Correlation analysis of serum cystatin C, uric acid and lactate dehydrogenase levels before chemotherapy on the prognosis of small-cell lung cancer

HAOCHENG WANG, DONGFENG SHAN, YA DONG, XUE YANG, LINWEI ZHANG and ZHUANG YU

Department of Oncology, The Affiliated Hospital of Qingdao University, Qingdao, Shandong 266003, P.R. China

Received March 21, 2020; Accepted October 26, 2020

DOI: 10.3892/ol.2020.12334

Abstract. Related studies have reported that cystatin C (Cys C), uric acid (UA) and lactate dehydrogenase (LDH) affect tumor growth and invasion; however, the correlation between them and the prognosis of patients with small-cell lung cancer (SCLC) remains unclear. The present study aimed to investigate the effects of serum Cys C, UA and LDH concentrations on the prognosis of patients with SCLC prior to initial treatment, in order to identify potential targets for determining the clinical outcome of patients with SCLC. A total of 205 patients with SCLC were enrolled in the present study, and the clinical and laboratory data were obtained from the medical records. The receiver operating characteristic curve was used to determine the optimal cut-off values of Cys C, UA and LDH, while the Kaplan-Meier method was used for survival analysis. The Cox proportional hazard model was used for univariate and multivariate analyses to identify independent prognostic factors. The optimal cut-off values for Cys C, UA and LDH were 0.775 mg/l, 296.45 μmol/l and 198.5 U/l, respectively. The survival curves demonstrated that progression-free survival (PFS) and overall survival (OS) time were shorter in patients with high levels of Cys C, UA and LDH prior to chemotherapy. Univariate and multivariate analyses indicated that LDH concentration prior to chemotherapy may be an independent prognostic factor for both PFS and OS in patients with SCLC, while Cys C concentration may be an independent prognostic factor for PFS in patients with SCLC. The concentrations of Cys C, UA and LDH prior to chemotherapy were associated with prognosis of patients with SCLC. PFS and OS time were shorter, and the prognosis was poor in patients with elevated serum levels of Cys C, UA and LDH. Taken together, the results of the present study suggest that high concentrations of LDH and Cys C prior to chemotherapy may indicate rapid disease progression, thus it is important to focus on the progression and recurrence of the disease. High LDH concentration may also indicate a shorter survival time.

Correspondence to: Professor Zhuang Yu, Department of Oncology, The Affiliated Hospital of Qingdao University, 16 Jiangsu Road, Qingdao, Shandong 266003, P.R. China
E-mail: yuzhuang2002@163.com

Key words: SCLC, cystatin C, uric acid, lactate dehydrogenase, prognosis

Introduction

The incidence and mortality rates of lung cancer are one of the highest amongst all types of malignant tumors, which affects the physical and mental health of patients (1). According to the pathological subtypes, lung cancer is divided into non-small cell lung cancer (NSCLC) and small-cell lung cancer (SCLC), of which ~15% of all cases are SCLC. The common characteristics of this subtype include high malignancy, a short tumor doubling time, rapid growth, and strong invasive ability (1). Thus, patients with SCLC are prone to distant metastasis in the early stage and poor prognosis (2,3). Several histological and immunohistochemical markers are used in the diagnosis of SCLC, including thyroid transcription factor-1, cytokeratin 7, Leu-7, chromogranin A and synaptophysin (4). These tests have guided significance and are beneficial for the diagnosis of SCLC; however, they are expensive, the waiting time is long, and they are only used as diagnostic indicators, and so cannot be used to determine prognosis. Thus, it remains critical to identify sensitive and accurate indicators to assess prognosis and guide early clinical treatment.

Hematological assessment is extensively used in clinical settings due to its convenience, production of fast results, low costs, and low invasiveness. Currently, several studies have implemented serum indexes to predict the prognosis of patients with SCLC, including carcinoembryonic antigen, neuron specific enolase and gastrin releasing peptide precursor; however, their sensitivity and specificity are poor (5-7). Biochemical tests are commonly used for serum index tests, and the results of serum cystatin C (Cys C), uric acid (UA) and lactate dehydrogenase (LDH) are included in the biochemical tests.

Cys C is a non-glycosylated basic protein encoded by the CST3 gene (8). It has been hypothesized that Cys C may be overexpressed in tumor cells, resulting in increased circulating levels (8,9). UA is a product of xanthine oxidoreductase (XOR) oxidation between xanthine and hypoxanthine (10). Ames *et al* (10) hypothesized that UA is a key antioxidant that acts against different types of human cancer; however, Vona-Davis *et al* (11) proposed that the proinflammatory effect

of UA promotes the occurrence and development of cancer. LDH catalyzes the conversion of pyruvate and lactate during glycolysis and gluconeogenesis, respectively (12). A previous study has confirmed that increased glycolysis promotes the progression of malignant tumors (12).

It is well-known that Cys C, UA and LDH all affect tumor growth and invasion (11,13,14); however, the association between Cys C, UA and LDH and prognosis of patients with SCLC remains unclear. Thus, the present study aimed to investigate the association between serum concentrations of Cys C, UA and LDH prior to chemotherapy and the prognosis of patients with SCLC, with the potential to discover independent prognostic factors. The results presented here can be applied in clinical settings to assess patients at an early stage of the disease, and provide novel strategies for identifying the changes in SCLC.

Materials and methods

Patients. The present study was a retrospective analysis that selected patients pathologically diagnosis with SCLC at the Affiliated Hospital of Qingdao University between April 2015 and December 2018. The inclusion criteria were as follows: SCLC was diagnosed by fiberoptic bronchoscopy or puncture biopsy and no antitumor treatment was administered prior to diagnosis. The exclusion criteria were as follows: i) SCLC was diagnosed by postoperative pathology; ii) History of other malignancies; iii) Patients with renal insufficiency (serum creatinine, >1.5 times the upper limit of normal value or creatinine clearance rate <50 ml/min) and iv) Patients with liver dysfunction [alanine aminotransferase (ALT)/aspartate aminotransferase (AST) >2.5 times upper limit of the normal value, or ALT/AST >5 times upper limit of normal value in liver metastasis]. Based on these criteria, 230 patients were selected and followed-up. A total of 25 patients were lost to follow-up, with a loss rate of 10.87%; thus, 205 patients with SCLC were included in the final cohort, including 161 men and 44 women (mean age, 62 years; age range, 41-76 years). The present study was approved by the Ethics Committee of Qingdao University Hospital (Shandong, China; approval. no. QYFYWZLL25870) and informed consent was provided by all patients prior to the study start.

