Decreased complement 4d increases poor prognosis in patients with non-small cell lung cancer combined with gastrointestinal lymph node metastasis

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Abstract. Lung cancer is a common malignancy that is difficult to treat and has a high risk of mortality. Although gastrointestinal lymph node metastasis has long been known to exert major impact on the prognosis of lung cancer, the mechanism of its occurrence and potential biological markers remain elusive. Therefore, the present study retrospectively analyzed data from 132 patients with non-small cell lung cancer (NSCLC) combined with lymph node metastasis between February 2010 and April 2019 from the First Affiliated Hospital of Soochow University (Suzhou, China) and Sichuan Cancer Hospital (Chengdu, China). Overall survival was assessed using Kaplan-Meier analysis and Cox logistic regression model. In addition, a prediction model was constructed based on immune indicators such as complement C3b and C4d (measured by ELISA), before the accuracy of this model was validated using calibration curves for 5-year OS. Among the 132 included patients, a total of 92 (70.0%) succumbed to the disease within 5 years. Multifactorial analysis revealed that complement C3b deficiency increased the risk of mortality by nearly two-fold [hazard ratio (HR)=2.23; 95% CI=1.20-4.14; P=0.017], whilst complement C4d deficiency similarly increased the risk of mortality by two-fold (HR=2.14; 95% CI=1.14-4.00; P=0.012). The variables were subsequently screened using Cox model to construct a prediction model based on complement C3b and C4d levels before a Nomogram plotted. By internal validation for the 132 patients, the Nomogram accurately estimated the risk of mortality, with a corrected C-index of 0.810. External validation of the model in another 50 patients from Sichuan Cancer Hospital revealed an accuracy of 77.0%. Overall, this mortality risk prediction model constructed based on complement levels showed accuracy in assessing the prognosis of patients with metastatic NSCLC. Therefore, complement C3b and C4d have potential for use as biomarkers to predict the risk of mortality in such patients.

Introduction

Lung cancer is a major cause of mortality in middle-aged and elderly patients (1). In addition, it has one of the highest incidence rates among all tumor types (1). A total of 2.1 million newly diagnosed and 1.8 million deaths were reported in 2018 from 322 population-based registries in 71 countries, making it the number one cause of cancer-associated mortalities (2). Among all lung cancer subtypes, adenocarcinoma is the most invasive and heterogeneous, with an abnormally high tumor mutation burden (3). Despite advances in the development of lung cancer treatment methods over the past decade, the prevention, early diagnosis and management of patients with lung cancer remain challenging (4).

The complement pathway is an integral part of the innate immune system that serves to clear microbes and impaired cells by driving inflammation (5). This process in turn recruits innate and adaptive immune cells to attack the cell membrane of pathogens (6). Activation of the complement pathway serves an important role in the development of tumors (7). It can be activated by classical, lectin or alternative pathways, all of which converge onto lead the activation of C3b. For example, lung cancer could be activated by the classical complement pathway (6). This then forms the membrane attack complex to mediate cell lysis (7). Complement C3 and C4 are key components in this pathway that are important for complement activation (8). C3 is the mediator molecule in the process of complement activation, whilst C4 is the terminal by-product, the level of which provides an indication of complement activation in the body (8). Previous studies have revealed the

presence of complement-associated proteins such as complement factor H in the tumor microenvironment, in which tumor cells (such as lung cancer cells) can exhibit multiple effects (such as activating the complement) on complement proteins (9,10). The complement-activated lectin pathway plays an important role in human solid tumors, including those of the female reproductive system, the lungs and the digestive tract (11). Therefore, the present study selected these two molecules as markers.

Accumulating evidence suggest that the complement system can serve a role in tumor progression by promoting cancer cell angiogenesis, proliferation and antitumor immunity (11,12). The presence of data supporting complement activation and C5b-9 in deposition-related data in multiple types of malignancies, such as lung and pancreatic cancer, support this notion (13). To the best of our knowledge, Niculescu *et al* (14) first identified abnormal complement activation and elevated sC5b-9 levels in patients with breast cancer. However, the association between serum complement C3b and C4d levels and the prognosis of patients with NSCLC combined with lymph node metastasis remains unclear.

The present study examined serum complement C3b and C4d expression levels in patients with NSCLC combined with lymph node metastasis before exploring their potential as prognostic factors in such patients. A predictive model of mortality risk was constructed based on complement C3b and C4d expression levels. This was presented through Nomograms, which can be readily calculated and would provide a beneficial tool to support the decision-making of clinicians.

