A nomogram prediction model for brain metastases in patients with lung adenocarcinoma

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Abstract. Patients with lung adenocarcinoma (LUAD) with brain metastasis (BM) frequently present without central nervous system (CNS) symptoms at initial diagnosis, underscoring the clinical need for early predictive tools. This study aimed to evaluate the role of epidermal growth factor receptor (EGFR) mutation subtypes in BM risk and to develop a nomogram prediction model combining EGFR mutation status with serum biomarkers in patients with LUAD. A retrospective cohort of 615 patients with LUAD-401 with BM and 214 without BM, matched by age and sex-was recruited from a single center in China between June 2021 and June 2024. Logistic regression, receiver operating characteristic (ROC) curve analysis and nomogram modeling were performed, with internal validation via 10-fold cross-validation. Although the overall distribution of EGFR mutations did not differ significantly between BM and non-BM groups, the proportion of exon 19 deletion (19del) mutations was significantly higher in the BM group (P=0.013), with an odds ratio of 2.10 in the univariate analysis, and 19del was confirmed as an independent risk factor in the multivariate analysis. Serum concentrations of neuron-specific enolase, CEA, CA125, CA153 and cytokeratin 19 fragment were markedly elevated in patients with BM (all P<0.0001); ROC analyses stratified by 19del mutation status demonstrated further improvement in predictive performance for selected biomarkers. A nomogram integrating 19del mutation status with the five serum biomarkers achieved an area under the ROC curve (AUC) of 0.835 in the training cohort and a cross-validated AUC of 0.814, with good calibration confirmed by the Hosmer-Lemeshow test (P=0.151). These findings indicate that LUAD patients harboring EGFR 19del mutations have an elevated BM risk and the combined nomogram provides an effective tool for BM risk stratification in clinical practice.

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Abbreviations: AIC, Akaike information criterion; AUC, area under the curve; BM, brain metastasis; CA125, cancer antigen 125; CA153, cancer antigen 153; CEA, carcinoembryonic antigen; CNS, central nervous system; CYFRA21-1, cytokeratin 19 fragment; DCA, decision curve analysis; EGFR, epidermal growth factor receptor; IDI, integrated discrimination improvement; LC, lung cancer; LUAD, lung adenocarcinoma; NRI, net reclassification improvement; NSCLC, non-small cell lung cancer; NSE, neuron-specific enolase; ROC, receiver operating characteristic; TKI, tyrosine kinase inhibitor; 19del, exon 19 deletion

Key words: biomarkers, brain metastasis, EGFR mutations, lung adenocarcinoma, nomogram model

Introduction

Lung cancer (LC) is one of the most prevalent malignant tumors in China. In 2022, ~1.06 million new cases of LC were diagnosed in China, with an incidence rate of 149.54 per 100,000 and a mortality rate of 102.97 per 100,000, ranking first among malignant tumors in terms of cancer-related mortality (1). Brain metastasis (BM) is one of the most serious complications affecting patient prognosis and quality of life, and represents the most common form of distant metastasis in LC, occurring in ~20-65% of patients with LC during the course of disease (2,3). Lung adenocarcinoma (LUAD), a subtype of non-small cell lung cancer (NSCLC) arising from the pulmonary glandular epithelium, accounts for ~50% of all LC cases (4). Approximately 30% of patients with LUAD develop BM, which is a leading cause of treatment failure (5). Early detection and intervention of BM is therefore considered essential for improving the prognosis of patients with LUAD.

Epidermal growth factor receptor (EGFR) mutation is among the most frequently identified driver gene alterations in NSCLC, particularly in Asian populations (6). A total of ~40-50% of patients with LUAD in Asia harbor deleterious mutations in the EGFR gene (7). The specific mutation site and type are key determinants for selecting targeted therapies, such as EGFR tyrosine kinase inhibitors (TKIs) (8). Third-generation EGFR TKIs have demonstrated definite efficacy in treating BM in patients with NSCLC with EGFR mutations (9). Several clinical studies have suggested that certain EGFR mutation subtypes may increase the risk of BM in LC, although most were limited by small sample sizes (10-12). However, evidence directly confirming the relationship between specific EGFR mutations and BM development in LUAD remains scarce, and qualitative assessment of EGFR mutation status alone cannot

serve as a real-time quantitative marker for monitoring disease progression (13). In the present study, the impact of EGFR mutation subtypes on BM risk in LUAD was analyzed and EGFR mutation status was integrated with serum LC-related biomarkers to develop a combined predictive approach. This combined method was evaluated for its utility in auxiliary diagnosis and risk stratification of BM in LUAD, with the aim of providing a quantitative and dynamic tool for early detection and clinical intervention.

Materials and methods

Study population. This study retrospectively enrolled 615 patients with LUAD, including 401 with BM and 214 without BM, matched by age and sex. Between June 2021 and June 2024, patients with LUAD who developed BM were consecutively identified from the medical records of the First Affiliated Hospital of Guangzhou Medical University (Guangzhou, China); a control group of patients with LUAD without BM was subsequently selected from the same institution during the same period, matched to the BM group by age and sex. LUAD diagnosis was established by a pathologist and respiratory physicians based on histopathological examination of lung biopsy specimens. BM diagnosis was established by a multidisciplinary team comprising neurologists, radiologists and respiratory physicians, based on findings from contrast-enhanced 3.0T MRI or detection of tumor cells in the cerebrospinal fluid. All patients in the non-BM group also underwent contrast-enhanced 3.0T MRI, with the absence of BM confirmed on the basis of negative imaging findings, thereby ensuring that BM status was determined by standardized imaging assessment across all enrolled patients. The primary inclusion criterion was a confirmed diagnosis of primary LC in the absence of other concurrent malignancies. The exclusion criteria were as follows: i) History of psychiatric complaints, disturbance of consciousness, dementia, central nervous system (CNS) infection or other conditions associated with impaired brain function; ii) severe hepatic or renal dysfunction; and iii) absence of key diagnostic examinations required for clinical diagnosis. All participants provided written informed consent for the collection, processing and analysis of blood samples and clinical data for research and publication purposes. This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University (approval no. GZMC2021-06-1237; Fig. S1).

