respectively. In dynamic analysis of serum CEA levels, class 1 (normal-level trajectory) had the best prognosis, with a MST of

23 months (95% CI: 22–25); class 2 (baseline-high trajectory) had the worst prognosis, with a MST of 20 months (95% CI: 17–23); and class 3 (persistently-rising trajectory) had an intermediate prognosis, with a median time of 23 months (95% CI: 20–26) (*Figure 2D-2F*).

Multivariate Cox regression analysis and subgroup analysis

After excluding confounding factors using multivariate Cox regression analysis, dynamic changes of STMs were not significantly correlated with survival. In either scenario, status of driver genes (the mutation status of *ALK* and *EGFR*), receiving targeted therapy, baseline NSE level were independent prognostic factors for patients with BM (*Figure 2G-2I*). The joining of STM analysis showed that for both NSCLC and SCLC, there was no statistically significant difference in the dynamic trajectory of STMs (CEA, CYFRA21-1 and NSE), while NSCLC patients with positive driver genes (P<0.05) have better survival, and SCLC patients with chemotherapy (P<0.001) have better survival (*Figure 3*). Further analysis indicated that among BM patients lacking key driver genes, NSE trajectory(P<0.05), CYFRA21-1 trajectory (P<0.05) and receiving chemotherapy (P<0.001) were independent prognostic factors (Figure S5).

Discussion

We report for the first time the relationship between baseline levels and dynamic changes of STMs (CEA, CYFRA21-1 and NSE) in lung cancer patients with BM and prognosis. We found that serum CEA, CYFRA21-1 as well as NSE at baseline levels were correlated with OS of patients, serving as independent prognostic factors. Moreover, the performance of the GPA model was significantly improved with the addition of STMs at baseline levels. More specifically, we delineated the dynamic

Table 3 Baseline characteristics of participants with different STMs dynamics during follow-up

Features	Level	Total	CYFRA21-1				NSE				CEA			
			1	2	3	P	1	2	3	P	1	2	3	P
n		1,169	933	62	174		48	1,014	107		914	67	188	
Histology	AD	782 (66.9)	602 (64.5)	39 (62.9)	141 (81.0)	<0.001	5 (10.4)	735 (72.5)	42 (39.3)	<0.001	567 (62.0)	57 (85.1)	158 (84.0)	<0.001
	AD_SCC	3 (0.3)	3 (0.3)	0 (0.0)	0 (0.0)		0 (0.0)	3 (0.3)	0 (0.0)		2 (0.2)	0 (0.0)	1 (0.5)	
	SAR	1 (0.1)	1 (0.1)	0 (0.0)	0 (0.0)		0 (0.0)	1 (0.1)	0 (0.0)		1 (0.1)	0 (0.0)	0 (0.0)	
	SCC	127 (10.9)	92 (9.9)	17 (27.4)	18 (10.3)		2 (4.2)	114 (11.2)	11 (10.3)		118 (12.9)	2 (3.0)	7 (3.7)	
	SCLC	169 (14.5)	164 (17.6)	1 (1.6)	4 (2.3)		34 (70.8)	88 (8.7)	47 (43.9)		154 (16.8)	2 (3.0)	13 (6.9)	
	NOS	87 (7.3)	71 (7.6)	5 (8.1)	11 (6.3)		7 (14.6)	73 (7.2)	7 (6.5)		72 (7.9)	6 (9.0)	9 (4.8)	
Age (years)	<50	323 (27.6)	258 (27.7)	20 (32.3)	45 (25.9)	0.75	8 (16.7)	286 (28.2)	29 (27.1)	0.29	246 (26.9)	14 (20.9)	63 (33.5)	0.03
	>60	427 (36.6)	341 (36.5)	24 (38.7)	62 (35.6)		21 (43.8)	361 (35.6)	45 (42.1)		323 (35.3)	31 (46.3)	73 (38.8)	
	50–60	419 (35.8)	334 (35.8)	18 (29.0)	67 (38.5)		19 (39.6)	367 (36.2)	33 (30.8)		345 (37.7)	22 (32.8)	52 (27.7)	
Gender	Male	702 (60.1)	545 (58.4)	51 (82.3)	106 (60.9)	0.001	42 (87.5)	577 (56.9)	83 (77.6)	<0.001	559 (61.2)	41 (61.2)	102 (54.3)	0.21
	Female	467 (39.9)	388 (41.6)	11 (17.7)	68 (39.1)		6 (12.5)	437 (43.1)	24 (22.4)		355 (38.8)	26 (38.8)	86 (45.7)	
Smoking	No	591 (76.7)	487 (78.5)	21 (56.8)	83 (72.8)	0.006	12 (50.0)	549 (79.2)	30 (55.6)	<0.001	441 (75.6)	35 (76.1)	115 (81.0)	0.40
	Yes	180 (23.3)	133 (21.5)	16 (43.2)	31 (27.2)		12 (50.0)	144 (20.8)	24 (44.4)		142 (24.4)	11 (23.9)	27 (19.0)	
KPS	<70	78 (6.7)	58 (6.2)	5 (8.1)	15 (8.6)	0.49	2 (4.2)	73 (7.2)	3 (2.8)	0.28	68 (7.4)	4 (6.0)	6 (3.2)	0.24
	>90	33 (2.8)	28 (3.0)	0 (0.0)	5 (2.9)		0 (0.0)	30 (3.0)	3 (2.8)		25 (2.7)	1 (1.5)	7 (3.7)	
	70–90	1,058 (90.5)	847 (90.8)	57 (91.9)	154 (88.5)		46 (95.8)	911 (89.8)	101 (94.4)		821 (89.8)	62 (92.5)	175 (93.1)	
EM	No	212 (18.1)	191 (20.5)	4 (6.5)	17 (9.8)	<0.001	6 (12.5)	195 (19.2)	11 (10.3)	0.043	191 (20.9)	8 (11.9)	13 (6.9)	<0.001
	Yes	957 (81.9)	742 (79.5)	58 (93.5)	157 (90.2)		42 (87.5)	819 (80.8)	96 (89.7)		723 (79.1)	59 (88.1)	175 (93.1)	
MT	>3	560 (47.9)	439 (47.1)	30 (48.4)	91 (52.3)	0.08	22 (45.8)	478 (47.1)	60 (56.1)	0.42	428 (46.8)	31 (46.3)	101 (53.7)	0.17
	1	441 (37.7)	369 (39.5)	21 (33.9)	51 (29.3)		20 (41.7)	385 (38.0)	36 (33.6)		359 (39.3)	22 (32.8)	60 (31.9)	
	2–3	168 (14.4)	125 (13.4)	11 (17.7)	32 (18.4)		6 (12.5)	151 (14.9)	11 (10.3)		127 (13.9)	14 (20.9)	27 (14.4)	
BMI		22.32 [20.34, 24.45]	22.46 [20.50, 24.49]	21.63 [19.48, 23.24]	21.97 [19.84, 24.13]	0.02	21.76 [18.93, 25.09]	22.41 [20.40, 24.44]	21.83 [20.54, 24.78]	0.78	22.36 [20.39, 24.44]	21.62 [19.53, 24.52]	22.45 [20.54, 24.48]	0.33
EGFR	Negative	238 (20.4)	161 (17.3)	22 (35.5)	55 (31.6)	<0.001	2 (4.2)	212 (20.9)	24 (22.4)	<0.001	186 (20.4)	16 (23.9)	36 (19.1)	0.001
	Positive	265 (22.6)	206 (22.1)	12 (19.4)	47 (27.0)		2 (4.2)	253 (25.0)	10 (9.3)		183 (20.0)	22 (32.8)	60 (31.9)	
	Unknown	666 (57.0)	566 (60.7)	28 (45.2)	72 (41.4)		44 (91.7)	549 (54.1)	73 (68.2)		545 (59.6)	29 (43.3)	92 (48.9)	