Materials and methods

Patients. The present study included data from 132 patients with NSCLC collected from the First Affiliated Hospital of Soochow University (Suzhou, China) and Sichuan Cancer Hospital (Chengdu, China) between February 1, 2010 and April 1, 2019. The median age of the patients was 65 years (range, 57-69 years), and the cohort included 45 (34.1%) men. In addition, data from 50 patients [mean age, 64.5±10.92; male, 16 (32.0%); female, 34 (68.0%)] with NSCLC from the Sichuan Cancer Hospital between June 2012 and May 2019 were collected as an external validation cohort for subsequent modeling with the same inclusion and exclusion criteria as for the 132 patients above. NSCLC was diagnosed by pathological analysis. Patients who lacked information on complement composition data, those with SLE and renal dysfunction, and those who withdrew from treatment or had missing follow-up information were excluded (Fig. 1). Patient data, including age, sex, serum carcinoembryonic antigen (CEA) levels, body mass index, albumin levels, lymphocyte count, C-reactive protein (CRP) level, neutrophils, hemoglobin, prognostic nutritional index (PNI), platelet count, neutrophil-lymphocyte ratio, surgery, staging of documented lung cancer, radiotherapy, tyrosine kinase inhibitor application, diabetes mellitus, Karnofsky performance status (KPS) score (15), smoking, heart failure, hyperlipidemia (plasma total cholesterol concentration >5.17 mmol/l OR plasma triacylglycerol concentration >2.3 mmol/l), were all selected for analysis. The data distributions of C3 and C4 were tested for normality and were revealed to be skewed by normality test. According to these statistical

principles, data from skewed distributions are suitable for analysis using the median (16). Therefore, the median was selected as the cut-off value of continuous variables. Informed written consent was obtained from all patients or their immediate family members. All research programs are in line with the guidelines of the Ethics Committee of Soochow University and followed the Declaration of Helsinki. Only the medical records of the 182 patients in total were collected from the hospital database.

The inclusion criteria were as follows: i) Patients can understand the study and agree to sign a written informed consent document; ii) patients are aged 18-75 years and must have a life expectancy of >3 months; iii) patients must have a confirmed histological or cytological diagnosis of NSCLC; iv) Eastern Cooperative Oncology Group score standard of 0-2; v) patients must have normal organ and marrow function within 2 weeks prior to the study. Normal organ and marrow function was defined as absolute neutrophil count >1,500/ml; platelets >100,000/ml; total bilirubin within normal institutional limits (1.71-17.1 μmol/l); aspartate transaminase/alanine aminotransferase <2.5X institutional upper limit of normal; creatinine ≤1.5X institutional upper limit of normal; and urine dipstick for proteinuria of <1+. If urine dipstick is >1+, a 24-h urine for protein must demonstrate <500 mg protein in 24 h to allow participation in the present study. Exclusion criteria: i) Women who were pregnant due to concerns their complement values may be affected by the fetus; ii) if during the treatment, a serious active infection from which an intravenous injection of antibiotics was required; iii) the patient has symptoms of brain metastases or suffers from severe mental or cognitive impairment; iv) patients who had congestive heart failure, arrhythmia, myocardial infarction, unstable angina, stroke or transient ischemic attack in 6 months; and v) patients with other malignancies within 5 years, except for those with cervical carcinoma *in situ*, skin squamous cell carcinoma of the skin or the basic control of skin basal cell carcinoma.

Complement C3b and C4d detection. Blood samples were collected from patients with NSCLC combined with lymph node metastasis at the time of diagnosis. Peripheral blood samples were collected and anti-coagulated with EDTA. Samples were then centrifuged at 800 x g for 10 min at room temperature to collect the supernatants. All blood plasma specimens were stored at -80°C in a specimen refrigerator for further study. Complement detection was performed within 3 days after plasma collection. According to the manufacturer's protocols, the complement C3b and C4d levels in plasma were detected using ELISA kits (cat. nos. WLS11421 and ZY-E67-44H; Shanghai Yuanye Biotechnology Co., Ltd.).

Statistical analysis. NCSS-PASS software version 10.0 (NCSS, LLC) was used for sample size assessment. Power was set to 0.99 and α to 0.5. P<0.05 was considered to indicate a statistically significant difference. Missing values (≤5.0%) were estimated by the random forest method using the 'mice' package (17) in RStudio (R version 3.5.0; RStudio, Inc.) (18). Categorical variables were represented as proportions and matched using the χ^2 test. Commonly and skewed distributed variables were presented as the median with interquartile range. Group comparisons were performed using either one-way
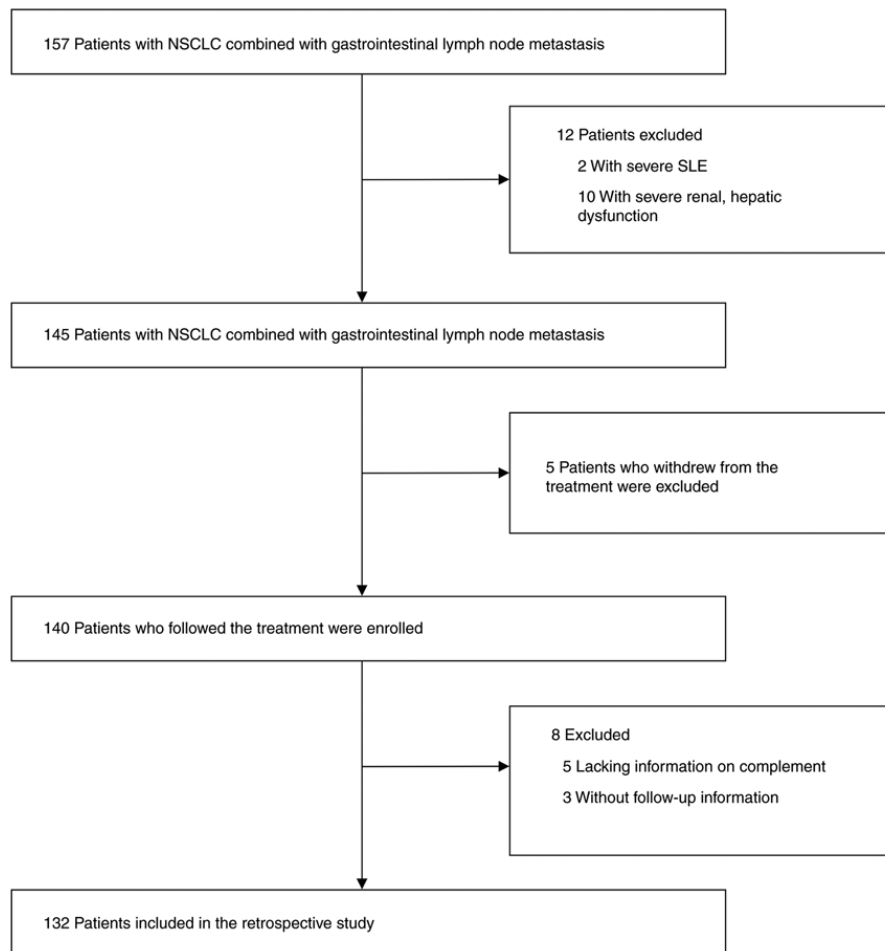
Figure 1. A flow chart for patient screening in the present study. Patients were screened for enrollment according to the details in the flowchart. Patients who dropped out of therapy and those with missing crucial information were excluded, resulting in 132 cases being enrolled into the present study. SLE, systemic lupus erythematosus.