Peripheral blood collection and detection. Blood samples from patients in the BM group were collected within 3 days of BM diagnosis; this sampling window was intended to ensure that collection preceded the initiation of BM-specific treatments, including corticosteroids, whole-brain radiotherapy and stereotactic radiosurgery. Blood samples from patients in the non-BM group were collected at the time of initial LUAD diagnosis, prior to the initiation of any antitumor therapy, representing a treatment-naive baseline state. All samples were collected in the morning under fasting conditions and stored in anticoagulant-containing polypropylene tubes. Plasma was separated by centrifugation at 500 x g for 10 min at 4˚C. Serum samples were processed for LC biomarker detection within 24 h of collection; remaining samples were stored at -80˚C. The LC biomarkers assessed included neuron-specific enolase (NSE), carcinoembryonic antigen (CEA), cancer antigen 125 (CA125), CA153 and cytokeratin 19 fragment (CYFRA21-1). CEA concentrations were measured by electrochemiluminescence immunoassay using a commercial kit (Architect CEA Reagent Kit; Abbott Diagnostics Division), whereas NSE, CA125, CA153 and CYFRA21-1 concentrations were determined by immunoradiometric assay using commercially available kits (Cytokeratin 19 Fragment IRMA Kit and NSE IRMA Kit; Beckman Coulter, Inc.). The procedure followed the manufacturer's instructions, as reported previously (14). The upper reference limits were as follows: NSE, 16.30 ng/ml; CEA, 5.00 ng/ml; CA125, 35.00 U/ml; CA153, 25.00 U/ml; and CYFRA21-1, 3.30 ng/ml. Each sample was measured in triplicate and the mean value was used for subsequent analyses. No samples were subjected to repeated freeze-thaw cycles.

DNA extraction and sequencing. Total DNA was extracted from formalin-fixed, paraffin-embedded tumor tissue using a QIAamp DNA Tissue Kit (Qiagen GmbH), following previously described methods (15). DNA purity was assessed by spectrophotometric measurement of the 260/280 nm absorbance ratio using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Inc.). Targeted next-generation sequencing was performed by Burning Rock Biotech, a commercial clinical laboratory certified by the Clinical Laboratory Improvement Amendments and accredited by the College of American Pathologists (15). A commercial capture panel targeting 520 genes of the human genome (OncoScreen Plus; Burning Rock Biotech) was used for target enrichment according to the manufacturer's protocol. DNA fragments of 200-400 bp were purified using a Bioanalyzer 2100 (Agilent Technologies, Inc.), hybridized with capture probes, selected with magnetic beads and amplified. Library sequencing was performed on the NextSeq 500 platform (Illumina, Inc.), with a mean sequencing depth of ~1,000 x. Further methodological details were reported previously (15).

Nomogram construction and validation. A total of 9 candidate variables were initially entered into a multivariate analysis, encompassing TNM staging components (T stage, N stage and other tissue metastases as a surrogate for distant metastatic burden), EGFR exon 19 deletion (19del) mutation status, and five serum biomarkers (NSE, CEA, CA125, CA153 and CYFRA21-1). M stage was excluded to avoid collinearity with the outcome variable, as BM itself constitutes M1 disease by definition. Bidirectional stepwise selection based on the Akaike information criterion (AIC) was applied to identify the optimal variable subset; T stage was eliminated during this process and eight variables were retained in the final model (AIC=439.42). A nomogram was subsequently constructed using these variables to predict BM risk in patients with LUAD (16). Calibration curve and receiver operating characteristic (ROC) curve analyses were used to evaluate the calibration and discriminatory ability of the nomogram, respectively, and decision curve analysis (DCA) was performed to assess its clinical utility. Subsequently, 10-fold cross-validation was performed for the internal validation of