Data collection. The clinicopathological data of the patients were collected and analyzed at diagnosis, including sex, age, smoking status, primary tumor site, stage (according to Veterans Administration Lung Study Group) (15), Cys C, UA, LDH, urea nitrogen, creatinine, alkaline phosphatase, adenosine deamination enzymes, neutrophil to lymphocyte ratio and platelet to lymphocyte ratio. During follow-up, the treatment plan was assessed, and first-line chemotherapy was recorded. In addition, administration of radiotherapy and the imaging assessment results were also recorded. The clinicopathological characteristics of patients with SCLC are presented in Table I.

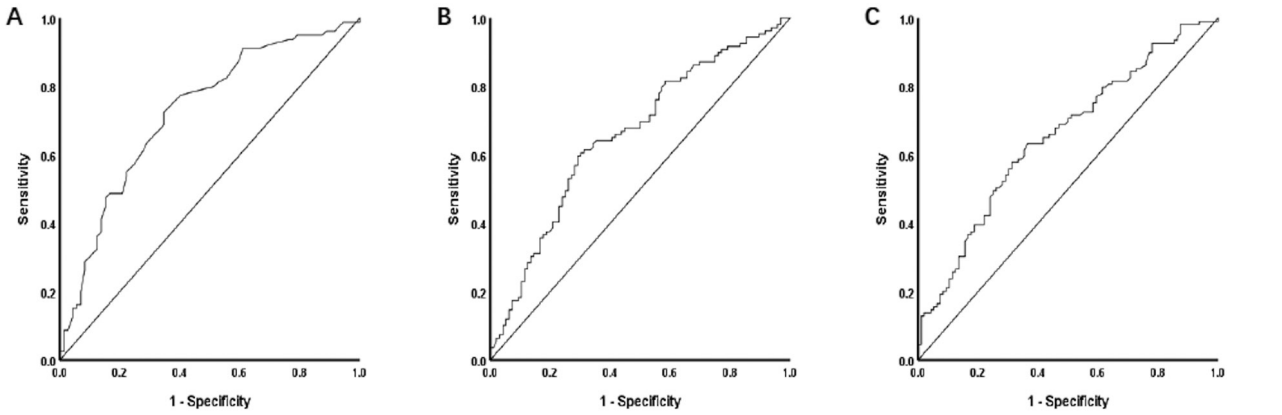
Progression-free survival (PFS) and overall survival (OS) were the observation indicators that were recorded. Follow-up methods included review of the electronic medical record system via the telephone. The follow-up period was between September 2019 to December 2019.

Table I. Clinicopathological characteristics of 205 patients with small-cell lung cancer.

Characteristic	Number of patients, % or median (range)
Sex	
Male	161, 78.54
Female	44, 21.46
Age, years	
<60	87, 42.44
≥60	118, 57.56
Smoking status	
Yes	142, 69.27
No	63, 30.73
Primary lesion	
Right lung	126, 61.46
Left lung	79, 38.54
Clinical stage	
LS	107, 52.20
ES	98, 47.80
First-line chemotherapy	
Etoposide and platinum	175, 85.37
Irinotecan and platinum	30, 14.63
Radiotherapy	
Yes	110, 53.66
No	95, 46.34
Cys C (mg/l)	0.79 (0.34-1.77)
UA (umol/l)	285.00 (77.30-579.00)
LDH (U/l)	199.00 (102.30-837.00)
Urea nitrogen (mmol/l)	5.10 (1.53-11.77)
Creatinine (umol/l)	80.00 (31.00-126.10)
Alkaline phosphatase (U/l)	76.00 (38.00-248.67)
Adenosine deaminase (U/l)	10.00 (2.00-26.00)
Neutrophil to lymphocyte ratio	2.44 (0.56-20.94)
Platelet to lymphocyte ratio	158.60 (55.21-516.19)

LS, limited stage; ES, extensive stage; Cys C, cystatin C; UA, uric acid; LDH, lactate dehydrogenase.

Statistical analysis. Statistical analysis was performed using SPSS v25.0 software (IBM Corp.). Receiver operating characteristic (ROC) curve and the area under the curve (AUC) graphs were constructed to determine diagnostic accuracy. The larger the area, the greater the diagnostic accuracy is to determine the optimal cut-off value for the serum index. Survival analysis was performed using the Kaplan-Meier method and log-rank test. The Cox proportional hazards model was used to identify factors associated with prognosis, while univariate and multivariate analyses was performed to identify independent prognostic factors. The χ^2 test was used to assess the association between UA and LDH concentrations and different disease stages. $P<0.05$ was considered to indicate a statistically significant difference.



AUC

Test Result Variable(s): Cys C

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% CI Lower Bound	Asymptotic 95% CI Upper Bound
.725	.041	.000	.644	.806

AUC

Test Result Variable(s): UA

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% CI Lower Bound	Asymptotic 95% CI Upper Bound
.658	.038	.000	.583	.733

AUC

Test Result Variable(s): LDH

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% CI Lower Bound	Asymptotic 95% CI Upper Bound
.653	.038	.000	.579	.728

Figure 1. ROC curves of serum Cys C, UA and LDH concentrations in patients with small-cell lung cancer, prior to chemotherapy. (A) ROC curve of serum Cys C concentration. (B) ROC curve of serum UA concentration. (C) ROC curve of serum LDH concentration. ROC, receiver operating characteristic; Cys C, cystatin C; UA, uric acid; LDH, lactate dehydrogenase; AUC, area under the curve; CI, confidence interval.