Table 3 (*continued*)

Table 3 *(continued)*

Features	Level	CYFRA21-1					NSE				CEA			
		Total	1	2	3	P	1	2	3	P	1	2	3	P
ALK	Negative	369 (31.6)	269 (28.8)	25 (40.3)	75 (43.1)	0.001	3 (6.2)	341 (33.6)	25 (23.4)	<0.001	284 (31.1)	24 (35.8)	61 (32.4)	0.65
	Positive	60 (5.1)	46 (4.9)	3 (4.8)	11 (6.3)		0 (0.0)	54 (5.3)	6 (5.6)		48 (5.3)	5 (7.5)	7 (3.7)	
	Unknown	740 (63.3)	618 (66.2)	34 (54.8)	88 (50.6)		45 (93.8)	619 (61.0)	76 (71.0)		582 (63.7)	38 (56.7)	120 (63.8)	
ROS	Negative	311 (26.6)	224 (24.0)	23 (37.1)	64 (36.8)	0.002	3 (6.2)	287 (28.3)	21 (19.6)	0.004	246 (26.9)	16 (23.9)	49 (26.1)	0.67
	Positive	14 (1.2)	11 (1.2)	0 (0.0)	3 (1.7)		0 (0.0)	13 (1.3)	1 (0.9)		13 (1.4)	0 (0.0)	1 (0.5)	
	Unknown	844 (72.2)	698 (74.8)	39 (62.9)	107 (61.5)		45 (93.8)	714 (70.4)	85 (79.4)		655 (71.7)	51 (76.1)	138 (73.4)	
GPA	0–1	413 (35.3)	318 (34.1)	24 (38.7)	71 (40.8)	0.008	24 (50.0)	345 (34.0)	44 (41.1)	0.043	308 (33.7)	29 (43.3)	76 (40.4)	0.16
	1.5–2	518 (44.3)	403 (43.2)	31 (50.0)	84 (48.3)		12 (25.0)	456 (45.0)	50 (46.7)		410 (44.9)	28 (41.8)	80 (42.6)	
	2.5–3.0	217 (18.6)	192 (20.6)	6 (9.7)	19 (10.9)		11 (22.9)	194 (19.1)	12 (11.2)		176 (19.3)	9 (13.4)	32 (17.0)	
	3.5–4.0	21 (1.8)	20 (2.1)	1 (1.6)	0 (0.0)		1 (2.1)	19 (1.9)	1 (0.9)		20 (2.2)	1 (1.5)	0 (0.0)	

Data are presented as n (%) or median [IQR]. CYFRA21-1: class 1, normal-level trajectory; class 2, early-rising trajectory; class 3, later-rising trajectory. CEA: class 1, normal-level trajectory; class 2, baseline-high trajectory; class 3, persistently-rising trajectory. NSE: class 1, persistently-rising trajectory; class 2, normal-level trajectory; class 3, persistently-descending. STMs, serum tumor markers; CYFRA21-1, cytokeratin-19 fragment; NSE, neuron-specific enolase; CEA, carcinoembryonic antigen; AD, adenocarcinoma; SCC, squamous cell carcinoma; SAR, sarcomatoid; SCLC, small cell lung cancer; NOS, not otherwise specified; KPS, Karnofsky Performance Status; EM, extracranial metastases; MT, brain metastasis; BMI, body mass index; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; ROS, reactive oxygen species; GPA, Graded Prognostic Assessment; IQR, interquartile range.
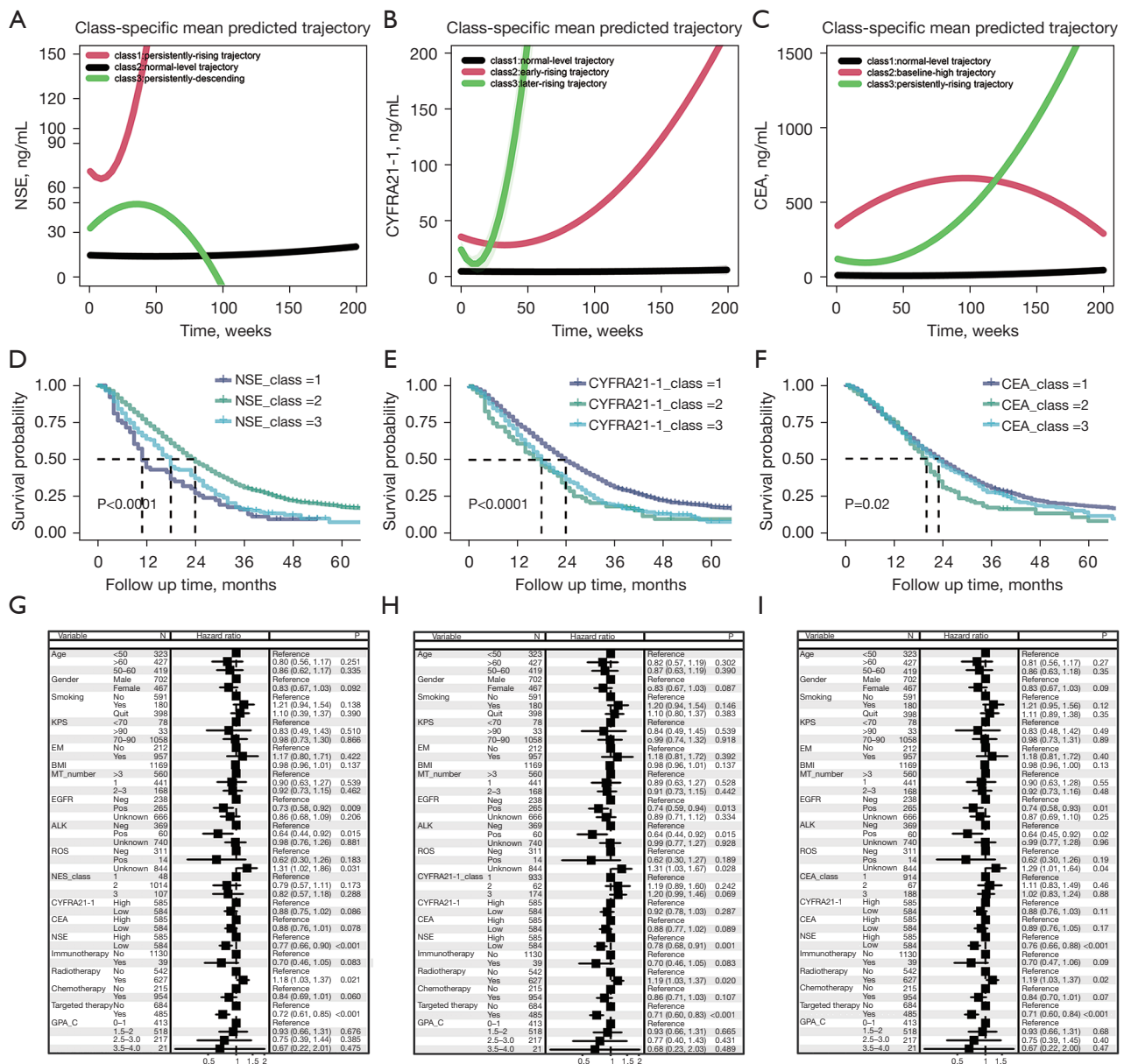
Figure 2 Trajectory curves, Kaplan-Meier curves and multivariate Cox's forest plots of overall survival in lung cancer patients with brain metastases based on dynamic changes of different serum tumor markers. (A) Dynamic trajectories based on serum NSE stratification. (B) Dynamic trajectories based on serum CYFRA21-1 stratification. (C) Dynamic trajectories based on serum CEA stratification. (D) Kaplan-Meier curves of patients stratified by NSE trajectories. (E) Kaplan-Meier curves of patients stratified by CYFRA21-1 trajectories. (F) Kaplan-Meier curves of patients stratified by CEA trajectories. (G) Multivariable Cox regression analysis of patients stratified by NSE trajectories. (H) Multivariable Cox regression analysis of patients stratified by CYFRA21-1 trajectories. (I) Multivariable Cox regression analysis of patients stratified by CEA trajectories. NSE, neuron-specific enolase; CYFRA21-1, cytokeratin-19 fragment; CEA, carcinoembryonic antigen; KPS, Karnofsky Performance Status; EM, extracranial metastases; MT_number, brain metastasis number; BMI, body mass index; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; ROS, reactive oxygen species; GPA_C, Graded Prognostic Assessment score.