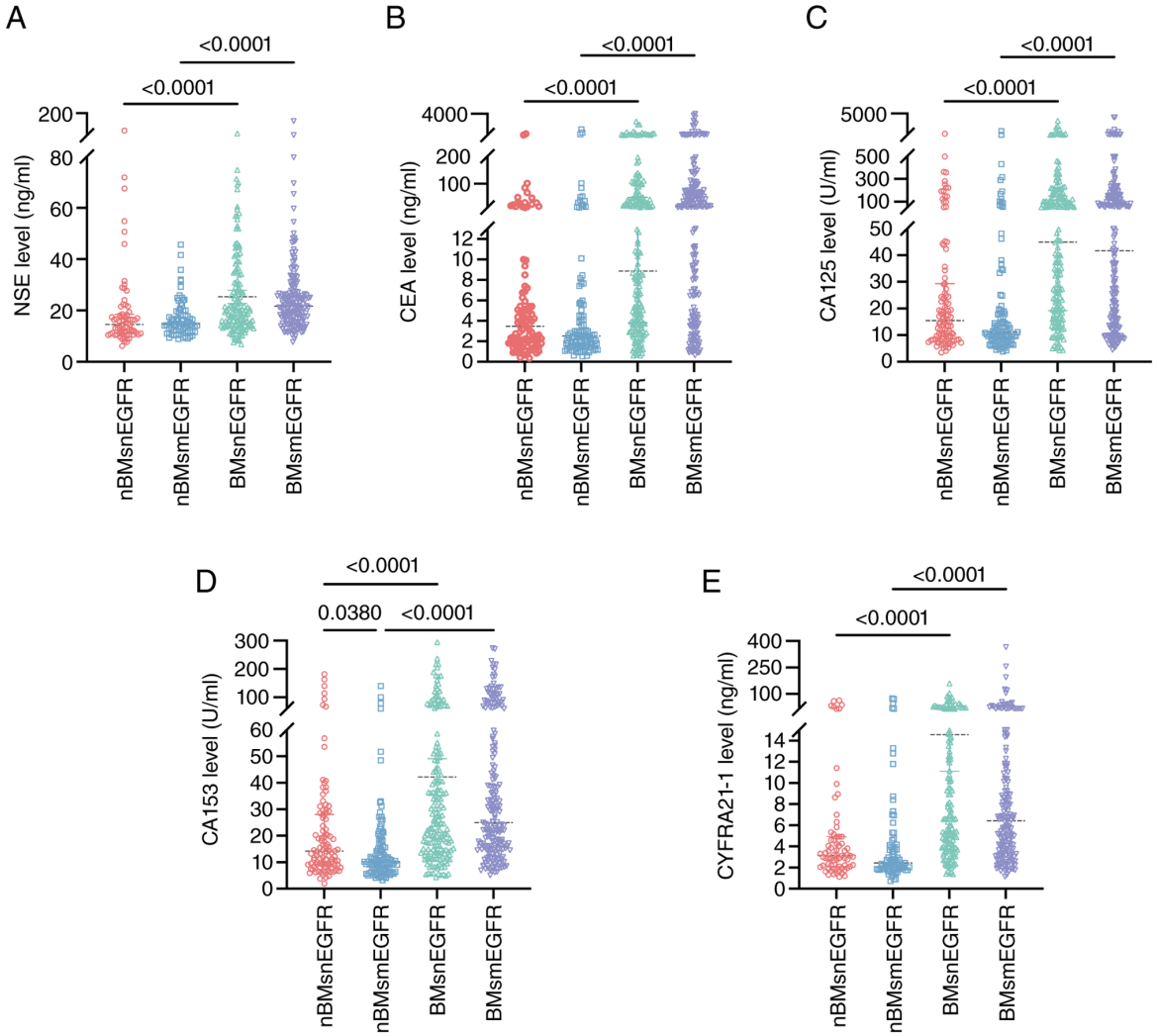

Figure 1. Lung cancer biomarker concentrations in patients with LUAD with EGFR mutations. The concentrations of (A) NSE, (B) CEA, (C) CA125, (D) CA153 and (E) CYFRA21-1 in the serum of different LUAD groups were detected. Patients were stratified into four groups according to BM and EGFR mutation status: nBMsnEGFR, non-BM without EGFR mutation; nBMsmEGFR, non-BM with EGFR mutation; BMsnEGFR, BM without EGFR mutation; BMsmEGFR, BM with EGFR mutation. The data are presented in scatter plots and the median is shown by a horizontal line. P-values are shown above the comparison brackets. Comparisons between groups were performed with the Kruskal-Wallis test and Dunn's multiple-comparisons test. LUAD, lung adenocarcinoma; NSE, neuron-specific enolase; CYFRA21-1, cytokeratin 19 fragment; BM, brain metastasis.

the nomogram. The original dataset was randomly partitioned into 10 equal subsets; at each iteration, 9 subsets were pooled for model training and the remaining subset was withheld for validation, with this process repeated 10 times. The mean area under the ROC curve (AUC), sensitivity and specificity across the 10 validation iterations were calculated to assess the generalizability of the model. A web-based calculator was also developed (https://clinicalnomogram.shinyapps.io/BMsPredictionApp/) to facilitate the clinical application of the nomogram, providing individualized BM probability estimates based on patient-specific variable inputs.

Statistical analysis. Statistical analyses were performed using GraphPad Prism (version 8.0.2; Dotmatics), SPSS (version 19.0; SPSS, Inc.) and R (version 4.1.0; R Foundation for Statistical Computing). Key R packages used included rms, pROC, ResourceSelection and caret. Non-normally distributed data are presented as median (interquartile range), while normally distributed data are expressed as mean ± standard deviation. Categorical data were expressed as n (%). Group comparisons were performed using the Mann-Whitney U test, Kruskal-Wallis test or chi-square test, as appropriate for the data distribution. Logistic regression was used to identify factors independently associated with BM in patients with LUAD. ROC curves and the corresponding area under the curve (AUC) values were generated to evaluate and compare the predictive performance of individual and combined markers for BM. The net reclassification improvement (NRI) and integrated discrimination improvement (IDI) were calculated to quantitatively compare the discriminative ability of the nomogram with that of the TNM staging model. The optimal cut-off value for each serum biomarker was determined by maximizing the Youden index (sensitivity + specificity-1) on the training set ROC curves: NSE, 18.18 ng/ml; CEA, 6.01 ng/ml; CA125, 19.59 U/ml; CA153, 14.82 U/ml; and CYFRA21-1, 4.17 ng/ml. A post-hoc power analysis was conducted for the primary comparison of BM incidence between 19del and non-19del groups within a chi-square test framework (Cohen's w=0.10; N=615; achieved