By consulting the literature, it was found that the mOS of patients with SCLC was 18 months (1). Disease progression is used as a grouping standard to draw ROC curve. The patients were divided into the disease progressive group and the non-progressive disease group. In addition, according to the cut-off value of each index, patients were also divided into the high value group and the low value group.

Results

Clinicopathological characteristics. As presented in Table I, 205 patients diagnosed with SCLC were enrolled in this retrospective study, including 161 men (78.54%) and 44 women (21.46%). A total of 87 patients (42.44%) were <60 years old, while 118 patients (57.56%) were ≥60 years old. In addition, 142 patients (69.27%) had a history of smoking, while 63 patients (30.73%) did not. Primary lesions in the right lung was observed in 126 patients (61.46%), while 79 patients (38.54%) had primary lesions in the left lung. At initial diagnosis, 107 patients (52.20%) had limited stage, while 98 patients (47.80%) had extensive stage SCLC. For the application of first-line chemotherapy following diagnosis, 175 patients (85.37%) were treated with etoposide and platinum, while 30 patients (14.63%) were treated with irinotecan and platinum. During treatment, 110 patients (53.66%) received chest radiotherapy, while 95 patients (46.34%) did not. Notably, detection of Cys C concentration was not included in the routine assessment of patients before July 2016 at the Affiliated Hospital of Qingdao University, thus Cys C concentration was only measured in 152/205 patients included. Data of the other indicators were collected from all 205 patients.

ROC and survival curve analyses. The optimal cut-off value for the serum index was determined according to the most approximate index, and the optimal sensitivity and specificity were exhibited. Disease progression was used as the grouping standard, and patients were divided into non-progressive disease, including 96 patients (46.83%) and disease progressive groups, including 109 patients (53.17%). For the ROC curve, the continuous variables, Cys C, UA and LDH were used, and the binary variable was whether the disease was progressing. The optimal cut-off value for serum Cys C was 0.775 mg/l (sensitivity, 0.725 and specificity, 0.653), while the AUC was 0.725 [95% confidence interval (CI), 0.644-0.806; P<0.001; Fig. 1A]. Based on these values, Cys C concentration was lower than the cut-off value in 69 patients (45.39%), who were classified into the low Cys C group, while Cys C concentration was higher than or equal to the cut-off value in 83 patients (54.61%), who were classified into the high Cys C group. The optimal cut-off value for UA was 296.45 μmol/l (sensitivity, 0.596 and specificity, 0.708), while the AUC was 0.658 (95% CI, 0.583-0.733; P<0.001; Fig. 1B). Based on these values, UA concentration was lower than the cut-off value in 112 patients (54.63%), who were classified into the low UA group, while UA concentration was higher than or equal to the cut-off value in 93 patients (45.37%), who were classified into the high UA group. The optimal cut-off value for LDH was 198.5 U/l (sensitivity, 0.633 and specificity, 0.635), while the AUC was 0.653 (95% CI, 0.579-0.728; P<0.001; Fig. 1C). Based on these values, LDH concentration was lower than the cut-off value in 101 patients (49.27%), who were classified into the low LDH group, while LDH concentration was higher than or equal to the cut-off value in 104 patients (50.73%), who were classified into the high LDH group (Table II).

Survival analysis was performed using the Kaplan-Meier method and log-rank test. The results demonstrated that the survival time of patients in the high Cys C group (≥0.775 mg/l) was significantly shorter compared with the low Cys C group (<0.775 mg/l), for both mean (m) PFS (5.70 months vs. 8.57 months; P<0.001) and mOS (14.67 months vs. 19.57 months; P<0.001) (Fig. 2A and B). Similarly, the survival time of patients in the high UA group (≥296.45 μmol/l)

Table II. Diagnostic value of ROC curves for Cys C, UA and LDH.

Variable	AUC	P-value	95% CI	Sensitivity	Specificity	Cut-off value	Low group, n (%)	High group, n (%)
Cys C	0.725	<0.001	0.644-0.806	0.725	0.653	0.775 mg/l	69 (45.39)	83 (54.61)
UA	0.658	<0.001	0.583-0.733	0.596	0.708	296.450 μmol/l	112 (54.63)	93 (45.37)
LDH	0.653	<0.001	0.579-0.728	0.633	0.635	198.500 U/l	101 (49.27)	104 (50.73)

ROC, receiver operating characteristic; AUC, area under the curve; Cys C, cystatin C; UA, uric acid; LDH, lactate dehydrogenase; CI, confidence interval.