ANOVA or Kruskal-Wallis test followed by Tukey's test for each of the pairwise comparisons.

Survival data was displayed by Kaplan-Meier (KM) curves on a cumulative basis and compared using a log-rank test. The univariate and multivariate survival responses of OS were adjusted using Cox regression models to estimate OS. Forest plots were used for the visualization of the importance of prognosis by the covariate. Using Harrell's regression modeling R package of 'rms' (R software, version 5.1-2; https://www.rdocumentation.org/ packages/rms/versions/5.1-2).

To establish the prognostic risk, risk factors were identified using Cox multifactor regression models (variants with P-values <0.05 were included in the model). The weight of each variant was quantified, before nomograms were generated and internal validation was performed using 1,001 bootstrapping (R version 3.5.0, 'rms' package) (19). A calibration test of 5-year OS to an ideal curve estimated the concordance of the derived model. Log-rank tests and KM curves were applied to analyze the associations of C3b and C4d with survival outcomes. Spearman's correlation test was performed to analyze the association between PNI and C3b as well as C4d. C-statistics was calculated by 'rms' package in R software. A dot plot was created based on the accuracy of the predictions, with different colors used to indicate correct and incorrect

predictions, and to calculate the percentage correct. Statistical analyses were performed using RStudio (R version 3.5.0) with the following R packages: 'rms', 'ggplot2', 'risk regression', 'PredictABLE' and 'survminer' (20-22).

Results

Baseline characteristics. The characteristics of the patients included in the present study are listed in Table I. Specifically, the present study included 132 patients who suffered from NSCLC combined with lymph node metastasis diagnosed between February 2010 and April 2019. By the follow-up endpoint (December 2021), the overall mortality rate was 70.0%. The median serum CEA and CRP levels were 8.28 ng/ml and 3.80 μmol/l, respectively. A total of 23 (17.4%) patients in the included population were diagnosed with stage I, 19 (14.3%) with stage II, 30 (22.7%) with stage III and 60 (45.4%) with stage IV according to TNM staging (23). In terms of treatment, 59 (45.0%) patients underwent surgery and 34 (26.0%) patients received radiation therapy. The KPS score was also evaluated, with 116 (88.0%) patients obtaining a score of ≥80. The comorbidities of these patients were also examined. There were 12 (9.0%) with type II diabetes mellitus and nine (7.0%) cases of hyperlipidemia. A total of 52 (39.0%)

Table I. Study participant characteristics at enrollment.