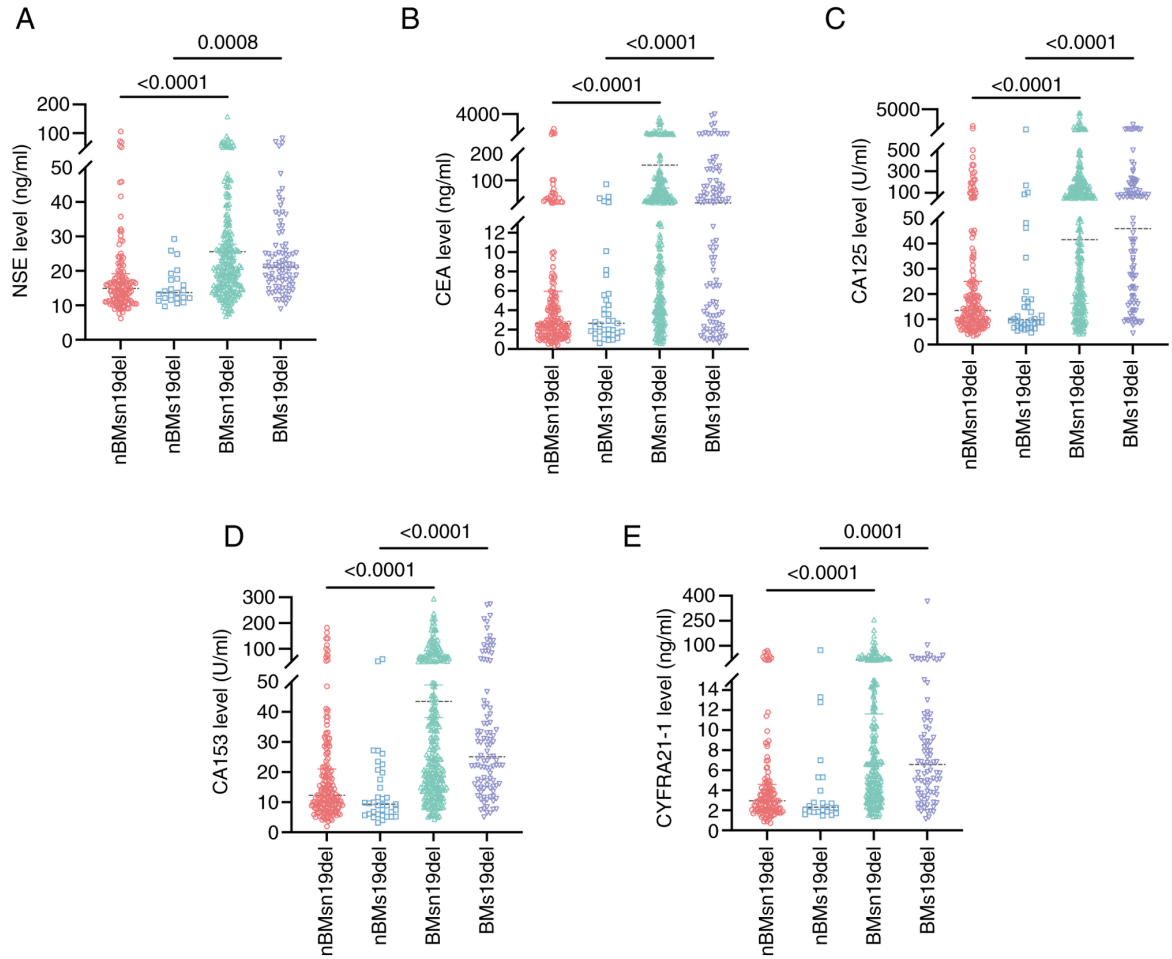

Figure 2. Lung cancer biomarker concentrations in patients with lung adenocarcinoma with the 19del mutation. The concentrations of (A) NSE, (B) CEA, (C) CA125, (D) CA153 and (E) CYFRA21-1 in the serum of patients with 19del mutation or BM were detected. Patients were stratified into four groups according to BM and 19del mutation status: nBMsn19del, non-BM without 19del; nBMs19del, non-BM with 19del; BMsn19del, BM without 19del; BMs19del, BM with 19del. The data are presented in scatter plots and the median is shown by a horizontal line. P-values are shown above the comparison brackets. Comparisons between groups were performed with the Kruskal-Wallis test and Dunn's multiple comparisons test. NSE, neuron-specific enolase; CYFRA21-1, cytokeratin 19 fragment; BM, brain metastasis; 19del, exon 19 deletion.

power, 70.3%; α=0.05). A two-sided P<0.05 was considered to indicate statistical significance for all analyses.

Results

The distribution of patients with EGFR mutations does not change in LUAD with BM. In accordance with the inclusion and exclusion criteria, 401 patients with LUAD with BM and 214 patients without BM were recruited, matched by age [BM group: Median, 61 years (range, 24-88 years); non-BM group: Median, 60 years (range, 30-93 years)] and sex [BM group: 55.4% male; non-BM group: 49.5% male] and were well-balanced with respect to smoking history [BM group: 9.2% (37/401); non-BM group: 9.3% (20/214); P=0.961; Table SI]. Demographic and clinicopathological characteristics, including pathologic stage, primary tumor status, lymph node metastasis and number of BMs, were recorded for all enrolled patients. The proportion of patients with other tissue metastases was significantly higher in the BM group than in the non-BM group (54.1 vs. 22.0%, P<0.001), whereas the overall distribution of EGFR mutations did not differ significantly between groups (P>0.999; Table SI). These findings suggest that BM development in LUAD may be associated with enhanced tumor invasiveness; however, the lack of a significant difference in overall EGFR mutation distribution indicates that distinct mutation subtypes may exert differential effects on BM risk, warranting further subtype-level analysis. Serum concentrations of NSE, CEA, CA125, CA153 and CYFRA21-1 were subsequently measured in all patients. As shown in Fig. S2, serum levels of NSE, CEA, CA125, CA153 and CYFRA21-1 were significantly elevated in patients with BM compared with those in the non-BM group, by 1.44-, 4.93-, 3.33-, 2.13- and 2.30-fold, respectively (all P<0.0001), suggesting potential predictive value for BM in clinical settings. To further characterize these relationships, patients were stratified into four subgroups according to BM and EGFR mutation status. As shown in Fig. 1A-E, serum levels of all five biomarkers were significantly elevated in both BM subgroups compared with both non-BM subgroups (all P<0.0001), while EGFR mutation status exerted no significant effect within either the non-BM or BM stratum (all P>0.9999). The sole exception was CA153 (Fig. 1D), for which a modest but statistically significant difference was observed between EGFR-mutated and non-mutated patients within the non-BM