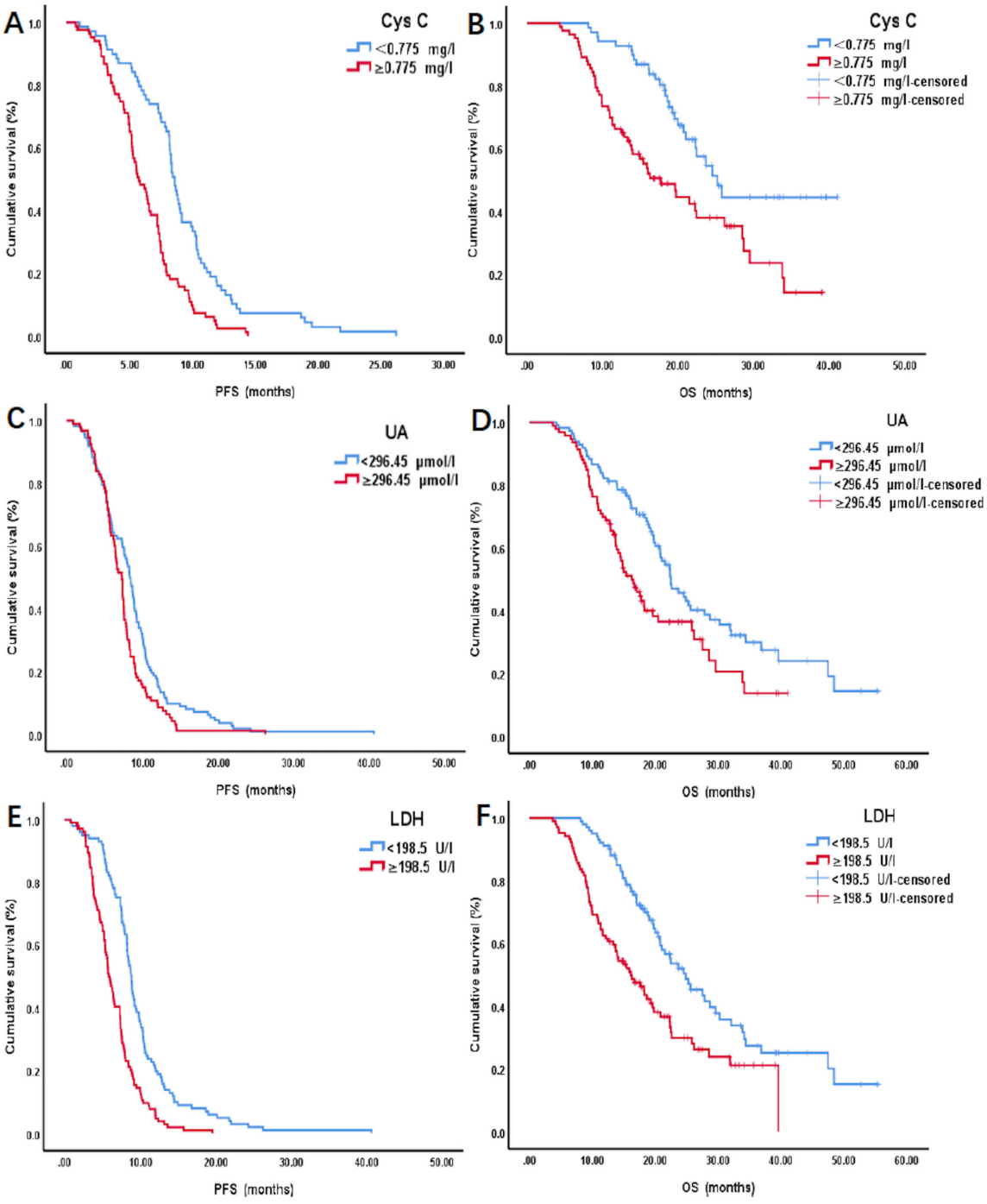


Figure 2. Kaplan-Meier survival curves of serum Cys C, UA and LDH concentrations, based on PFS and OS time of patients with small-cell lung cancer, prior to chemotherapy. (A) Survival curve of Cys C concentration and PFS. (B) Survival curve of Cys C concentration and OS. (C) Survival curve of UA concentration and PFS. (D) Survival curve of UA concentration and OS. (E) Survival curve of LDH concentration and PFS. (F) Survival curve of LDH concentration and OS. Cys C, cystatin C; UA, uric acid; LDH, lactate dehydrogenase; PFS, progression-free survival; OS, overall survival.

Table III. Survival rates of patients.

	OS (Low group)			OS (High group)		
Variable	>1 year, %	>2 years, %	>3 years, %	>1 year, %	>2 years, %	>3 years, %
Cys C	92.75	26.09	8.70	66.27	20.48	2.41
UA	82.14	31.25	10.71	69.89	17.20	4.30
LDH	91.09	32.67	12.87	62.50	17.31	2.88

OS, overall survival; Cys C, cystatin C; UA, uric acid; LDH, lactate dehydrogenase.

Table IV. Univariate analysis of clinicopathological characteristics of 205 patients with small-cell lung cancer.

	PFS			OS		
Variable	P-value	HR	95% CI	P-value	HR	95% CI
Sex						
Male vs. Female	0.079	0.741	0.530-1.035	0.137	0.724	0.473-1.109
Age, years						
<60 vs. ≥60	0.372	1.136	0.859-1.503	0.599	0.911	0.642-1.291
Smoking status						
Yes vs. No	0.013	0.685	0.507-0.924	0.011	0.598	0.402-0.890
Primary lesion						
Right lung vs. Left lung	0.552	1.089	0.822-1.445	0.345	0.840	0.585-1.206
Clinical stage						
LS vs. ES	<0.001	1.756	1.324-2.330	0.001	1.775	1.251-2.519
First-line chemotherapy						
Etoposide vs. Irinotecan	0.012	1.659	1.116-2.466	0.087	1.518	0.941-2.451
Radiotherapy						
Yes vs. No	<0.001	2.289	1.727-3.035	<0.001	2.054	1.442-2.926
Cys C	<0.001	3.649	1.792-7.430	0.020	3.171	1.198-8.390
UA	0.068	1.001	1.000-1.003	0.027	1.002	1.000-1.004
LDH	<0.001	1.005	1.004-1.007	<0.001	1.005	1.003-1.007
Urea nitrogen	0.930	1.000	1.000-1.001	0.250	1.000	1.000-1.001
Creatinine	0.714	1.002	0.993-1.011	0.615	1.003	0.991-1.051
Alkaline phosphatase	0.001	1.011	1.005-1.017	0.495	1.002	0.996-1.009
Adenosine deaminase	0.346	1.019	0.980-1.059	0.570	1.014	0.967-1.063
Neutrophil to lymphocyte ratio	0.909	1.003	0.956-1.052	0.382	1.026	0.968-1.088
Platelet to lymphocyte ratio	0.258	1.001	0.999-1.003	0.989	1.000	0.998-1.002

PFS, progression-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; LS, limited stage; ES, extensive stage; Cys C, cystatin C; UA, uric acid; LDH, lactate dehydrogenase.

was significantly shorter compared with the low UA group (<296.45 μmol/l), for both mPFS (6.67 vs. 8.27 months; P=0.010) and mOS (14.77 vs. 19.64 months; P=0.003) (Fig. 2C and D). In addition, the survival time of patients in the high LDH group (≥198.5 U/l) was significantly shorter compared with the low LDH group (<198.5 U/l), for both mPFS (5.75 vs. 8.73 months; P<0.001) and mOS (14.64 vs. 19.60 months; P<0.001) (Fig. 2E and F).