Variables	Total (n=132)	Stage I (n=23)	Stage II (n=19)	Stage III (n=30)	Stage IV (n=60)	P-value
Median age (IQR), years	65.00 (57.00-69.00)	66.00 (61.00-71.00)	63.00 (59.50-66.50)	65.00 (58.25-69.00)	63.00 (53.75-69.00)	0.5
Sex, n (%)						0.101
Male	45 (34)	11 (48)	9 (47)	6 (20)	19 (32)	
Female	87 (66)	12 (52)	10 (53)	24 (80)	41 (68)	
Surgery, n (%)						<0.001
No	73 (55)	5 (22)	4 (21)	14 (47)	50 (83)	
Yes	59 (45)	18 (78)	15 (79)	16 (53)	10 (17)	
Radiation, n (%)						0.426
No	98 (74)	19 (83)	15 (79)	19 (63)	45 (75)	
Yes	34 (26)	4 (17)	4 (21)	11 (37)	15 (25)	
Chemotherapy, n (%)						0.262
AP	106 (80)	22 (96)	16 (84)	22 (73)	46 (77)	
DP	17 (13)	1 (4)	1 (5)	6 (20)	9 (15)	
EP	5 (4)	0 (0)	1 (5)	0 (0)	4 (7)	
GP	1 (1)	0 (0)	0 (0)	0 (0)	1 (2)	
NP	1 (1)	0 (0)	0 (0)	1 (3)	0 (0)	
TP	2 (2)	0 (0)	1 (5)	1 (3)	0 (0)	
Target therapy (tyrosine kinase inhibitors), n (%)						0.082
No	88 (67)	20 (87)	14 (74)	19 (63)	35 (58)	
Yes	44 (33)	3 (13)	5 (26)	11 (37)	25 (42)	
Karnofsky Performance Status, n (%)						0.13
50	2 (2)	0 (0)	1 (5)	0 (0)	1 (2)	
60	3 (2)	0 (0)	0 (0)	1 (3)	2 (3)	
70	11 (8)	2 (9)	1 (5)	1 (3)	7 (12)	
80	21 (16)	1 (4)	4 (21)	3 (10)	13 (22)	
90	51 (39)	8 (35)	4 (21)	14 (47)	25 (42)	
100	44 (33)	12 (52)	9 (47)	11 (37)	12 (20)	
Smoking, n (%)						0.014
No	68 (52)	15 (65)	15 (79)	12 (40)	26 (43)	
Yes	64 (48)	8 (35)	4 (21)	18 (60)	34 (57)	
Hypertension, n (%)						0.103
No	80 (61)	13 (57)	10 (53)	14 (47)	43 (72)	
Yes	52 (39)	10 (43)	9 (47)	16 (53)	17 (28)	

Table I. Continued.

Variables	Total (n=132)	Stage I (n=23)	Stage II (n=19)	Stage III (n=30)	Stage IV (n=60)	P-value
Diabetes, n (%)						0.016
No	120 (91)	17 (74)	19 (100)	27 (90)	57 (95)	
Yes	12 (9)	6 (26)	0 (0)	3 (10)	3 (5)	
Hypertlipemia, n (%)						0.315
No	123 (93)	20 (87)	18 (95)	27 (90)	58 (97)	
Yes	9 (7)	3 (13)	1 (5)	3 (10)	2 (3)	
OS Status, n (%)						<0.001
Alive	40 (30)	17 (74)	13 (68)	3 (10)	7 (12)	
Deceased	92 (70)	6 (26)	6 (32)	27 (90)	53 (88)	
Median OS time (IQR), months	25.10 (10.65-61.30)	61.30 (23.70-73.80)	61.50 (50.30-69.45)	26.00 (11.10-51.45)[a,b]	14.20 (8.23-34.95)[c,d]	<0.001
Mean ± SD body mass index	23.03±3.25	23.18±3.02	23.10±3.16	22.56±3.46	23.19±3.30	0.842[e]
Median (IQR) serum carcinoembryonic antigen, ng/ml	8.28 (2.57, 39.05)	6.96 (2.19, 51.80)	6.02 (2.85, 27.73)	3.89 (2.18, 16.64)	11.30 (2.84, 40.16)	0.334[f]
Mean ± SD C-reactive protein, μmol/l	5.98±5.46	5.19±5.87	2.14±3.05	5.24±5.66	7.68±5.09	<0.001[e]
Mean ± SD serum albumin, g/l	40.95±4.86	41.66±4.93	43.15±5.33	41.25±4.82	39.84±4.50	0.052[e]
Median (IQR) neutrophils, 10⁹/l	4.34 (3.32-5.53)	3.45 (3.05-4.34)	4.44 (2.67-5.05)	3.98 (3.57-4.64)	5.01 (3.66-6.38)[c]	0.006[f]
Median (IQR) lymphocytes, 10⁹/l	1.77 (1.26-2.33)	2.31 (1.75- 2.52)	2.34 (2.00-2.58)	1.56 (1.21-1.99)[a,b]	1.54 (1.10-1.95)[c,d]	<0.001[e]
Mean ± SD hemoglobin, g/l	132.98±16.72	132.39±18.23	136.11±15.17	131.70±17.76	132.85±16.35	0.836[e]
Median (IQR) platelets, 10⁹/l	216.00 (174.00-259.25)	207.00 (154.50-240.50)	204.00 (190.50-243.00)	222.00 (187.75-252.50)	219.50 (171.00-272.00)	0.696[f]
Median (IQR) prognostic nutritional index	48.85 (44.95-53.38)	47.45 (45.27-54.67)	51.40 (48.17-55.08)	49.55 (45.01-53.24)	48.05 (44.83-51.46)	0.162[f]
Median (IQR) neutrophil lymphocyte ratio	2.52 (1.71-3.95)	1.71 (1.16-2.21)	2.05 (1.22-2.58)	2.45 (1.82-3.65)	3.27 (2.24-4.90)[c]	<0.005[e]
Median (IQR) C3, μmol/l	366.10 (201.32-448.69)	461.85 (374.92-500.84)	444.03 (395.73-498.42)	247.26 (171.94-405.44)[a,b]	316.56 (186.92-404.25)[c,d]	<0.001[e]
Median (IQR) C4, μmol/l	408.56 (315.79-652.06)	665.84 (605.91-704.94)	642.61 (480.41-672.95)	411.74 (365.68-574.30)	331.41 (278.67-482.72)[c,d]	<0.001[e]

[a]$P<0.05$ stage III vs. I. [b]$P<0.05$ stage III vs. II. [c]$P<0.05$ stage IV vs. I. [d]$P<0.05$ stage IV vs. II. [e]One-way ANOVA. [f]Kruskal-Wallis. IQR, interquartile range; BMI, Body Mass Index; OS, overall survival; C3, complement C3; C4, complement C4; AP, pemetrexed + cis-platinum; DP docetaxel + cis-platinum; EP, etoposide + cis-platinum; GP, gemcitabine + cis-platinum; NP, vinorelbine + cis-platinum; TP, paclitaxel + cis-platinum.