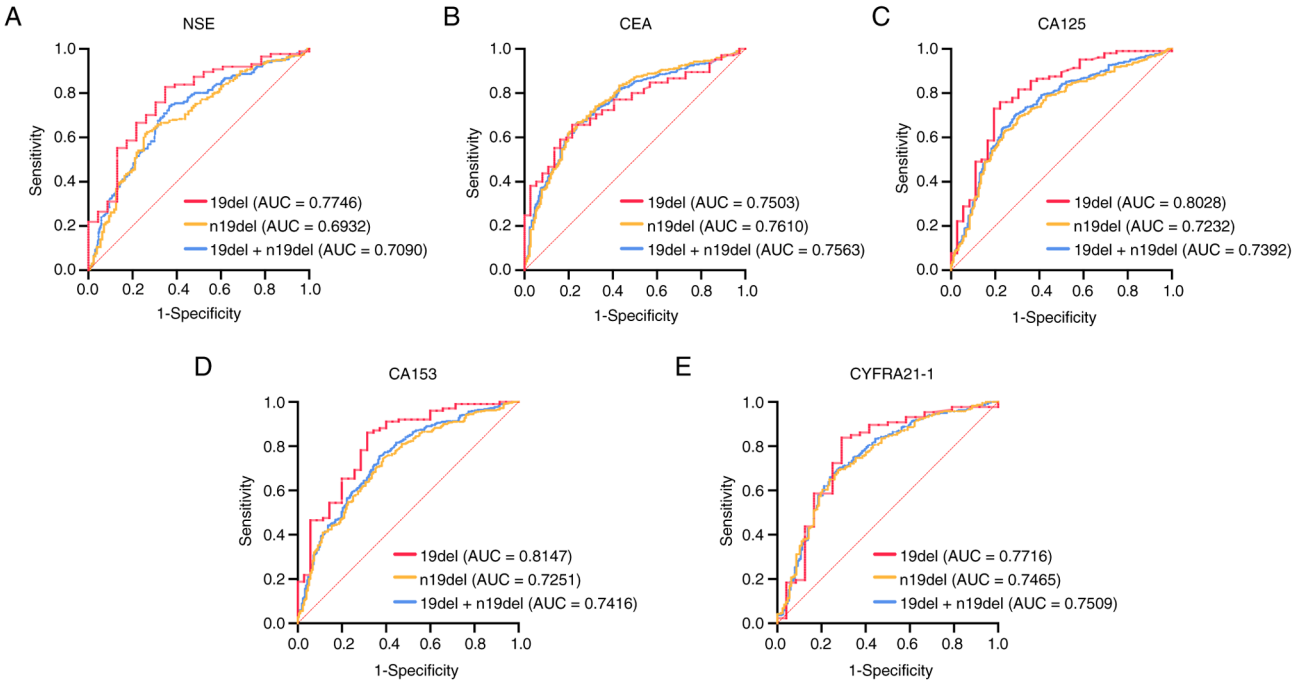

Figure 3. ROC curves of 19del combined with lung cancer biomarkers for the prediction of BM in patients with LUAD. ROC curves of serum levels of (A) NSE, (B) CEA, (C) CA125, (D) CA153 and (E) CYFRA21-1 were generated to distinguish BM in patients with LUAD. The three subgroups are shown: 19del, patients harboring the exon 19 deletion; n19del, patients without the exon 19 deletion; 19del+n19del, the overall cohort. ROC, receiver operating characteristic; AUC, area under the ROC curve; NSE, neuron-specific enolase; CYFRA21-1, cytokeratin 19 fragment; BM, brain metastasis; 19del, exon 19 deletion; LUAD, lung adenocarcinoma.

group (P=0.038), but not within the BM group. Collectively, these results indicate that elevation of all five serum biomarkers was primarily driven by BM status rather than EGFR mutation status. Nevertheless, the complex interplay among BM, EGFR mutations and serum biomarkers suggests that subtype-level analysis of EGFR mutations may help elucidate underlying mechanisms.

The 19del mutation is associated with increased risk of BM in patients with LUAD. All 615 patients with LUAD were classified into three subgroups according to EGFR genotype: 19del, 21L858R and other genotypes. Baseline clinicopathological characteristics, including pathologic stage, primary tumor status, lymph node metastasis and other tissue metastases, were comparable among the three genotype groups (Table SII). Compared with other genotypes, the 19del mutation was associated with a significantly higher BM rate (105/142, 73.9% vs. 296/473, 62.6%, P=0.013), while the 21L858R mutation showed a trend toward a lower BM rate that did not reach statistical significance (P=0.083; Table SII).

Patients were further stratified into four subgroups according to BM and 19del mutation status. As shown in Fig. 2A-E, serum levels of all five biomarkers were significantly elevated in both BM subgroups compared with both non-BM subgroups (all P<0.0001), whereas no significant difference was observed between 19del and non-19del subjects within either the non-BM or BM stratum (all P>0.999).

Univariate logistic regression analysis was performed with BM status as the dependent variable and nine candidate variables as independent variables, including primary tumor stage, lymph node metastasis, other tissue metastasis, EGFR 19del status and five serum biomarkers (NSE, CEA, CA125, CA153 and CYFRA21-1). All nine variables were significantly associated with BM in the univariate analysis (Table SIII). Among these, lymph node metastasis showed a stepwise increase in BM risk with advancing N stage (vs. N0: N1, OR=4.24; N2, OR=10.01; N3, OR=12.31; all P<0.001), and EGFR 19del was independently associated with elevated BM risk (OR=2.10, 95% CI: 1.25–3.51, P=0.005). All five serum biomarkers were also significantly associated with BM (all P<0.001; Table SIII). In the multivariate analysis, five variables were independently associated with BM: N stage (N1, N2 and N3 vs. N0), EGFR 19del, NSE, CEA and CYFRA21-1 (Table SIII). These results suggest that the EGFR 19del mutation is independently associated with an elevated risk of BM in LUAD, and that the concurrent elevation of serum NSE, CEA, CA125, CA153 and CYFRA21-1 reflects increased tumor burden at the time of BM development.

EGFR mutations combined with LC biomarkers for the prediction of BM in LUAD. Building on the above findings, the predictive performance of serum biomarkers for BM was further evaluated within EGFR mutation-defined subgroups. In the overall cohort, ROC curve analysis yielded AUC values of 0.709, 0.756, 0.739, 0.742 and 0.751 for NSE, CEA, CA125, CA153 and CYFRA21-1, respectively, as individual biomarkers (Fig. S3). Following stratification by EGFR mutation status, AUC values in the EGFR-mutated subgroup increased substantially for NSE, CEA and CA153, reaching 0.746, 0.781 and 0.800, respectively, while those for CA125 and CYFRA21-1 showed modest changes, reaching 0.753 and 0.763. Further stratification by the 19del subtype yielded additional AUC