A — NSCLC

Variable		N	Hazard ratio (95% CI)	P
Age	<50	266	Reference	
	>60	317	0.91 (0.59, 1.40)	0.656
	50–60	326	0.94 (0.65, 1.37)	0.762
Gender	Male	494	Reference	
	Female	415	0.74 (0.58, 0.93)	0.011
Smoking	No	521	Reference	
	Yes	120	0.94 (0.71, 1.26)	0.695
	Quit	268	0.97 (0.76, 1.24)	0.800
KPS	<70	61	Reference	
	>90	26	0.71 (0.37, 1.35)	0.298
	70–90	822	0.93 (0.66, 1.30)	0.661
EM	No	150	Reference	
	Yes	759	1.38 (0.88, 2.16)	0.160
BMI		909	0.98 (0.96, 1.01)	0.191
MT_number	>3	430	Reference	
	1	351	0.78 (0.52, 1.18)	0.242
	2–3	128	0.90 (0.69, 1.18)	0.452
CYFRA21-1_class	1	694	Reference	
	2	56	1.21 (0.87, 1.67)	0.257
	3	159	1.22 (0.98, 1.52)	0.082
CEA_class	1	685	Reference	
	2	59	0.97 (0.70, 1.33)	0.843
	3	165	0.96 (0.77, 1.20)	0.737
NSE_class	1	7	Reference	
	2	849	1.13 (0.46, 2.79)	0.789
	3	53	1.05 (0.41, 2.71)	0.916
CYFRA21-1	High	488	Reference	
	Low	421	0.82 (0.68, 1.00)	0.047
CEA	High	488	Reference	
	Low	421	0.91 (0.76, 1.09)	0.305
NSE	High	412	Reference	
	Low	497	0.82 (0.69, 0.99)	0.039
EGFR	Neg	228	Reference	
	Pos	261	0.73 (0.57, 0.93)	0.011
	Unknown	420	0.87 (0.68, 1.12)	0.276
ALK	Neg	357	Reference	
	Pos	58	0.61 (0.42, 0.89)	0.011
	Unknown	494	0.96 (0.74, 1.25)	0.772
ROS	Neg	299	Reference	
	Pos	14	0.65 (0.30, 1.29)	0.202
	Unknown	596	1.29 (1.01, 1.66)	0.044
Immunotherapy	No	879	Reference	
	Yes	30	0.65 (0.40, 1.06)	0.082
Radiotherapy	No	434	Reference	
	Yes	475	1.15 (0.97, 1.35)	0.102
Chemotherapy	No	163	Reference	
	Yes	746	0.98 (0.79, 1.22)	0.885
Targeted therapy	No	444	Reference	
	Yes	465	0.74 (0.62, 0.89)	0.001
GPA_C	0–1	309	Reference	
	1.5–2	417	0.98 (0.66, 1.47)	0.931
	2.5–3.0	169	0.98 (0.45, 2.12)	0.960
	3.5–4.0	14	0.76 (0.20, 2.88)	0.681

B — SCLC

Variable		N	Hazard ratio (95% CI)	P
Age	<50	38	Reference	
	>60	69	0.43 (0.13, 1.46)	0.177
	50–60	62	0.52 (0.17, 1.54)	0.237
Gender	Male	140	Reference	
	Female	29	1.29 (0.57, 2.88)	0.540
Smoking	No	39	Reference	
	Yes	29	1.79 (0.81, 3.97)	0.153
	Quit	101	1.39 (0.69, 2.80)	0.355
KPS	<70	4	Reference	
	>90	3	0.92 (0.15, 5.66)	0.931
	70–90	162	0.69 (0.19, 2.55)	0.574
EM	No	35	Reference	
	Yes	134	0.53 (0.15, 1.79)	0.304
BMI		169	1.00 (0.95, 1.05)	0.944
MT_number	>3	86	Reference	
	1	54	1.86 (0.56, 6.19)	0.309
	2–3	29	0.91 (0.51, 1.63)	0.759
CYFRA21-1_class	1	164	Reference	
	2	1	0.84 (0.10, 6.91)	0.872
	3	4	5.66 (1.67, 19.17)	0.005
CEA_class	1	154	Reference	
	2	2	1.77 (0.41, 7.55)	0.441
	3	13	1.55 (0.71, 3.38)	0.276
NSE_class	1	34	Reference	
	2	88	0.65 (0.38, 1.13)	0.127
	3	47	0.89 (0.54, 1.46)	0.636
CYFRA21-1	High	45	Reference	
	Low	124	1.25 (0.78, 2.00)	0.360
CEA	High	54	Reference	
	Low	115	0.70 (0.44, 1.10)	0.123
NSE	High	121	Reference	
	Low	48	0.90 (0.55, 1.47)	0.681
Immunotherapy	No	166	Reference	
	Yes	3	1.40 (0.37, 5.24)	0.618
Radiotherapy	No	48	Reference	
	Yes	121	1.75 (1.12, 2.74)	0.014
Chemotherapy	No	6	Reference	
	Yes	163	0.16 (0.06, 0.41)	<0.001
Targeted therapy	No	164	Reference	
	Yes	5	1.25 (0.37, 4.20)	0.723
GPA_C	0–1	72	Reference	
	1.5–2	61	0.56 (0.18, 1.78)	0.329
	2.5–3.0	33	0.21 (0.02, 1.91)	0.164
	3.5–4.0	3	0.12 (0.00, 4.35)	0.246

C — Total

Variable		N	Hazard ratio (95% CI)	P
Age	<50	323	Reference	
	>60	427	0.81 (0.56, 1.18)	0.274
	50–60	419	0.86 (0.63, 1.18)	0.358
Gender	Male	702	Reference	
	Female	467	0.84 (0.67, 1.04)	0.101
Smoking	No	591	Reference	
	Yes	180	1.20 (0.93, 1.53)	0.155
	Quit	398	1.10 (0.89, 1.37)	0.386
KPS	<70	78	Reference	
	>90	33	0.85 (0.49, 1.46)	0.551
	70–90	1058	0.98 (0.73, 1.31)	0.901
EM	No	212	Reference	
	Yes	957	1.17 (0.82, 1.47)	0.413
BMI		1169	0.98 (0.96, 1.01)	0.166
MT_number	>3	560	Reference	
	1	441	0.89 (0.63, 1.27)	0.526
	2–3	168	0.90 (0.72, 1.14)	0.383
CYFRA21-1_class	1	933	Reference	
	2	62	1.20 (0.89, 1.62)	0.229
	3	174	1.20 (0.98, 1.47)	0.070
CEA_class	1	914	Reference	
	2	67	1.07 (0.80, 1.44)	0.633
	3	188	1.01 (0.83, 1.24)	0.902
NSE_class	1	48	Reference	
	2	1014	0.78 (0.56, 1.08)	0.133
	3	107	0.78 (0.54, 1.13)	0.186
CYFRA21-1	High	585	Reference	
	Low	584	0.91 (0.78, 1.07)	0.269
CEA	High	585	Reference	
	Low	584	0.89 (0.76, 1.04)	0.143
NSE	High	585	Reference	
	Low	584	0.80 (0.68, 0.93)	0.005
EGFR	Neg	238	Reference	
	Pos	265	0.74 (0.58, 0.93)	0.011
	Unknown	666	0.89 (0.70, 1.12)	0.317
ALK	Neg	369	Reference	
	Pos	60	0.63 (0.44, 0.91)	0.014
	Unknown	740	0.98 (0.76, 1.27)	0.902
ROS	Neg	311	Reference	
	Pos	14	0.62 (0.30, 1.27)	0.192
	Unknown	844	1.31 (1.03, 1.67)	0.029
Immunotherapy	No	1130	Reference	
	Yes	39	0.70 (0.46, 1.05)	0.085
Radiotherapy	No	542	Reference	
	Yes	627	1.18 (1.02, 1.36)	0.023
Chemotherapy	No	215	Reference	
	Yes	954	0.85 (0.71, 1.02)	0.087
Targeted therapy	No	684	Reference	
	Yes	485	0.71 (0.61, 0.84)	<0.001
GPA_C	0–1	413	Reference	
	1.5–2	518	0.93 (0.66, 1.31)	0.680
	2.5–3.0	217	0.76 (0.39, 1.47)	0.416
	3.5–4.0	21	0.67 (0.22, 2.02)	0.478