The 1-, 2- and 3-year survival rates of the patients were subsequently assessed. As presented in Table III, the survival rates of the low value groups were higher compared with the high value groups, and treatment in the low value groups was more effective compared with that in the high value groups.

Univariate and multivariate analyses. Univariate analysis of the clinicopathological characteristics demonstrated that PFS was significantly associated with: Smoking status (P=0.013), clinical stage (P<0.001), first-line chemotherapy (P=0.012), radiotherapy (P<0.001), Cys C (P<0.001), LDH (P<0.001) and alkaline phosphatase (P=0.001). In addition, OS was

Table V. Multivariate analysis of clinicopathological characteristics of 205 patients with small-cell lung cancer.

		PFS			OS		
Variable		P-value	HR	95% CI	P-value	HR	95% CI
Smoking status	Yes vs. No	0.033	0.675	0.471-0.968	0.271	0.750	0.449-1.252
Clinical stage	LS vs. ES	0.884	0.972	0.669-1.414	0.533	1.166	0.719-1.890
First-line chemotherapy	Etoposide vs. Irinotecan	0.478	1.184	0.742-1.891	-	-	-
Radiotherapy	Yes vs. No	<0.001	2.234	1.551-3.218	0.034	1.648	1.039-2.615
Cys C		0.005	3.153	1.413-7.037	0.256	1.954	0.615-6.213
UA		-	-	-	0.522	0.999	0.996-1.002
LDH		<0.001	1.004	1.002-1.005	<0.001	1.005	1.003-1.007
Alkaline phosphatase		<0.001	1.012	1.006-1.019	-	-	-

PFS, progression-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; LS, limited stage; ES, extensive stage; Cys C, cystatin C; UA, uric acid; LDH, lactate dehydrogenase.

Table VI. χ^2 test of different stages, and UA and LDH concentrations.

	UA				LDH			
Variable	Low group, n	High group, n	χ^2	P-value	Low group, n	High group, n	χ^2	P-value
LS-SCLC	67	40	5.755	0.016	64	43	9.957	0.002
ES-SCLC	45	53			37	61		

UA, uric acid; LDH, lactate dehydrogenase; LS, limited stage; ES, extensive stage; SCLC, small-cell lung cancer.

significantly associated with: Smoking status (P=0.011), clinical stage (P=0.001), radiotherapy (P<0.001), Cys C (P=0.020), UA (P=0.027) and LDH (P<0.001) (Table IV).

These factors were included in the multivariate analysis and the results demonstrated that smoking status (P=0.033), radiotherapy (P<0.001), Cys C (P=0.005), LDH (P<0.001) and alkaline phosphatase (P<0.001) were independent prognostic factors for PFS, while radiotherapy (P=0.034) and LDH (P<0.001) were independent prognostic factors for OS (Table V).

χ^2 test. Indicators such as LDH and UA are associated with tumor burden (14,16). The χ^2 test was used to assess the association between the concentrations of UA or LDH, and different disease stages. For UA, χ^2=5.755, P=0.016, the concentration was associated with the stage of the disease. The concentration will increase in the ES. For LDH, χ^2=9.957, P=0.002, the concentration was also associated with the stage of the disease. The concentration will increase in the ES.

Discussion

The results of the present study confirmed that elevated serum levels of Cys C, UA and LDH prior to chemotherapy were significantly associated with shorter PFS and OS times in patients with SCLC. The serum LDH concentration prior to chemotherapy may be an independent prognostic factor for PFS and OS in patients with SCLC, while Cys C concentration prior to chemotherapy may be independent prognostic factor for PFS in patients with SCLC.

LDH consists of two subunits, A and B, which are encoded by LDH-A and LDH-B, respectively (17-19). LDH-A has been identified as a direct target gene of c-Myc oncogenic transcription factor (17-19). LDH is a key enzyme in glycolysis and gluconeogenesis that catalyzes the mutual conversion of pyruvate and lactic acid; thus, it is essential for energy metabolism. LDH is released during tissue damage and has been reported to be involved in tumor growth, metastasis and metabolism (17). Previous studies have demonstrated that LDH concentration is an important prognostic factor for tumor progression and metastasis in different types of cancer, including colon, nasopharyngeal, breast, prostate, and germ cell cancers and melanoma (20-28).

In the present study, ROC curves were used to determine the optimal cut-off value of LDH concentrations prior to chemotherapy, and the patients were divided into high and low groups for survival analysis. The results demonstrated that patients in the high LDH group had significantly shorter mPFS and mOS times compared with patients in the low LDH group. Thus, increased LDH concentration prior to chemotherapy was associated with shorter PFS and OS times in patients with SCLC. Univariate and multivariate analyses demonstrated that LDH concentration prior to chemotherapy may be an independent prognostic factor. Increased LDH

concentration was associated with rapid disease progression, a short survival time and poor prognosis. This may be due to factors such as LDH, hypoxia-inducible factor 1 (HIF-1) and vascular endothelial growth factor (VEGF), which associate tumor metabolism with angiogenesis (14,29-31). Under the regulation of HIF-1 and VEGF, tumor cells rapidly proliferate and contribute to disease progression (14,29-31). In addition, previous studies focusing on malignant tumors have demonstrated that increased LDH levels are associated with the tolerance of patients to chemoradiotherapy (32,33). Taken together, these results suggest that the concentration of LDH prior chemotherapy is associated with the prognosis of patients with SCLC.