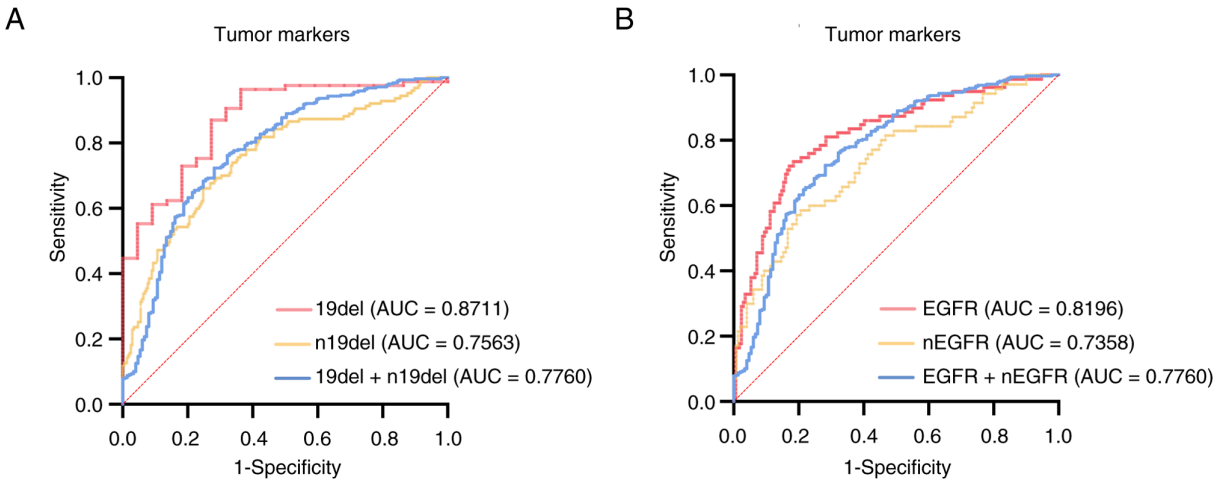

Figure 4. ROC curves of the combined method for the prediction of brain metastasis in lung adenocarcinoma. ROC curves of combined tumor markers, including NSE, CEA, CA125, CA153 and CYFRA21-1, were analyzed in subgroups of (A) 19del and (B) EGFR mutations. ROC, receiver operating characteristic; AUC, area under the ROC curve; NSE, neuron-specific enolase; CYFRA21-1, cytokeratin 19 fragment; 19del, exon 19 deletion.

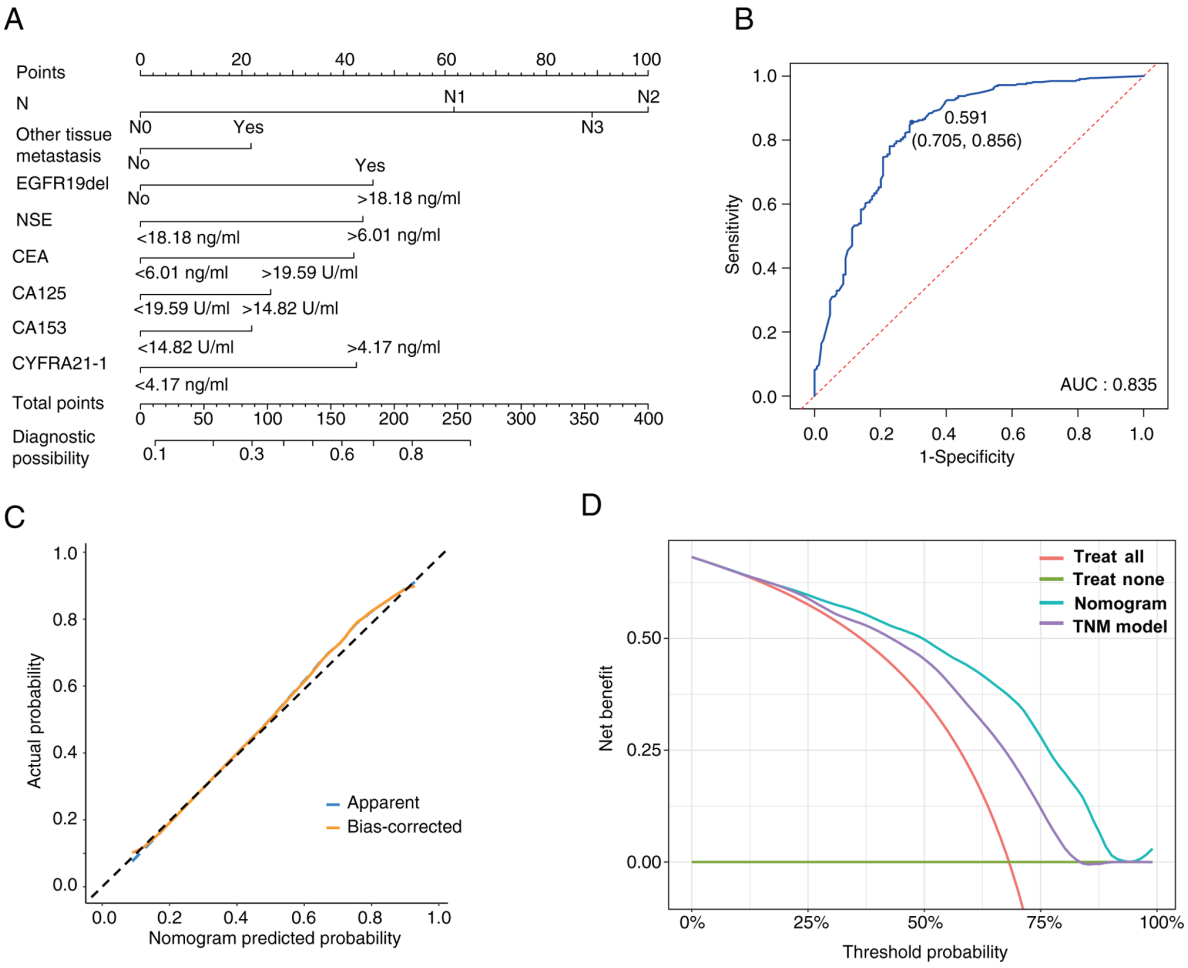

Figure 5. Construction and validation of the nomogram for BM. (A) Nomogram was constructed for predicting BM in patients with lung adenocarcinoma, and (B) ROC curves and (C) calibration plots were analyzed. (D) Decision curve analysis was used to evaluate the clinical value of the nomogram model and TNM model for the prediction of BM. ROC, receiver operating characteristic; AUC, area under the ROC curve; NSE, neuron-specific enolase; BM, brain metastasis; CYFRA21-1, cytokeratin 19 fragment; 19del, exon 19 deletion.

gains for CA125 and CA153 in the 19del subgroup, reaching 0.803 and 0.815, respectively, while AUC values for NSE, CEA and CYFRA21-1 showed modest changes, reaching 0.775, 0.750 and 0.772, respectively (Fig. 3). These results indicate that stratifying patients by EGFR mutation status improves the BM predictive performance of serum biomarkers compared

with biomarkers alone, and that further stratification by the 19del subtype yields additional gains for selected biomarkers. These findings suggest that distinct EGFR mutation subtypes exert differential effects on biomarker-based BM prediction in LUAD.