Figure 3 Joint multivariate Cox's forest plots of overall survival in lung cancer patients with brain metastases. (A) Joint multivariate Cox's forest plots in non-small cell lung cancer (NSCLC) patients with brain metastases. (B) Joint multivariate Cox's forest plots in small cell lung cancer (SCLC) patients with brain metastases; (C) Joint multivariate Cox's forest plots in all patients with brain metastases. KPS, Karnofsky Performance Status; EM, extracranial metastases; BMI, body mass index; MT_number, brain metastasis number; CYFRA21-1, cytokeratin-19 fragment; CEA, carcinoembryonic antigen; NSE, neuron-specific enolase; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; ROS, reactive oxygen species; GPA_C, Graded Prognostic Assessment score.
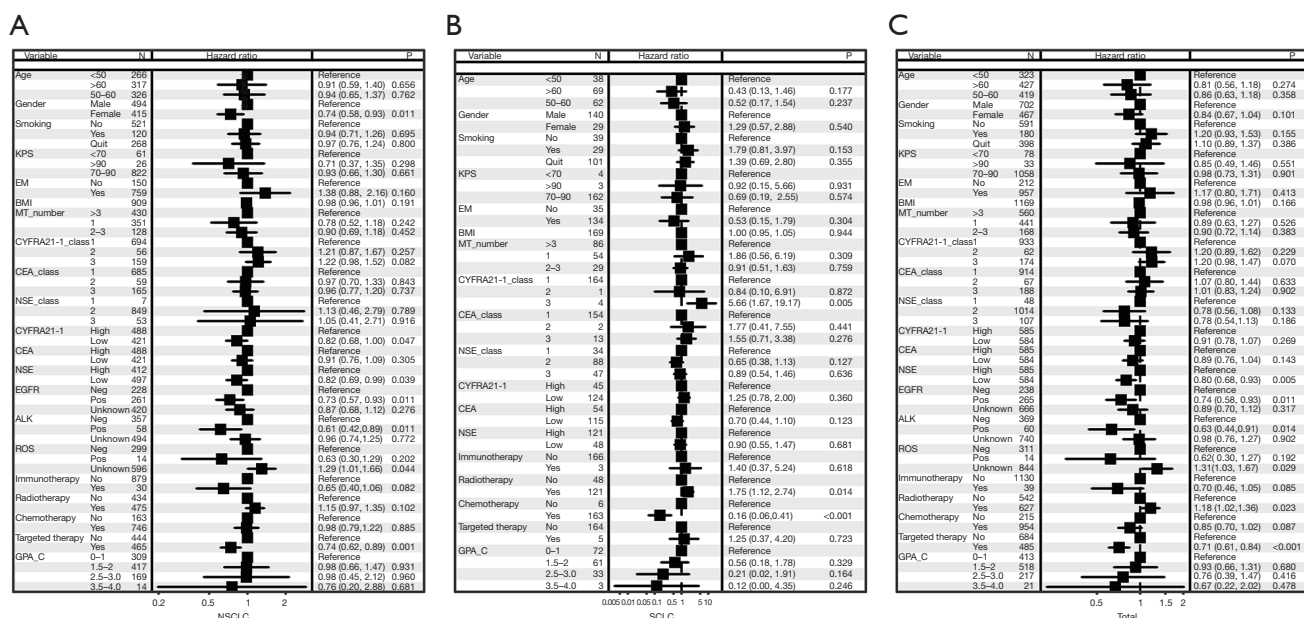
evolution trajectories of STMs. Each type of STMs can be divided into three different types based on changes in disease course. However, the dynamic alterations of STMs levels lacked prognostic significance. For patients with non-driver gene, the dynamic trajectory of CYFRA21-1 and NSE, as well as receiving chemotherapy were independent prognostic factors.

Several studies have shown that STMs are biomarkers and associated with BM development in lung cancer (14,15). After reviewing data from over 1,000 patients with BM from lung cancer from December 2013 and September 2020, we found that, the baseline levels of serum CEA (cut-off: 9.35 ng/mL), CYFRA21-1 (cut-off: 4.66 ng/mL) and NSE (cut-off: 15.5 ng/mL) were independent prognostic factors related to the outcomes of BM, representing a previously unexplored discovery. Normal reference values of CEA, CYFRA21-1 and NSE are <3.4, <3.0, and <15.0 ng/mL, respectively. In our study, the cutoff values of CYFRA21-1 and NSE did not differ much from previous normal reference ranges. However, the situation is different for CEA (which is elevated). This also explains why, after including STMs as continuous variables in Cox

regression, only CEA did not show statistical significance, which suggesting that the baseline level of CEA should have a higher value when used as a prognostic indicator. Furthermore, the status of key driver gene mutations (*EGFR* and *ALK*) was considered an independent prognostic indicator for patients with BM, consistent with previous reports (16,17). Individuals with positive mutation of driver gene benefit from personalized targeted therapy, which has the characteristics of low molecular weight and effective penetration of the blood-brain barrier, thereby enhancing therapeutic efficacy in brain tissue (18,19). Moreover, this analysis showed that GPA scores did not demonstrate predictive significance. GPA was first published in 2008 (20), including age, Karnofsky Performance Status (KPS), number of BM, and extracranial metastases, based on data from 1,960 patients from five randomly assigned Radiation Therapy Oncology Group (RTOG) trials. However, we collected data from patients with BM after 2010, with an increasing proportion receiving targeted therapy, radiotherapy and immunotherapy. The advancements in targeted therapy and immunotherapy have improved patient outcomes, necessitating further updates and research into

prognostic models for BM.

A LCGMM was used for the first time to determine the dynamic trajectory of STMs of lung cancer patients with BM using a large sample of cohort. Indeed, we found that after for lung cancer patients with BM receiving treatments, serum STM level showed dynamic changes. And we identified for the first time the distinct dynamic trajectories of CEA, CYFRA21-1, and NSE in lung cancer patients with BM, and analyzed their relationship with prognosis. Li *et al.* reported that the simultaneously dynamic measurements of CEA, CA199, and CA125 can be as independent prognostic factors of colorectal cancer patients (11), indicating the importance of dynamic monitoring of STMs. Interestingly, for patients with BM, no matter whether CYFRA21-1 rises dynamically earlier or later, patients with high baseline levels have poor prognosis, which is consistent with the close relationship between baseline levels of CYFRA21-1 and prognosis. After excluding confounding factors, dynamic changes of STMs during disease course were not significantly associated with the final outcome of BM patients, while status of driver gene and receiving targeted therapy is an absolute prognostic factor for BM patients. The mechanisms of these interactions identified are not yet clear and need further exploration in future studies. For patients with lung cancer BM, the combined use of anti-angiogenic targeted drugs has shown promising anti-tumor activity and tolerable safety (21). Of course, with the widespread adoption of next-generation sequencing (NGS) over the past decade, a positive driver gene status means the accessibility to targeted therapy and better control of BM disease progression (22-24). This is consistent with the findings of our study, emphasizing the importance of driver gene testing and targeted therapy in the management of BM patients, as well as the need for future research to delve into exploring their interaction mechanisms. In subgroup analysis, we observed that the dynamic trajectory of CYFRA21-1 and NSE as well as receiving chemotherapy were closely related to prognosis in driver gene-negative patients. Due to the limitation of the number of research subjects, this conclusion still needs further verification in future studies.