Table II. Cox regression analysis of hazard ratios in terms of patients with NSCLC with digestive disease (univariate analysis).

Variation	Non-adjustment		Model 1+	
	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
Age, ≥65 vs. <65 years	1.15 (0.76-1.73)	0.507	-	-
Sex, male vs. female	1.77 (1.13-2.78)	0.013	-	-
Surgery, yes vs. no	0.26 (0.17-0.41)	<0.001	0.27 (0.17-0.43)	<0.001
Radiation therapy, yes vs. no	1.21 (0.77-1.88)	0.405	1.26 (0.80-1.96)	0.315
Target therapy, yes vs. no	1.13 (0.74-1.73)	0.561	1.06 (0.69-1.65)	0.784
Smoking, yes vs. no	2.28 (1.50-3.47)	<0.001	2.17 (1.26-3.74)	0.005
Hypertension, yes vs. no	1.23 (0.81-1.86)	0.328	1.23 (0.80-1.89)	0.356
Diabetes, yes vs. no	1.15 (0.55-2.37)	0.714	1.29 (0.62-2.69)	0.492
Hyperlipemia, yes vs. no	0.73 (0.32-1.66)	0.45	0.72 (0.31-1.68)	0.451
Body mass index, <24.0 vs. ≥24.0	0.88 (0.58-1.35)	0.572	0.83 (0.54-1.27)	0.39
Stage of non-small cell lung cancer, IV+III vs. II+I	5.98 (3.23-11.07)	<0.001	5.90 (3.16-11.03)	<0.001
Serum carcinoembryonic antigen level, >8.28 ng/ml vs. ≤8.28 ng/ml	1.09 (0.73-1.65)	0.665	1.13 (0.75-1.70)	0.565
Serum C-reactive protein level, >3.80 μmol/l vs. ≤3.80 μmol/l	3.10 (2.01-4.78)	<0.001	2.90 (1.87-4.50)	<0.001
Chemotherapy, AP vs. others	0.64 (0.39-1.04)	0.07	0.67 (0.41-1.11)	0.121
Albumin level, >40.95 g/l vs. ≤40.95 g/l	0.45 (0.30-0.69)	<0.001	0.44 (0.29-0.68)	<0.001
Neutrophils count, >4.34x10^9/l vs. ≤4.34x10^9/l	2.03 (1.34-3.08)	0.001	2.07 (1.37-3.15)	0.001
Lymphocytes count, >1.77x10^9/l vs. ≤1.77x10^9/l	0.27 (0.17-0.43)	<0.001	0.28 (0.18-0.45)	<0.001
Hemoglobin level, >133 g/l vs. ≤133 g/l	0.72 (0.47-1.08)	0.114	0.54 (0.35-0.85)	0.008
Platelet count, >216x10^9/l vs. ≤216x10^9/l	1.66 (1.10-2.51)	0.017	1.73 (1.14-2.62)	0.011
Prognostic nutritional index score, >48.9 vs. ≤48.9	0.58 (0.38-0.88)	0.01	0.54 (0.35-0.82)	0.004
Neutrophil lymphocyte ratio, >2.52 vs. ≤2.52	3.62 (2.33-5.62)	<0.001	3.49 (2.25-5.44)	<0.001
Complement C4 level, ≤408.56 vs. >408.56 μmol/l	5.51 (3.43-8.84)	<0.001	5.52 (3.41-8.93)	<0.001
Complement C3 level, ≤366.10 vs. >366.10 μmol/l	3.96 (2.53-6.20)	<0.001	3.76 (2.38-5.92)	<0.001
Karnofsky Performance Status, ≥80 vs. <80	0.45 (0.26-0.79)	0.005	0.45 (0.25-0.79)	0.005

Model 1+, adjusted by age and sex. AP, pemetrexed + cis-platinum.

patients had hypertension. In addition, 64 (48.0%) of all patients were smokers.

Regression analysis. According to single-factor analysis, C3b levels (≤366.10 μmol/l, median) were a predictor of cancer-associated mortality [hazard ratio (HR) 3.96; 95% confidence interval (CI) 2.53-6.20; P<0.001; Table II]. KM curves revealed that those in the low C3b group had an increased cumulative incidence of mortality compared with patients in their high-level group (log-rank P<0.001; Fig. 2A). In addition, patients with low C4d levels (≤408.56 μmol/l, median) demonstrated a higher incidence of mortality on the survival curve compared with patients in the high-level group (P<0.001; Fig. 2B). The correlation between complement C3b/C4d levels and neutrophil-lymphocyte ratio (NLR) levels was next investigated as both were continuous variables, but no statistically significant correlation could be found (Fig. 2C and D).