Combining biomarkers for the prediction of BM in LUAD. A combined approach incorporating EGFR mutation status and serum concentrations of NSE, CEA, CA125, CA153 and CYFRA21-1 was evaluated for BM prediction. ROC curve analysis of the combined approach in the overall cohort yielded an AUC of 0.776. Following stratification by EGFR mutation status, the AUC increased to 0.820 in the EGFR-mutated subgroup and further to 0.871 in the 19del subgroup (Fig. 4). These findings demonstrate that the combined approach outperforms individual biomarkers for BM prediction in LUAD, with the performance further enhanced in EGFR mutation-enriched subgroups.

Verification of the prediction formulation of BM in LUAD. Multivariate logistic regression analysis identified eight variables independently associated with BM in patients with LUAD, including N stage, other tissue metastasis, EGFR 19del, NSE, CEA, CA125, CA153 and CYFRA21-1. The resulting risk prediction formula was as follows: Logit (P)=-2.4028 + 1.0930(N1) + 1.7697(N2) + 1.5743(N3) + 0.3853 (other tissue metastasis) + 0.8107(EGFR 19del) + 0.7753 (NSE >18.18 ng/ml) + 0.7440 (CEA > 6.01 ng/ml) + 0.4539 (CA125 >19.59 U/ml) + 0.3879 (CA153 >14.82 U/ml) + 0.7526 (CYFRA21-1 >4.17 ng/ml), with an optimal probability cutoff of 0.591. A nomogram incorporating these eight variables was constructed for individualized BM risk prediction (Fig. 5A). In the 10-fold cross-validation, the nomogram achieved a mean AUC of 0.814, sensitivity of 0.564 and specificity of 0.906 across the 10 iterations (Table SIV). The nomogram achieved an AUC of 0.835 on the training set ROC curve (Fig. 5B). Calibration curves demonstrated close agreement between predicted and observed BM frequencies across the cohort, confirming good model calibration (Fig. 5C). DCA demonstrated that the nomogram provided greater net clinical benefit than the TNM staging model across a wide range of threshold probabilities (Fig. 5D). Furthermore, compared with the TNM-only model, the nomogram yielded an NRI of 0.210 (P<0.001) and an IDI of 0.147 (P<0.001), indicating that incorporation of EGFR 19del mutation status and the five serum biomarkers significantly improved the predictive performance over TNM staging alone.

Discussion

BM is a critical complication that significantly affects the survival and prognosis of patients with LC (2,17). Early identification and risk monitoring of BM may provide a critical window for timely intervention and help prevent disease progression in patients with LC (18,19). On the basis of its distinct pathological characteristics and high BM incidence, LUAD was selected as the study population. Given that EGFR mutations contribute to tumor development and guide targeted therapy selection, it was hypothesized that specific mutation subtypes may differentially influence BM risk and accordingly, a subtype-level analysis was conducted. To identify a clinically applicable marker for BM risk assessment, the EGFR mutation status was integrated with serum LC-related biomarkers to develop a combined predictive approach and its performance was evaluated in patients with LUAD. A nomogram incorporating the identified predictors was subsequently constructed and internally validated. In summary, this study aimed to identify reliable biomarkers for early BM risk stratification in LUAD and to develop a combined approach integrating qualitative and quantitative indicators for individualized BM prediction.

EGFR plays a central physiological role in regulating epithelial tissue development and homeostasis, and serves as a key driver of tumorigenesis, particularly in LC and glioblastoma (20-22). As a member of the ErbB family of receptor tyrosine kinases, EGFR regulates multiple downstream signaling pathways, including the Ras/MAPK, PI3K/AKT, phospholipase C/protein kinase C and STAT pathways (20,23,24). These EGFR-mediated pathways are critical regulators of cell proliferation, apoptosis, migration and survival-processes central to LC progression (23,25). Clinical studies have demonstrated that patients with EGFR-mutated NSCLC face a substantially elevated risk of developing BM (26-28). In one cohort study, the cumulative BM incidence among patients with EGFR-mutated NSCLC reached 47% at 3 years and 53% at 5 years (29). Gene-targeted therapies, such as EGFR TKIs, suppress EGFR-mediated signaling and have become a cornerstone of LC treatment (30). EGFR TKIs have demonstrated efficacy in controlling CNS progression and are recommended for the treatment of BM, including leptomeningeal metastases (31-33). The efficacy of EGFR TKIs against BM has been further supported by several landmark phase III trials, including FLAURA, AENEAS and FURLONG (34-36). Collectively, these findings underscore the pivotal role of EGFR signaling in BM development in LC. An epidemiological study reported that activating EGFR mutations are present in at least 50% of Asian patients with NSCLC (37). EGFR mutation status is increasingly recognized as a clinically important biomarker in LC (38). In the present study, the distribution of EGFR mutations was compared between BM and non-BM groups in a Chinese LUAD cohort of 615 patients and no significant difference in the overall proportion of EGFR-mutated patients was found between groups. This finding suggests that different EGFR mutation subtypes may exert distinct functional effects on BM development, as individual mutations differ in their impact on EGFR signaling activity. Therefore, precise subtype-level classification of EGFR mutations is essential for a more nuanced understanding of their respective roles in BM development.

The 19del is among the most common activating mutations in EGFR, accounting for 85-90% of sensitizing mutations, and is strongly associated with LUAD histology (39,40). The most prevalent 19del variants are clustered within the 746-750 amino acid range, while other EGFR mutation types include the L858R point mutation and uncommon variants such as G719S, L861Q and C-helix deletions (41-43). LUAD patients harboring common 19del variants demonstrate a favorable response to TKI treatment in ~75% of cases, along with superior progression-free and overall survival, although response rates are lower in patients with the rare C-helix deletion

subtype (44-46). Compared with patients harboring L858R or uncommon mutations, those with 19del mutations generally achieve better clinical outcomes (44,45). A study using 19del-mutant LC cell lines demonstrated that EGFR-TKIs effectively suppress EGFR pathway activation, including downstream AKT and ERK signaling (47). These findings indicate that the 19del mutation exerts distinct effects on EGFR function in the pathogenesis of LUAD. Clinical studies have further demonstrated that 19del may serve as a favorable prognostic biomarker in LUAD (40). However, BM represents a well-established indicator of poor prognosis in LUAD, and its relationship with the 19del mutation has remained to be elucidated. In the present study, it was found that patients with LUAD harboring 19del mutations had a significantly higher BM rate than those with other EGFR genotypes.