Nevertheless, there are several limitations in this study. The data set evaluated was a heterogeneous cohort of patients with BM. We collected the survival data of patients with BM from different treatment models and histology, and we did not study the prognosis of patients with different treatment models separately. There would be selection bias in the final results.

Conclusions

Baseline levels of serum CEA, CYFRA21-1 and NSE are recommended for evaluating the prognosis of lung cancer patients with BM. Dynamic changes of STMs during disease course were not significantly associated with the final outcome of BM patients. More attention to driver gene testing and targeted therapy may be needed in the management of BM patients.

Acknowledgments

Footnote

Reporting Checklist: The authors have completed the

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Figure S1 A

Variable		N	Hazard ratio	p
NSE		1169	1.00 (1.00, 1.00)	0.033
Age	<50	323	Reference	
	>60	427	0.88 (0.61, 1.27)	0.501
	50–60	419	0.90 (0.66, 1.23)	0.509
Gender	Male	702	Reference	
	Female	467	0.84 (0.68, 1.05)	0.122
Smoking	No	591	Reference	
	Yes	180	1.27 (0.99, 1.62)	0.058
	Quit	398	1.19 (0.96, 1.47)	0.113
KPS	<70	78	Reference	
	>90	33	0.76 (0.45, 1.31)	0.326
	70–90	1058	0.94 (0.70, 1.25)	0.672
EM	No	212	Reference	
	Yes	957	1.27 (0.87, 1.84)	0.212
MT_number	>3	560	Reference	
	1	441	0.84 (0.59, 1.19)	0.328
	2–3	168	0.88 (0.70, 1.11)	0.279
BMI		1169	0.98 (0.96, 1.00)	0.089
EGFR	Neg	238	Reference	
	Pos	265	0.66 (0.53, 0.82)	<0.001
	Unknown	666	0.94 (0.75, 1.17)	0.570
ALK	Neg	369	Reference	
	Pos	60	0.58 (0.41, 0.83)	0.003
	Unknown	740	0.97 (0.75, 1.25)	0.806
ROS	Neg	311	Reference	
	Pos	14	0.61 (0.30, 1.25)	0.181
	Unknown	844	1.33 (1.04, 1.69)	0.021
GPA score	0–1	413	Reference	
	1.5–2	518	0.94 (0.67, 1.33)	0.734
	2.5–3.0	217	0.82 (0.43, 1.58)	0.554
	3.5–4.0	21	0.69 (0.23, 2.07)	0.512

Figure S1 B

Variable		N	Hazard ratio	p
CYFRA21-1		1169	1.00 (1.00, 1.01)	<0.001
Age	<50	323	Reference	
	>60	427	0.89 (0.62, 1.29)	0.553
	50–60	419	0.92 (0.67, 1.25)	0.582
Gender	Male	702	Reference	
	Female	467	0.85 (0.69, 1.06)	0.144
Smoking	No	591	Reference	
	Yes	180	1.26 (0.99, 1.61)	0.061
	Quit	398	1.19 (0.96, 1.48)	0.103
KPS	<70	78	Reference	
	>90	33	0.77 (0.45, 1.32)	0.339
	70–90	1058	0.94 (0.70, 1.25)	0.670
EM	No	212	Reference	
	Yes	957	1.27 (0.87, 1.84)	0.214
MT_number	>3	560	Reference	
	1	441	0.85 (0.60, 1.21)	0.363
	2–3	168	0.90 (0.72, 1.13)	0.359
BMI		1169	0.98 (0.96, 1.00)	0.097
EGFR	Neg	238	Reference	
	Pos	265	0.66 (0.53, 0.83)	<0.001
	Unknown	666	0.97 (0.78, 1.22)	0.809
ALK	Neg	369	Reference	
	Pos	60	0.59 (0.42, 0.85)	0.004
	Unknown	740	0.97 (0.76, 1.25)	0.836
ROS	Neg	311	Reference	
	Pos	14	0.62 (0.31, 1.27)	0.194
	Unknown	844	1.32 (1.04, 1.68)	0.024
GPA score	0–1	413	Reference	
	1.5–2	518	0.92 (0.66, 1.30)	0.647
	2.5–3.0	217	0.82 (0.43, 1.59)	0.563
	3.5–4.0	21	0.69 (0.23, 2.07)	0.512

Figure S1 C

Variable		N	Hazard ratio	p
CEA		1169	1.00 (1.00, 1.00)	0.089
Age	<50	323	Reference	
	>60	427	0.88 (0.61, 1.26)	0.481
	50–60	419	0.90 (0.66, 1.23)	0.498
Gender	Male	702	Reference	
	Female	467	0.85 (0.68, 1.05)	0.125
Smoking	No	591	Reference	
	Yes	180	1.29 (1.01, 1.64)	0.043
	Quit	398	1.22 (0.98, 1.51)	0.069
KPS	<70	78	Reference	
	>90	33	0.73 (0.43, 1.25)	0.253
	70–90	1058	0.94 (0.70, 1.25)	0.664
EM	No	212	Reference	
	Yes	957	1.27 (0.87, 1.84)	0.214
MT_number	>3	560	Reference	
	1	441	0.85 (0.60, 1.20)	0.358
	2–3	168	0.90 (0.71, 1.13)	0.345
BMI		1169	0.98 (0.96, 1.00)	0.058
EGFR	Neg	238	Reference	
	Pos	265	0.66 (0.53, 0.82)	<0.001
	Unknown	666	0.96 (0.77, 1.21)	0.739
ALK	Neg	369	Reference	
	Pos	60	0.60 (0.42, 0.86)	0.006
	Unknown	740	0.98 (0.77, 1.26)	0.893
ROS	Neg	311	Reference	
	Pos	14	0.61 (0.30, 1.24)	0.172
	Unknown	844	1.30 (1.03, 1.66)	0.030
GPA score	0–1	413	Reference	
	1.5–2	518	0.93 (0.66, 1.31)	0.690
	2.5–3.0	217	0.81 (0.42, 1.56)	0.534
	3.5–4.0	21	0.68 (0.23, 2.02)	0.485

Figure S1 Multivariate Cox's forest plots of overall survival in lung cancer patients with brain metastases based on baseline levels of different serum tumor markers as continuous variables.