Subsequently, Besides C3b and C4d, albumin level, sex, PNI score, neutrophil and platelet counts, NLR, NSCLC stage, surgery, KPS score and smoking status were associated with mortality (Table II). After correction for age and sex, patients with low C3b and C4d also exhibited a higher mortality incidence compared with those with high C3b and C4d levels.

Complement C3b levels (HR, 2.23; 95% CI, 1.20-4.14; P=0.012) and C4d levels (HR, 2.14; 95% CI, 1.14-4.00; P=0.017) were also positively associated with the risk of mortality after correction by Cox multifactorial regression analysis (Table III). In addition, surgery, albumin level and PNI score were independent risk factors for OS in patients with NSCLC.

Predictive model construction and validation. Subsequently, the independent risk factors (factors that were statistically significant after correction for multi-factor COX regression analysis) calculated by the multi-factor analysis were used to construct a prognostic model for mortality risk from NSCLC, using a Nomogram (Fig. 3A). This predictive model was validated internally using the bootstrap validation method. For validation, nomogram had a C-statistics (effect sizes that reflect prediction accuracy) of 0.810 for predicting mortality risk. In the validation cohort of 50 patients, the nomogram had
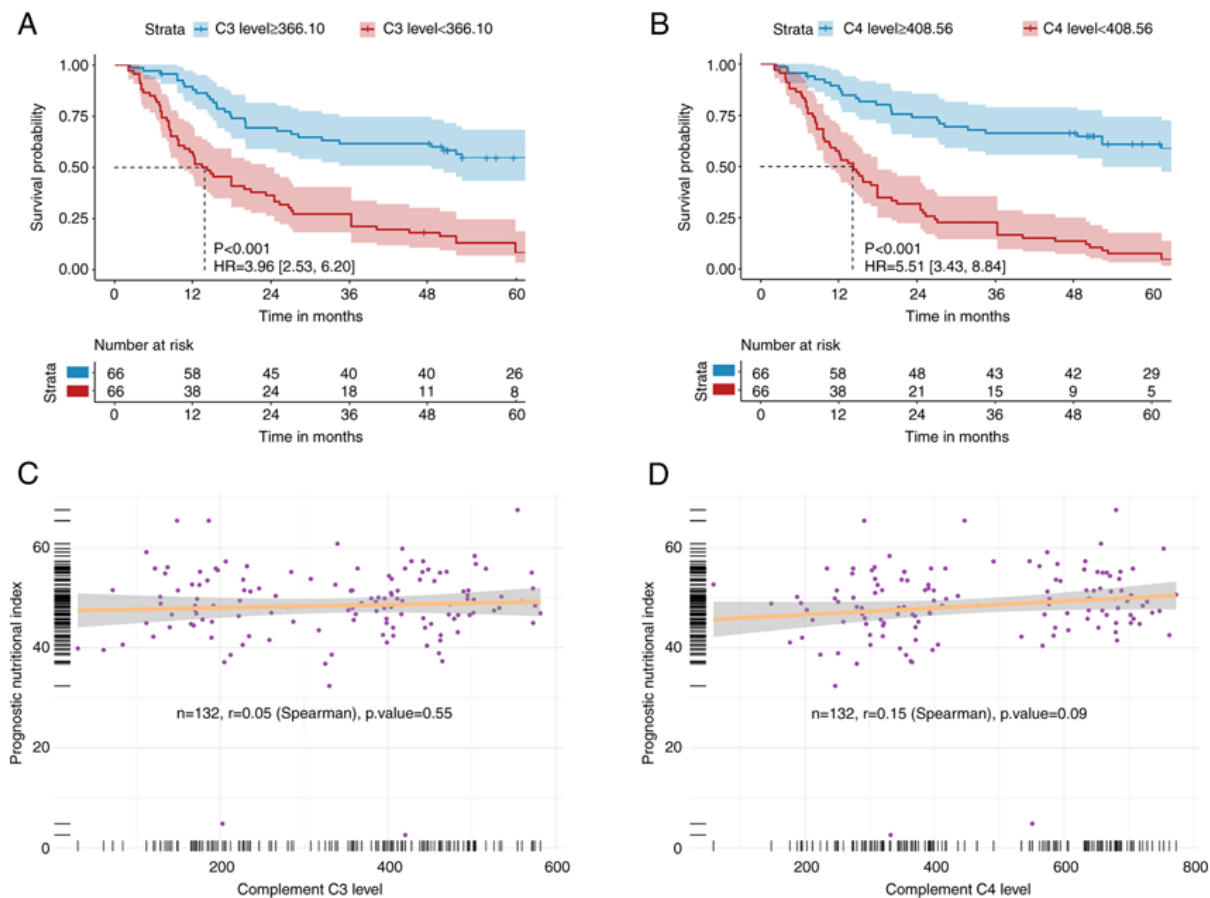
Figure 2. Kaplan-Meier curves for different groups of complement levels in patients with NSCLC combined with gastrointestinal lymph node metastasis. (A) Kaplan-Meier curves for overall survival of patients with different levels of C3b. (B) Kaplan-Meier curves for overall survival of patients with different levels of C4d. (C) Correlation curves between NLR and complement C3b. (D) Correlation curves between NLR and complement C4d. NSCLC, non-small-cell lung cancer; C3b, complement C3b; C4d, complement C4d; NLR, neutrophil-lymphocyte ratio.

an estimated C-statistics of 0.810 for OS, which also demonstrated a suitable calibration curve in estimation in Fig. 3B. Overall, 50 patients were collected from both hospitals as the external validation cohort for the model. The validation revealed that the prediction accuracy of the present constructed model was 77.0% (the number of correct predictions divided by the total number of points) (Fig. S1). Lower complement levels of C4d were revealed in patients with gastrointestinal lymph node metastases compared with those in patients without metastases in these 50 validated cases (362.1±117.3 vs. 584.5±136.7; P<0.05).