Cys C is a non-glycosylated basic protein encoded by the CST3 gene. Cys C is continuously transcribed and expressed in all nucleated cells (8). It is not affected by factors such as age, sex and weight, and does not change under inflammatory conditions (8). Previous studies have reported an association between Cys C and cancer; however, these findings are inconsistent. For example, Ervin *et al* (34) demonstrated that Cys C plays an important role in inhibiting melanoma lung metastasis, while Naumnik *et al* (13) reported that Cys C concentrations are significantly higher in patients with NSCLC compared with healthy individuals. A previous study by Sevier and Kaiser (35) demonstrated that there are no significant differences in Cys C expression between lung squamous cell carcinoma tissues and normal lung tissues. Thus, the association between Cys C expression and the prognosis of patients with SCLC was investigated in the present study. Notably, the effect of Cys C concentration on the prognosis of patients prior to treatment was assessed, thus Cys C levels were detected following diagnosis. Given that the patients did not receive any treatment, Cys C concentration was not affected by chemotherapy drugs. Based on the ROC curve, the optimal cut-off value of Cys C concentration prior to chemotherapy was 0.775 mg/l. Patients were subsequently divided into high and low Cys C groups for survival analysis. The results demonstrated that patients in the high Cys C had significantly shorter mPFS and mOS times compared with patients in the low Cys C group. Thus, increased Cys C concentration prior to chemotherapy was associated with shorter PFS and OS times in patients with SCLC. Univariate and multivariate analyses demonstrated that Cys C concentration prior to chemotherapy may be an independent prognostic factor for PFS of patients with SCLC. Notably, Cys C concentration was not identified as an independent prognostic factor for OS, and thus cannot be used alone to predict patient survival. Increased Cys C concentration was associated with rapid disease progression and poor prognosis. This may be due to the following reasons, the process of tumor cell division and proliferation requires folic acid as a coenzyme to participate in the synthesis of nucleic acids, leading to the accumulation of homocysteine in the body (36). Cys C is a well-known cysteine protease inhibitor C, which can bind with cysteine protease to inhibit homocysteine activity, which in turn increases Cys C concentration (36). In addition, the imbalance between cathepsin and protease inhibitor may lead to the invasion and metastasis of cancer cells, thus further promoting the concentration of Cys C (37). Increased Cys C concentration is associated with tumor infiltration and metastasis (37,38).

UA is a product of XOR oxidizing xanthine and hypoxanthine (10). Previous studies have reported an association between UA and cancer; however, these findings are inconsistent. For example, Ames *et al* (10) hypothesized that UA, as a powerful antioxidant, is a scavenger of free radicals, which can inhibit lipid peroxidation under high concentrations and exert anticancer effects. However, it has been demonstrated that UA promotes the development of inflammation, and plays a key role in the development of breast cancer (11). Elevated UA expression increases the risk of colorectal, breast and prostate cancers (39-43). In the present study, the optimal cut-off value of UA concentration prior to chemotherapy was 312.75 μmol/l. Based on this value, patients were divided into high and low UA groups for survival analysis and the results demonstrated that patients in the high UA group had significantly shorter mPFS and mOS times compared with patients in the low UA group. Notably, univariate and multivariate analyses demonstrated that UA concentration prior to chemotherapy was not an independent prognostic factor for PFS and OS, and thus cannot be used alone to predict disease progression and the survival time of patients with SCLC. Increased UA concentration prior to chemotherapy was associated with poor prognosis. This may be due to the proinflammatory nature of UA (16). Inflammation mediators and cellular effectors are an important part of the tumor's local environment, whereby the inflammatory response has been demonstrated to promote tumor proliferation and survival (16). In addition, UA has the ability to inhibit XOR expression, and decreased XOR expression regulates the secretion of COX-2 and MMP-1, which in turn induce the expression of differentiated protein inhibitors to increase the aggressiveness of cancer cells (44). Increased UA and decreased XOR expression levels contribute to the proliferation, migration and survival of tumor cells (44).

Indicators such as LDH and UA are associated with tumor burden (14,16). It is speculated that patients with extensive stage SCLC exhibit poor prognosis compared with other stages. The χ^2 test was used to assess the association between UA and LDH concentrations, and different disease stages (Table VI). Patients were divided into different groups based on their disease stage. Further analysis demonstrated that there were no significant associations between the assessed indexes and the prognosis of patients. Multivariate analysis demonstrated that staging was not an independent prognostic factor for PFS or OS; thus, staging analysis was not performed in the present study.

Given that SCLC is a tumor that is sensitive to radiotherapy and chemotherapy (45), the treatment-related factors were also assessed in the preset study. Etoposide or irinotecan combined with platinum is the most common chemotherapy regimen for first-line treatment of SCLC (46). Univariate and multivariate analyses demonstrated that there were no significant differences in the effects of both schemes on PFS or OS of patients with SCLC. Radiotherapy plays an important role in the treatment of SCLC (47). Consistent with previous findings (45,47), multivariate analysis in the present study demonstrated that radiotherapy was an independent prognostic factor for PFS and OS in patients with SCLC, which was associated with favorable prognosis.

The present study is not without limitations. First, all patients who participated were Chinese and predominantly

from coastal areas, thus this may cause selection bias. Secondly, the sample size was relatively small, which may have also caused selection bias. Thirdly, this was a retrospective study, which cannot completely exclude selection bias and information bias. In addition, even if some interfering factors were excluded, other confounding factors associated with Cys C, UA and LDH, such as dietary habits and lifestyle were not included as variables in the present study. Thus, large-scale prospective and multicenter studies are required to validate the results presented here.

In conclusion, the results of the present study demonstrated that Cys C, UA and LDH concentrations in patients with SCLC, prior to chemotherapy were associated with the prognosis of patients. Patients with elevated concentrations exhibited shorter PFS and OS times, and poor prognosis. Notably, high LDH concentration prior to chemotherapy may be an independent risk factor for patients with shorter PFS and OS times, while elevated Cys C levels prior to chemotherapy may be an independent risk factor for patients with a shorter PFS time. The identification of these factors will assist with the prediction of the differences in prognosis in different populations, and further provide new ideas for determining the changes in SCLC.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Natural Science Foundation of Shandong Province (grant no. ZR2017MH062) and the Science and Technology for People's Livelihood Project of Qingdao (grant no. 17-3-3-33-nsh).