Figure S2 A — Kaplan-Meier curve, NSCLC, CEA=high vs CEA=low, p = 0.00092

Figure S2 B — Kaplan-Meier curve, SCLC, CEA=high vs CEA=low, p = 0.25

Figure S2 C — Kaplan-Meier curve, Others, CEA=high vs CEA=low, p = 0.41

Figure S2 D

Variable		N	Hazard ratio	p
CEA	High	456	Reference	
	Low	453	0.80 (0.69, 0.94)	0.006
Age	<50	266	Reference	
	>60	317	0.91 (0.59, 1.40)	0.661
	50–60	326	0.96 (0.67, 1.39)	0.845
Gender	Male	494	Reference	
	Female	415	0.71 (0.57, 0.90)	0.004
Smoking	No	521	Reference	
	Yes	120	0.98 (0.74, 1.31)	0.907
	Quit	268	1.02 (0.80, 1.30)	0.885
KPS	<70	61	Reference	
	>90	26	0.72 (0.38, 1.36)	0.310
	70–90	822	0.93 (0.66, 1.32)	0.699
EM	No	150	Reference	
	Yes	759	1.45 (0.93, 2.26)	0.103
MT_number	>3	430	Reference	
	1	351	0.78 (0.52, 1.18)	0.234
	2–3	128	0.91 (0.70, 1.20)	0.512
BMI		909	0.97 (0.95, 1.00)	0.037
EGFR	Neg	228	Reference	
	Pos	261	0.72 (0.57, 0.92)	0.009
	Unknown	420	0.88 (0.69, 1.12)	0.307
ALK	Neg	357	Reference	
	Pos	58	0.62 (0.43, 0.91)	0.013
	Unknown	494	0.95 (0.73, 1.24)	0.724
ROS	Neg	299	Reference	
	Pos	14	0.59 (0.29, 1.22)	0.154
	Unknown	596	1.27 (0.99, 1.63)	0.060
GPA_C	0–1	309	Reference	
	1.5–2	417	0.99 (0.66, 1.47)	0.946
	2.5–3.0	169	0.95 (0.45, 2.05)	0.905
	3.5–4.0	14	0.72 (0.19, 2.73)	0.630
Immunotherapy	No	879	Reference	
	Yes	30	0.70 (0.43, 1.13)	0.146
Radiotherapy	No	434	Reference	
	Yes	475	1.15 (0.98, 1.35)	0.094
Chemotherapy	No	163	Reference	
	Yes	746	0.93 (0.75, 1.16)	0.538
Targeted therapy	No	444	Reference	
	Yes	465	0.76 (0.64, 0.91)	0.003

Figure S2 E

Variable		N	Hazard ratio	p
CEA	High	85	Reference	
	Low	84	0.85 (0.59, 1.23)	0.391
Age	<50	38	Reference	
	>60	69	0.47 (0.15, 1.51)	0.206
	50–60	62	0.56 (0.20, 1.59)	0.279
Gender	Male	140	Reference	
	Female	29	1.14 (0.52, 2.50)	0.750
Smoking	No	39	Reference	
	Yes	29	1.72 (0.78, 3.79)	0.179
	Quit	101	1.34 (0.68, 2.66)	0.399
KPS	<70	4	Reference	
	>90	3	1.19 (0.20, 6.97)	0.843
	70–90	162	0.83 (0.22, 3.05)	0.774
EM	No	35	Reference	
	Yes	134	0.73 (0.24, 2.28)	0.591
MT_number	>3	86	Reference	
	1	54	1.83 (0.59, 5.72)	0.298
	2–3	29	0.88 (0.49, 1.58)	0.673
BMI		169	0.99 (0.94, 1.05)	0.836
GPA_C	0–1	72	Reference	
	1.5–2	61	0.59 (0.20, 1.73)	0.338
	2.5–3.0	33	0.28 (0.03, 2.31)	0.236
	3.5–4.0	3	0.17 (0.01, 5.07)	0.310
Immunotherapy	No	166	Reference	
	Yes	3	1.07 (0.30, 3.82)	0.915
Radiotherapy	No	48	Reference	
	Yes	121	1.36 (0.90, 2.04)	0.140
Chemotherapy	No	6	Reference	
	Yes	163	0.24 (0.09, 0.61)	0.003
Targeted therapy	No	164	Reference	
	Yes	5	1.24 (0.37, 4.11)	0.731

Figure S2 F

Variable		N	Hazard ratio	p
CEA	High	46	Reference	
	Low	45	1.56 (0.88, 2.76)	0.132
Age	<50	19	Reference	
	>60	41	1.02 (0.33, 3.13)	0.977
	50–60	31	0.85 (0.30, 2.43)	0.769
Gender	Male	68	Reference	
	Female	23	4.30 (1.80, 10.26)	0.001
Smoking	No	31	Reference	
	Yes	31	4.43 (1.86, 10.55)	<0.001
	Quit	29	5.70 (2.19, 14.85)	<0.001
KPS	<70	13	Reference	
	>90	4	0.64 (0.12, 3.45)	0.602
	70–90	74	1.65 (0.71, 3.86)	0.247
EM	No	27	Reference	
	Yes	64	0.77 (0.20, 2.93)	0.707
MT_number	>3	44	Reference	
	1	36	0.96 (0.30, 3.08)	0.949
	2–3	11	1.13 (0.49, 2.60)	0.780
BMI		91	1.03 (0.96, 1.11)	0.368
GPA_C	0–1	32	Reference	
	1.5–2	40	0.67 (0.21, 2.08)	0.485
	2.5–3.0	15	0.24 (0.02, 2.84)	0.259
	3.5–4.0	4	1.03 (0.03, 31.88)	0.989
Immunotherapy	No	85	Reference	
	Yes	6	1.40 (0.41, 4.71)	0.589
Radiotherapy	No	60	Reference	
	Yes	31	0.96 (0.51, 1.79)	0.891
Chemotherapy	No	46	Reference	
	Yes	45	0.43 (0.24, 0.77)	0.005
Targeted therapy	No	76	Reference	
	Yes	15	0.41 (0.19, 0.89)	0.023

Figure S2 Kaplan-Meier curves and multivariate Cox's forest plots of overall survival based on baseline levels of CEA, stratified by non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) patients with brain metastases, respectively.

Figure S3 Kaplan-Meier curves and multivariate Cox's forest plots of overall survival based on baseline levels of NSE, stratified by non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) patients with brain metastases, respectively.