Discussion

The present study examined the levels of complement proteins, namely complement C3b and C4d, in patients with NSCLC combined with gastrointestinal lymph node metastasis between February 2010 and April 2019. Patients in the low-level C3b or C4d expression group displayed a lower OS compared with patients in the low-level group. Multivariate estimation revealed that C3b and C4d levels were independent risk factors for overall mortality. Subsequently, the independent risk factors (C3b, C4d, surgery, albumin level and PNI score) calculated using this multivariate analysis were incorporated into a predictive mortality risk model, specifically

as a nomogram. After internal validation, it was found to be accurate in predicting the mortality possibility.

Tumor development is a complex biological process that involves numerous genes (24). During this process, the immune system serves an important role (25). Complement is a part of the immune system that connects the adaptive and innate immune responses (26). Previous studies have revealed that the complement system is involved in the development of lung and pancreatic cancer, as well as metastasis (27,28). Osther et al (29) previously indicated that the complement system may be activated through the lectin pathway in patients with pancreatic cancer. It has also been revealed that complement-converting enzymes C5 and C3b are involved in lung cancer development, since these two mediators may affect all three known routes of complement activation pathways (30,31). CRP is an important biological marker of inflammation, which in turn correlates with complement activation (21). Therefore, increased CRP levels are frequently accompanied with increased complement activation and C4 levels (32).

Previous studies have demonstrated that a number of complement components can be used as biomarkers for lung cancer diagnosis and determination of prognosis (33,34). Complement components have recently been regarded as biomarkers in predicting mortality risk in NSCLC (35). Oner et al (36) demonstrated that C3b and C4d levels are

Table III. Multivariate analysis of the different risk factors for overall survival.

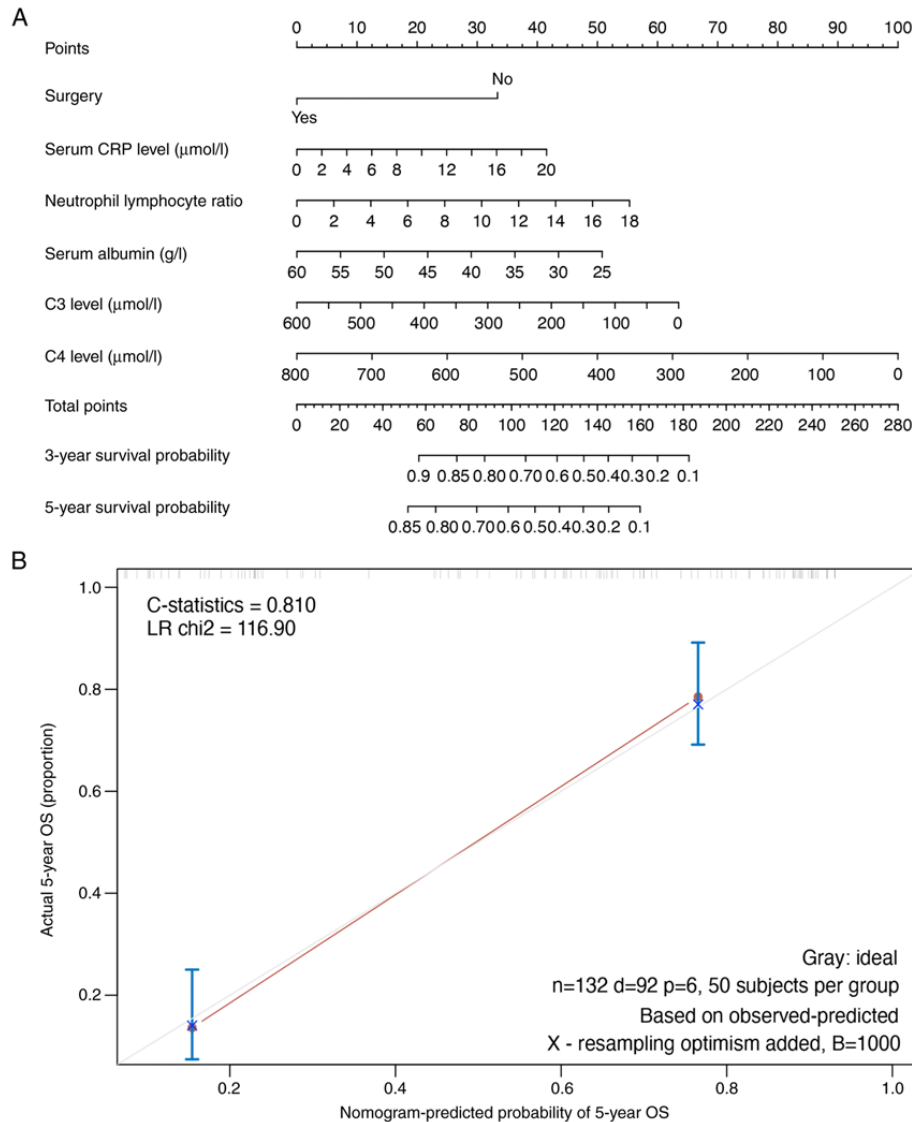| Variation	Hazard ratio (95% CI)	P-value
Sex, male vs. female	1.23 (0.61-2.46)	0.568
Surgery, yes vs. no	0.33 (0.18-0.60)	<0.001
Smoking, yes vs. no	1.22 (0.66-2.24)	0.524
Stage of non-small cell lung cancer, IV+III vs. II+I	1.34 (0.64-2.80)	0.436
Serum C-reactive protein level, >3.80 μmol/l vs. ≤3.80 μmol/l	1.67 (0.91-3.05)	0.097
Albumin level, >40.95 μmol/l vs. ≤40.95 μmol/l	0.48 (0.25-0.91)	0.026
Neutrophils count, >4.34x10^9/l vs. ≤4.34x10^9/l	0.92 (0.52-1.65)	0.786
Lymphocytes count, >1.77x10^9/l vs. ≤1.77x10^9/l	0.55 (0.30-1.01)	0.052
Platelet count, >216x10^9/l vs. ≤216x10^9/l	0.97 (0.61-1.54)	0.905
Prognostic nutritional index score, >48.9 vs. ≤48.9	1.94 (1.03-3.67)	0.042
Neutrophil lymphocyte ratio, >2.52 vs. ≤2.52	1.08 (0.53-2.21)	0.823
Complement C4 level, ≤408.56 vs. >408.56 μmol/l	2.14 (1.14-4.00)	0.017
Complement C3 level, ≤366.10 vs. >366.10 μmol/l	2.23 (1.20-4.14)	0.012
Karnofsky Performance Status, ≥80 vs. <80	0.69 (0.36-1.32)	0.266