Availability of data and materials

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

Authors' contributions

ZY, HW, DS, YD and LZ conceived and designed the present study. ZY provided administrative support. DS and YD provided the study materials and patient samples. LZ collected and assembled the data. HW and XY interpreted and analyzed the data. HW, DS and YD revised the manuscript for important intellectual content. All authors drafted the initial manuscript, and have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Qingdao University Hospital (Shandong, China; approval no. QYFYWZLL25870) and informed consent was provided by all patients prior to the study start.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. van Meerbeeck JP, Fennell DA and De Ruysscher DK: Small-Cell lung cancer. Lancet 378: 1741-1755, 2011.
2. Amini A, Byers LA, Welsh JW and Komaki RU: Progress in the management of limited-stage small cell lung cancer. Cancer 120: 790-798, 2014.
3. Kiani A, Khosravi A, Moghaddam AS, Jabbari H and Fakhri M: Long-Term survival of a small cell lung cancer patient with proper endobronchial management. Pneumologia 61: 245-248, 2012.
4. Poola I and Graziano SL: Expression of neuron-specific enolase, chromogranin A, synaptophysin and Leu-7 in lung cancer cell lines. J Exp Clin Cancer Res 17: 165-173, 1998.
5. Yang X, Wang D, Yang Z, Qing Y, Zhang Z, Wang G, Yang Z and Wang Z: CEA is an independent prognostic indicator that is associated with reduced survival and liver metastases in SCLC. Cell Biochem Biophys 59: 113-119, 2011.
6. Molina R, Auge JM, Escudero JM, Marrades R, Viñolas N, Carcereny E, Ramirez J and Filella X: Mucins CA 125, CA 19.9, CA 15.3 and TAG-72.3 as tumor markers in patients with lung cancer: Comparison with CYFRA 21-1, CEA, SCC and NSE. Tumour Biol 29: 371-380, 2008.
7. Winther B, Moi P, Paus E and Reubsaet JL: Targeted determination of the early stage SCLC specific biomarker pro-gastrin-releasing peptide (ProGRP) at clinical concentration levels in human serum using LC-MS. J Sep Sci 30: 2638-2646, 2007.
8. Nakai K, Kikuchi M, Fujimoto K, Kaneko Y, Omori S, Nakai K and Suwabe A: Serum levels of cystatin C in patients with malignancy. Clin Exp Nephrol 12: 132-139, 2008.
9. Kos J, Krasovec M, Cimerman N, Nielsen HJ, Christensen IJ and Brünner N: Cysteine proteinase inhibitors stefin A, stefin B, and cystatin C in sera from patients with colorectal cancer: Relation to prognosis. Clin Cancer Res 6: 505-511, 2000.
10. Ames BN, Cathcart R, Schwiers E and Hochstein P: Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: A hypothesis. Proc Natl Acad Sci USA 78: 6858-6862, 1981.
11. Vona-Davis L, Howard-McNatt M and Rose DP: Adiposity, type 2 diabetes and the metabolic syndrome in breast cancer. Obes Rev 8: 395-408, 2007.
12. Dang CV and Lewis BC: Role of oncogenic transcription factor c-myc in cell cycle regulation, apoptosis and metabolism. J Biomed Sci 4: 269-278, 1997.
13. Naumnik W, Niklińska W, Ossolińska M and Chyczewska E: Serum cathepsin K and cystatin C concentration in patients with advanced non-small-cell lung cancer during chemotherapy. Folia Histochem Cytobiol 47: 207-213, 2009.
14. Parks SK, Chiche J and Pouysségur J: Disrupting proton dynamics and energy metabolism for cancer therapy. Nat Rev Cancer 13: 611-623, 2013.
15. Micke P, Faldum A, Metz T, Beeh KM, Bittinger F, Hengstler JG and Buhl R: Staging small cell lung cancer: Veterans administration lung study group versus international association for the study of lung cancer-what limits limited disease? Lung Cancer 37: 271-276, 2002.
16. Ghaemi-Oskouie F and Shi Y: The role of uric acid as an endogenous danger signal in immunity and inflammation. Curr Rheumatol Rep 13: 160-166, 2011.
17. Fantin VR, St-Pierre J and Leder P: Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance. Cancer Cell 9: 425-434, 2006.
18. Lewis BC, Shim H, Li Q, Wu CS, Lee LA, Maity A and Dang CV: Identification of putative c-myc-responsive genes: Characterization of rcl, a novel growth-related gene. Mol Cell Biol 17: 4967-4978, 1997.
19. Shim H, Dolde C, Lewis BC, Wu CS, Dang G, Jungmann RA, Dalla-Favera R and Dang CV: C-Myc transactivation of LDH-A: Implications for tumor metabolism and growth. Proc Natl Acad Sci USA 94: 6658-6663, 1997.
20. Koukourakis MI, Giatromanolaki A, Sivridis E, Gatter KC, Trarbach T, Folprecht G, Shi MM, Lebwohl D, Jalava T, Laurent D, *et al*: Prognostic and predictive role of lactate dehydrogenase 5 expression in colorectal cancer patients treated with PTK787/ZK 222584 (vatalanib) antiangiogenic therapy. Clin Cancer Res 17: 4892-4900, 2011.