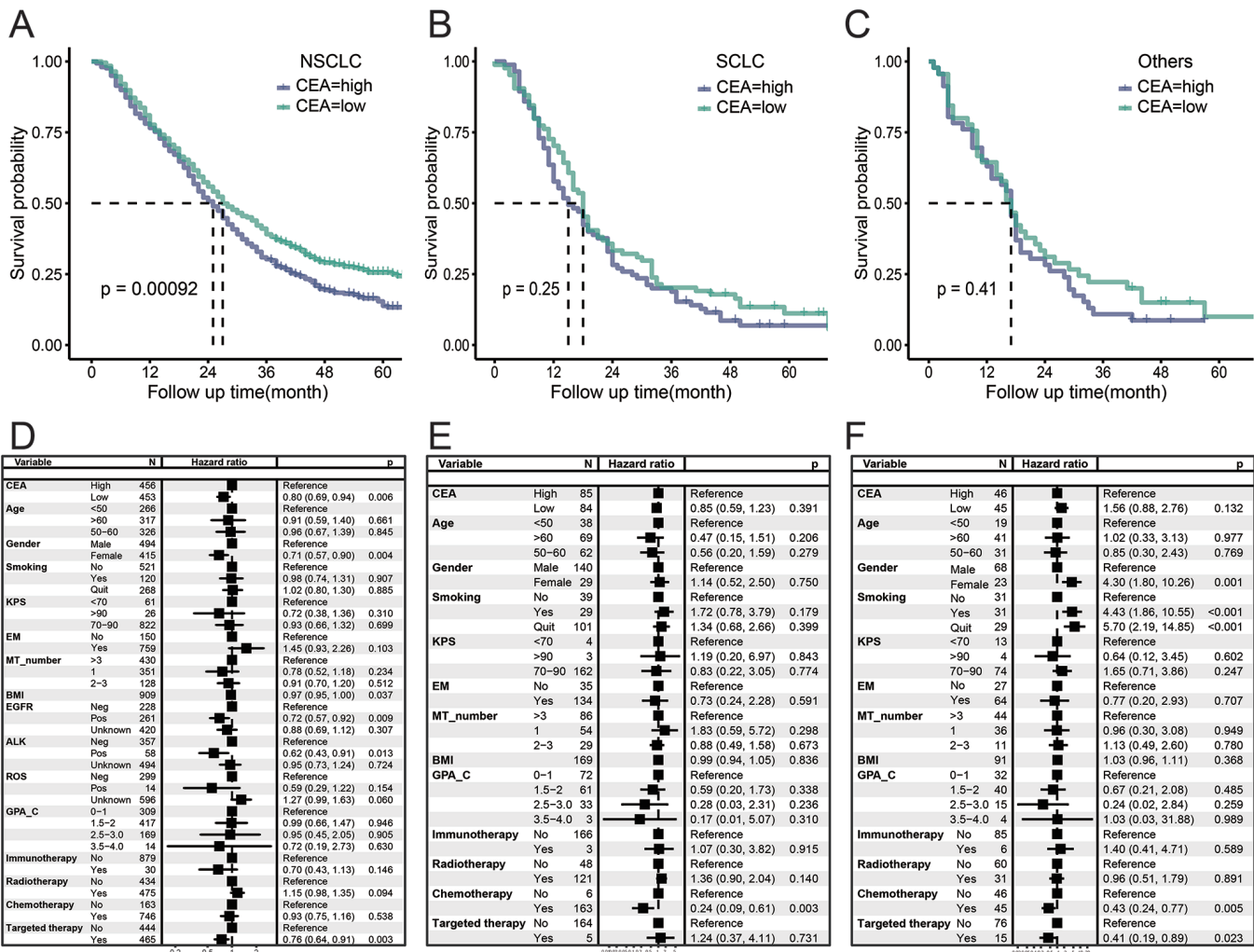**Figure S4** Kaplan-Meier curves and multivariate Cox's forest plots of overall survival based on baseline levels of serum levels of CYFRA21-1, stratified by non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) patients with brain metastases, respectively.
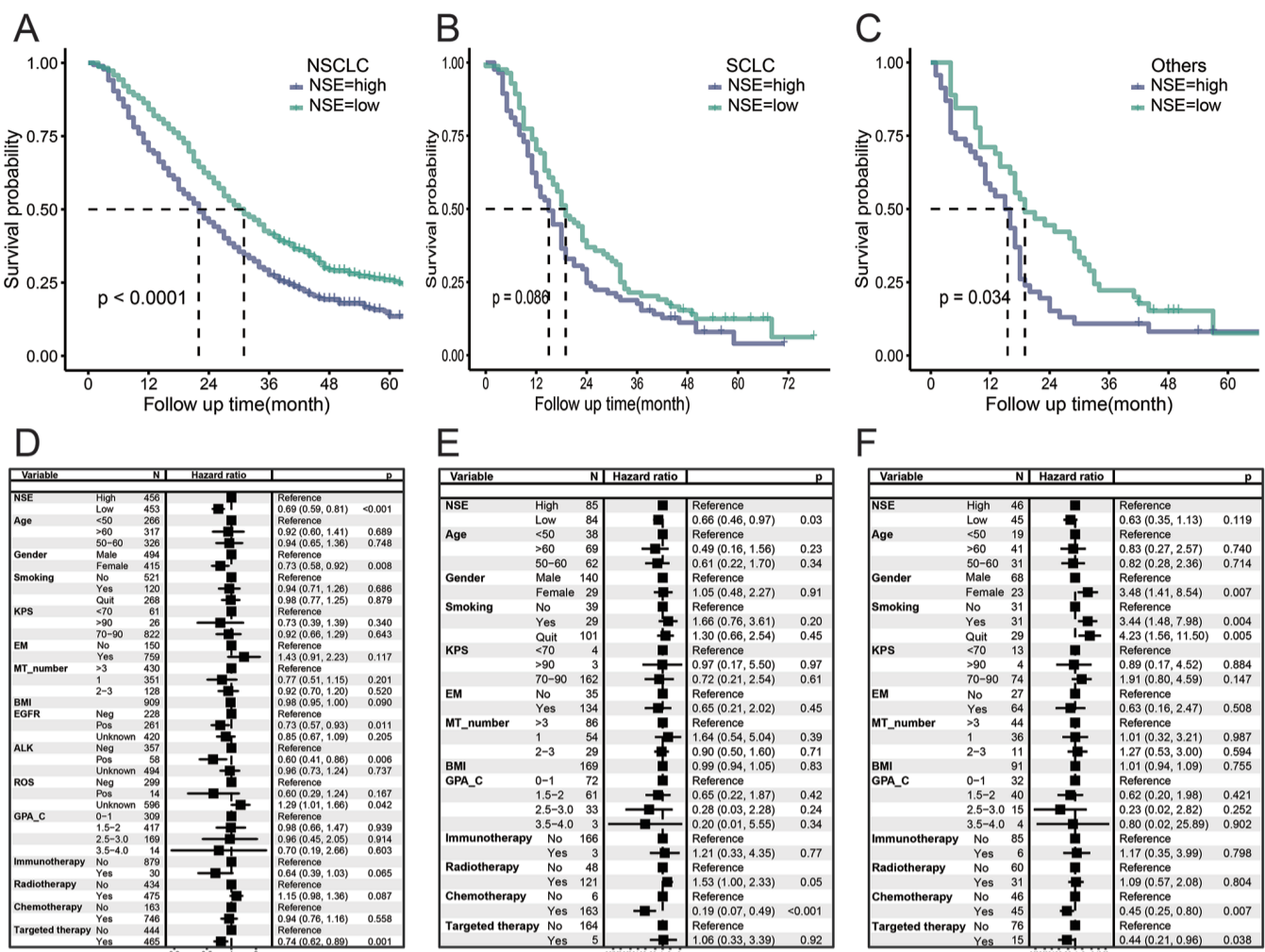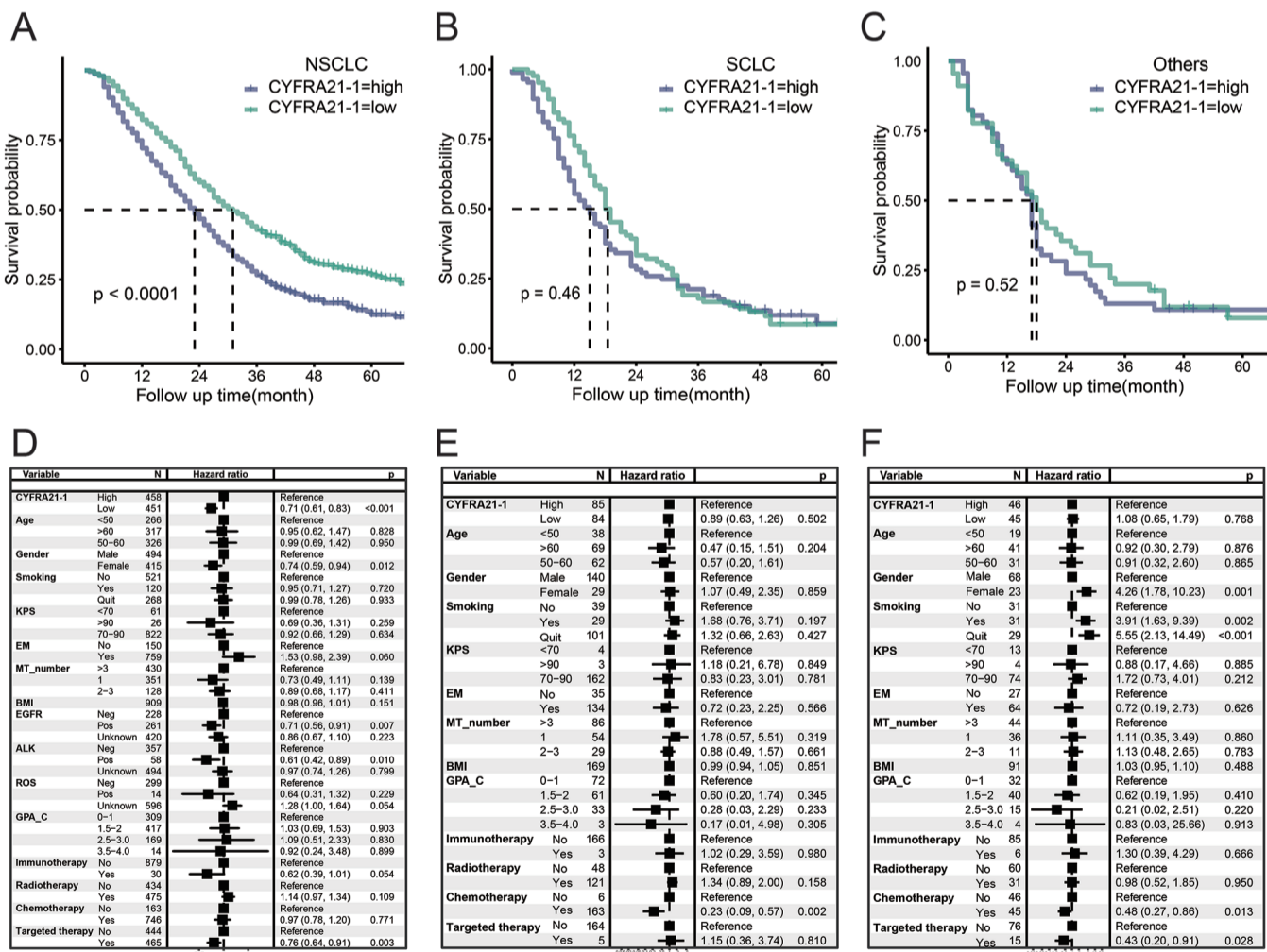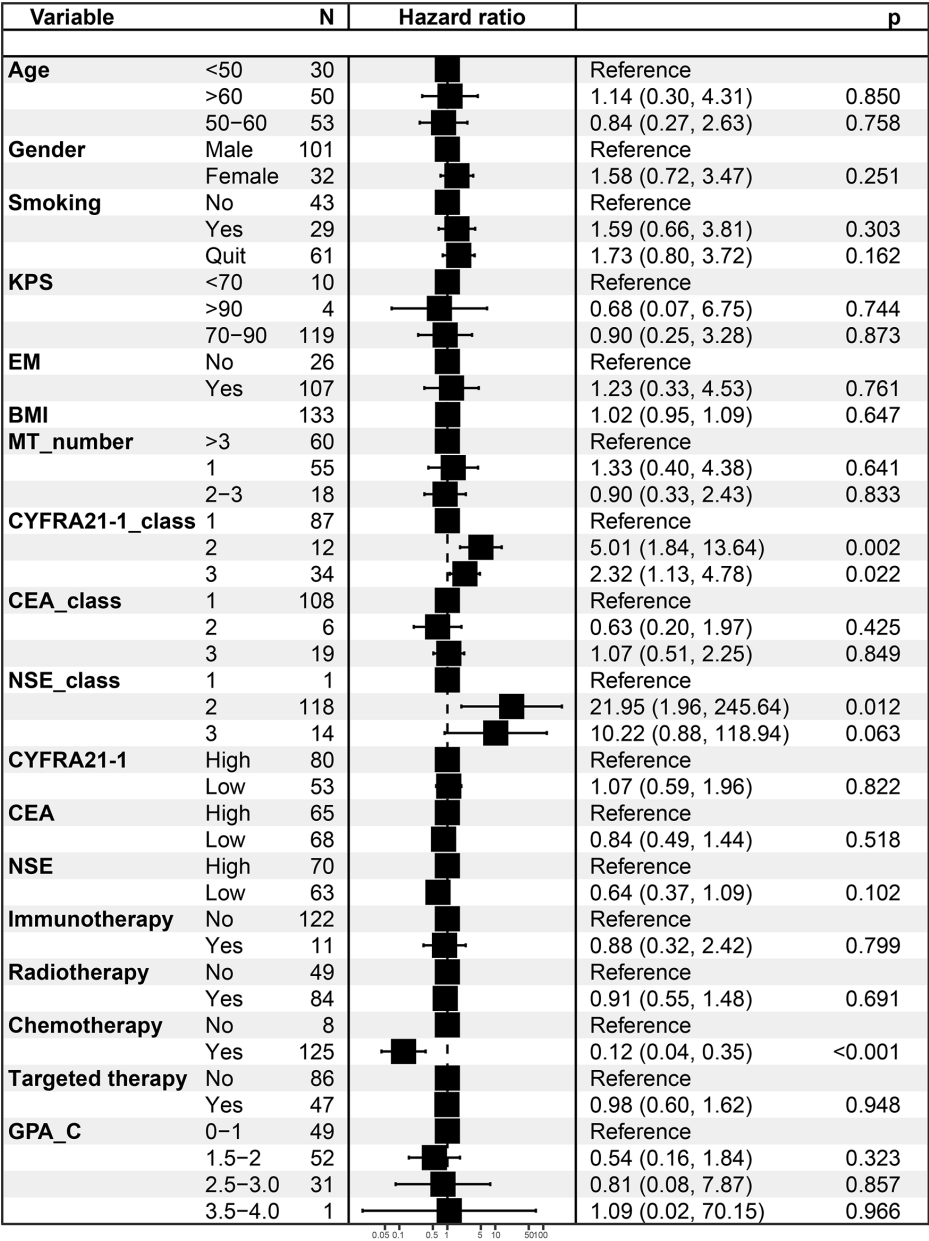
Variable		N	Hazard ratio		p
Age	<50	30		Reference	
	>60	50		1.14 (0.30, 4.31)	0.850
	50–60	53		0.84 (0.27, 2.63)	0.758
Gender	Male	101		Reference	
	Female	32		1.58 (0.72, 3.47)	0.251
Smoking	No	43		Reference	
	Yes	29		1.59 (0.66, 3.81)	0.303
	Quit	61		1.73 (0.80, 3.72)	0.162
KPS	<70	10		Reference	
	>90	4		0.68 (0.07, 6.75)	0.744
	70–90	119		0.90 (0.25, 3.28)	0.873
EM	No	26		Reference	
	Yes	107		1.23 (0.33, 4.53)	0.761
BMI		133		1.02 (0.95, 1.09)	0.647
MT_number	>3	60		Reference	
	1	55		1.33 (0.40, 4.38)	0.641
	2–3	18		0.90 (0.33, 2.43)	0.833
CYFRA21-1_class	1	87		Reference	
	2	12		5.01 (1.84, 13.64)	0.002