These findings suggest that 19del may enhance the invasive ability of LUAD cells. A prior *in vitro* study demonstrated that ectopic expression of mutated EGFR-including constructs harboring either the 19del or the L858R point mutation-induced epithelial-to-mesenchymal transition-like morphological changes and promoted cell migration in LUAD cells, effects not observed with wild-type EGFR; consistent with these findings, vimentin expression was significantly elevated in EGFR-mutated clinical tumor specimens compared with wild-type counterparts, supporting a biological basis for enhanced tumor cell dissemination (48). Notably, the two common EGFR mutation subtypes displayed comparable migratory potential *in vitro*, suggesting that differences in intrinsic invasive capacity alone may not fully account for the elevated BM risk associated with 19del observed in the present study. A complementary explanation is that the prolonged overall survival conferred by 19del mutations may extend the temporal window for intracranial dissemination and colonization, particularly given the limited blood-brain barrier penetration of first-generation EGFR-TKIs (48,49). At the molecular level, sustained activation of EGFR-mediated signaling pathways-including RAS/MAPK and PI3K/AKT-upregulates matrix metalloproteinase expression and promotes extracellular matrix degradation, potentially facilitating tumor cell transgression across the blood-brain barrier (49). The precise molecular mechanisms specifically linking 19del to elevated BM risk warrant dedicated investigation in future *in vitro* and *in vivo* studies (50). From a clinical perspective, the elevated BM risk associated with 19del appears to contradict the favorable prognosis previously reported for this mutation subtype; however, this discrepancy may be attributable to the pronounced efficacy of TKI therapy in 19del-mutated LUAD, whereby targeted treatment partially suppresses the invasive phenotype induced by this mutation, ultimately contributing to improved overall outcomes (51,52).

An ideal dynamic indicator for the real-time evaluation, prediction and monitoring of BM risk remains lacking in clinical practice. EGFR mutation status may assist in BM risk prediction in LUAD but cannot serve as a real-time quantitative monitoring index (53). Serum biomarkers-including NSE, CEA, CA125, CA153 and CYFRA21-1-have been employed as quantitative indices for LC risk assessment based on their measurable serum concentrations (54,55). Among single biomarkers-including CA-125, CA-199, CA-153, α-fetoprotein and CEA-CYFRA21-1 demonstrated the highest diagnostic performance for ocular metastasis in patients with LC with hypertension, achieving a sensitivity of 77.6%, specificity of 87.0% and AUC of 0.875 (56). Another study demonstrated that a combination of CA-125, CA-153 and CYFRA21-1 could facilitate the diagnosis of ocular metastasis in male patients with LC, achieving an AUC of 0.859 (57). In the context of BM, baseline serum concentrations of CEA, CYFRA21-1 and NSE, evaluated as individual indices, have also been associated with prognosis in patients with LC with BM (58,59). However, most prior studies focused on the predictive value of individual serum biomarkers for BM without incorporating the EGFR mutation genotype. In the present study, quantitative serum biomarker data were integrated with qualitative EGFR mutation genotype information to develop a combined approach for BM risk prediction. The results demonstrated that these serum biomarkers exhibited meaningful predictive value for BM in LUAD and their integration with EGFR mutation status-particularly 19del-yielded an improved predictive performance, as reflected by higher AUC values across subgroup analyses. These findings suggest that integrating EGFR mutation subtype information with serum biomarker profiling may provide a more precise approach for BM risk stratification in clinical practice.

Of note, the present study has several limitations. First, it was a single-center retrospective study in which matching was performed only for age and sex; prior treatment exposure before blood collection cannot be fully excluded in patients with metachronous BM, representing an inherent limitation of the retrospective design. Second, the timing of enhanced MRI was determined by attending clinicians based on individual clinical presentation rather than a standardized surveillance protocol, and the possibility of missed diagnoses in asymptomatic patients cannot be entirely excluded. Third, the sample size of the 19del subgroup was relatively limited (n=142), and subgroup-level ROC analyses should be interpreted as exploratory and hypothesis-generating, pending confirmation in larger prospective cohorts. Fourth, the model was validated internally through 10-fold cross-validation without an independent external validation cohort, which limits the definitive assessment of its generalizability. Fifth, this study was conducted in a Chinese cohort with a substantially higher EGFR mutation prevalence than Western populations, and the biomarker cutoff values were optimized using the Youden index in this cohort; both the model's applicability and the cutoff thresholds may require recalibration before use in populations with different genetic backgrounds, and prospective validation in multiethnic cohorts is warranted.

In conclusion, patients with LUAD harboring EGFR 19del mutations demonstrated a significantly elevated risk of BM compared with those carrying other mutation subtypes. Elevated serum concentrations of NSE, CEA, CA125, CA153 and CYFRA21-1 were associated with increased BM risk in patients with LUAD. The combined nomogram integrating EGFR 19del mutation status with five serum biomarkers demonstrated robust discriminatory performance and may serve as an effective tool for individualized BM risk stratification in LUAD. This approach offers a feasible framework combining quantitative serum indices with qualitative genetic information, and warrants prospective validation in multicenter cohorts to confirm its clinical applicability.

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Availability of data and materials

The data generated in this study may be requested from the corresponding author.

Authors' contributions

MM conceived and designed the study and interpreted the experiments. MM wrote the main manuscript text. HL and LW performed the data analysis and statistical modeling, constructed the nomogram and web-based calculator, and prepared the figures. XZ, YH, HD and ZL reviewed the medical records, extracted and verified the clinical and laboratory data, and maintained the study database. HL and MM checked and confirmed the authenticity of all the raw data. All authors reviewed the manuscript and have read and approved the final manuscript.

Ethics approval and consent to participate

The First Affiliated Hospital of Guangzhou Medical University Ethics Committee approved this study (approval no. GZMC2021-06-1237). All procedures were carried out in accordance with the hospital's policies. All participants provided written informed consent for the collection, processing and analysis of blood samples and clinical data for research and publication purposes.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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