Figure 3. Nomogram for predicting the risk of mortality in patients with NSCLC combined with gastrointestinal lymph node metastasis. (A) Nomogram for predicting the risk of mortality in patients. (B) Validity of the calibration curve in estimating patient prognosis. C3, complement C3; C4, complement C4; CRP, C-reactive protein; OS, overall survival.

aberrantly expressed in patients with lung cancer, which were then proposed to be biomarkers for long-term survival prediction in these patients. As a component of the complement component, sC5b-9 has been used as a therapeutic target in various complement activation-related diseases, such as thrombosis and viral infections (37-39). A number of studies have revealed that aberrant complement activation accompanied by elevated sC5b-9 levels can be seen in infection and inflammation (40,41). However, to the best of our knowledge, few studies have examined complement components C3b and C4d as potential indicators of disease prognosis in cancer, especially NSCLC combined with lymph node metastasis. Therefore, the present study attempted to test the viability of complement C3b and C4d as potential biomarkers to predict the long-term risk of mortality in patients with NSCLC combined with lymph node metastasis.

The present study first examined the expression levels of complement C3b and C4d in patients. Univariate analysis first demonstrated that low levels of complement C3b and C4d were strong predictors of cancer-associated mortality. In addition, sex, serum CRP, albumin, neutrophil, platelet count, PNI, NLR, lung cancer stage, surgery, smoking and KPS scores were associated with mortality. Subsequent multivariate analysis indicated that C3b, C4d, surgery, albumin and PNI were independent risk factors of NSCLC.

Nomograms are intuitive methods for visualizing risk prediction models (42,43). They have been widely used to predict survival and tumorigenesis risk (44,45). Recently, several studies have successfully developed risk prediction models combining miRNA expression levels with different clinical indicators of colon or esophageal cancer (46-48). However, few studies have used complement levels combined with other clinical risk factors of patients with lung cancer to build prognostic models. The present study developed a risk model capable of individualizing the long-term predictive risk of patients with lung cancer based on C3b and C4d and a number of clinicopathological characteristics. The model displayed accuracy in assessing the mortality possibility in patients. Therefore, to the best of our knowledge, this is the first prediction model that considered clinicopathological variables parallel to complement levels. Depending on this model, high-risk, low-survival patients at high risk can be selected for specific therapies.

There are limitations with the present study. The role of complement C3b and C4d needs to be validated in *in vitro* experiments. Therefore, studies on the molecular mechanisms of complement activation in patients with NSCLC combined with lymph node metastasis should also be continued. The predictive map also needs to be validated using a larger sample size. In addition, a prospective study should be launched before predictive models can be carried out.

In conclusion, the present study demonstrated that complement C3b and C4d are independent risk factors for the prediction of mortality in patients with NSCLC combined with gastrointestinal lymph node metastasis. In addition, a nomogram based on C3b and C4d levels was demonstrated to be accurate for assessing overall mortality.

Availability of data and materials

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

Authors' contributions

YW conducted the interpretation and analysis of data. YL conceived the study. MX and HZ analyzed the data. YL and YW confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Consent was obtained from all patients or their immediate family members. All research programs are in line with the guidelines of the Ethics Committee of Soochow University and follow the Declaration of Helsinki.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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