	3	34		2.32 (1.13, 4.78)	0.022
CEA_class	1	108		Reference	
	2	6		0.63 (0.20, 1.97)	0.425
	3	19		1.07 (0.51, 2.25)	0.849
NSE_class	1	1		Reference	
	2	118		21.95 (1.96, 245.64)	0.012
	3	14		10.22 (0.88, 118.94)	0.063
CYFRA21-1	High	80		Reference	
	Low	53		1.07 (0.59, 1.96)	0.822
CEA	High	65		Reference	
	Low	68		0.84 (0.49, 1.44)	0.518
NSE	High	70		Reference	
	Low	63		0.64 (0.37, 1.09)	0.102
Immunotherapy	No	122		Reference	
	Yes	11		0.88 (0.32, 2.42)	0.799
Radiotherapy	No	49		Reference	
	Yes	84		0.91 (0.55, 1.48)	0.691
Chemotherapy	No	8		Reference	
	Yes	125		0.12 (0.04, 0.35)	<0.001
Targeted therapy	No	86		Reference	
	Yes	47		0.98 (0.60, 1.62)	0.948
GPA_C	0–1	49		Reference	
	1.5–2	52		0.54 (0.16, 1.84)	0.323
	2.5–3.0	31		0.81 (0.08, 7.87)	0.857
	3.5–4.0	1		1.09 (0.02, 70.15)	0.966

0.05 0.1   0.5 1   5 10   50 100

Figure S5 Multivariate Cox's forest plots of overall survival in lung cancer patients with brain metastases lacking key driver genes based on dynamic changes of different serum tumor markers.