21. Turen S, Ozyar E, Altundag K, Gullu I and Atahan IL: Serum lactate dehydrogenase level is a prognostic factor in patients with locoregionally advanced nasopharyngeal carcinoma treated with chemoradiotherapy. Cancer Invest 25: 315-321, 2007.
22. Jin Y, Ye X, Shao L, Lin BC, He CX, Zhang BB and Zhang YP: Serum lactic dehydrogenase strongly predicts survival in metastatic nasopharyngeal carcinoma treated with palliative chemotherapy. Eur J Cancer 49: 1619-1626, 2013.
23. Brown JE, Cook RJ, Lipton A and Coleman RE: Serum lactate dehydrogenase is prognostic for survival in patients with bone metastases from breast cancer: A retrospective analysis in bisphosphonate-treated patients. Clin Cancer Res 18: 6348-6355, 2012.
24. Halabi S, Small EJ, Kantoff PW, Kattan MW, Kaplan EB, Dawson NA, Levine EG, Blumenstein BA and Vogelzang NJ: Prognostic model for predicting survival in men with hormone-refractory metastatic prostate cancer. J Clin Oncol 21: 1232-1237, 2003.
25. von Eyben FE, Madsen EL, Liu F, Amato R and Fritsche H: Serum lactate dehydrogenase isoenzyme 1 as a prognostic predictor for metastatic testicular germ cell tumours. Br J Cancer 83: 1256-1259, 2000.
26. Gerlinger M, Wilson P, Powles T and Shamash J: Elevated LDH predicts poor outcome of recurrent germ cell tumours treated with dose dense chemotherapy. Eur J Cancer 46: 2913-2918, 2010.
27. Egberts F, Kotthoff EM, Gerdes S, Egberts JH, Weichenthal M and Hauschild A: Comparative study of YKL-40, S-100B and LDH as monitoring tools for stage IV melanoma. Eur J Cancer 48: 695-702, 2012.
28. Agarwala SS, Keilholz U, Gilles E, Bedikian AY, Wu J, Kay R, Stein CA, Itri LM, Suciu S and Eggermont AM: LDH correlation with survival in advanced melanoma from two large, randomised trials (Oblimersen GM301 and EORTC 18951). Eur J Cancer 45: 1807-1814, 2009.
29. Seagroves TN, Ryan HE, Lu H, Wouters BG, Knapp M, Thibault P, Laderoute K and Johnson RS: Transcription factor HIF-1 is a necessary mediator of the pasteur effect in mammalian cells. Mol Cell Biol 21: 3436-3444, 2001.
30. Schofield CJ and Ratcliffe PJ: Oxygen sensing by HIF hydroxylases. Nat Rev Mol Cell Biol 5: 343-354, 2004.
31. Ostergaard L, Tietze A, Nielsen T, Drasbek KR, Mouridsen K, Jespersen SN and Horsman MR: The relationship between tumor blood flow, angiogenesis, tumor hypoxia, and aerobic glycolysis. Cancer Res 73: 5618-5624, 2013.
32. Ryberg M, Nielsen D, Osterlind K, Skovsgaard T and Dombernowsky P: Prognostic factors and long-term survival in 585 patients with metastatic breast cancer treated with epirubicin-based chemotherapy. Ann Oncol 12: 81-87, 2001.
33. Brizel DM, Schroeder T, Scher RL, Walenta S, Clough RW, Dewhirst MW and Mueller-Klieser W: Elevated tumor lactate concentrations predict for an increased risk of metastases in head-and-neck cancer. Int J Radiat Oncol Biol Physics 51: 349-353, 2001.
34. Ervin H and Cox JL: Late stage inhibition of hematogenous melanoma metastasis by cystatin C over-expression. Cancer Cell Int 5: 14, 2005.
35. Sevier CS and Kaiser CA: Formation and transfer of disulphide bonds in living cells. Nat Rev Mol Cell Biol 3: 836-847, 2002.
36. Choi SW and Mason JB: Folate and carcinogenesis: An integrated scheme. J Nutr 130: 129-132, 2000.
37. Saleh Y, Sebzda T, Warwas M, Kopec W, Ziólkowska J and Siewinski M: Expression of cystatin C in clinical human colorectal cancer tissues. J Exp Ther Oncol 5: 49-53, 2005.
38. Zhang X, Hou Y, Niu Z, Li W, Meng X, Zhang N and Yang S: Clinical significance of detection of cathepsin X and cystatin C in the sera of patients with lung cancer. Zhongguo Fei Ai Za Zhi 16: 411-416, 2013 (In Chinese).
39. Hammarsten J, Damber JE, Peeker R, Mellström D and Högstedt B: A higher prediagnostic insulin level is a prospective risk factor for incident prostate cancer. Cancer Epidemiol 34: 574-579, 2010.
40. Bjorge T, Lukanova A, Jonsson H, Tretli S, Ulmer H, Manjer J, Stocks T, Selmer R, Nagel G, Almquist M, *et al*: Metabolic syndrome and breast cancer in the me-can (metabolic syndrome and cancer) project. Cancer Epidemiol Biomarkers Prev 19: 1737-1745, 2010.
41. Rose DP, Haffner SM and Baillargeon J: Adiposity, the metabolic syndrome, and breast cancer in African-American and white American women. Endocr Rev 28: 763-777, 2007.
42. Giovannucci E: Metabolic syndrome, hyperinsulinemia, and colon cancer: A review. Am J Clin Nutr 86: s836-s842, 2007.
43. Siddiqui AA: Metabolic syndrome and its association with colorectal cancer: A review. Am J Med Sci 341: 227-231, 2011.
44. Linder N, Butzow R, Lassus H, Lundin M and Lundin J: Decreased xanthine oxidoreductase (XOR) is associated with a worse prognosis in patients with serous ovarian carcinoma. Gynecol Oncol 124: 311-318, 2012.
45. Herrmann MK, Bloch E, Overbeck T, Koerber W, Wolff HA, Hille A, Vorwerk H, Hess CF, Muller M, Christiansen H and Pradier O: Mediastinal radiotherapy after multidrug chemotherapy and prophylactic cranial irradiation in patients with SCLC-treatment results after long-term follow-up and literature overview. Cancer Radiother 15: 81-88, 2011.
46. Buyse M, Thirion P, Carlson RW, Burzykowski T, Molenberghs G, Piedbois P; Mata-analysis Group in Cancer: Re: A model to select chemotherapy regimens for phase III trials for extensive-stage small-cell lung cancer. J Natl Cancer Inst 93: 399-401, 2001.
47. Abdelwahab S, Abdulla H, Azmy A, Abdelfatah A, Abdel-Aziz H, Margerges M, Riad A, Sharma V and Dwedar I: Integration of irinotecan and cisplatin with early concurrent conventional radiotherapy for limited-disease SCLC (LD-SCLC). Int J Clin Oncol 14: 230-236, 2009.