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MiR-219-5p decrease the risk of cancer-related mortality in patients with small cell lung cancer

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-064700
Article Type:	Original research
Date Submitted by the Author:	13-May-2022
Complete List of Authors:	Zhang, Xiaohui; First Affiliated Hospital of Soochow University Zhang, Jigang; First Affiliated Hospital of Soochow University Xiang, Mengqi; Medical School of University of Electronic Science and Technology of China, Department of Medical Oncology Xu, Zhihua; First Affiliated Hospital of Soochow University, General surgery Wu, Xiangmei; Suzhou Xiangcheng People's Hospital
Keywords:	Risk management < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, ONCOLOGY, Gene therapy < ONCOLOGY, Molecular aspects < ONCOLOGY, Oncogenes < ONCOLOGY

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4 **MiR-219-5p decrease the risk of cancer-related mortality in**
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6 **patients with small cell lung cancer**
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8 Xiaohui Zhang^{2,#}, Jigang Zhang^{4,#}, Mengqi Xiang^{5,#}, Zhihua Xu^{3,*}, Xiangmei Wu^{1,*}
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11
12 ¹ Department of Endocrinology, Suzhou Xiangcheng People's Hospital, Suzhou,
13 China
14

15
16 ² Department of Medicine, Respiratory, Emergency and Intensive Care Medicine, The
17 First Affiliated Hospital of Soochow University, Suzhou, China
18

19
20 ³ Department of General Surgery, The First Affiliated Hospital of Soochow
21 University, Suzhou, China
22

23
24 ⁴ Department of Traumatology Surgery, The First Affiliated Hospital of Soochow
25 University, Suzhou, China
26

27
28 ⁵ Department of Medical Oncology, Sichuan Cancer Hospital, Medical School of
29 University of Electronic Science and Technology of China, Chengdu, Sichuan
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34 Running title: MiR-219-5p decrease the risk of SCLC patients
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40 # These authors contributed equally to this work
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42 * Correspondence to: dr_xiangmeiwu@163.com (Xiangmei Wu) or
43 dr_zhihuaxu@163.com (Zhihua Xu)
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ABSTRACT

Objectives Small cell lung cancer (SCLC) is a lethal human malignancy, and previous studies support the contribution of microRNA (miRNA) to cancer progression. The prognostic value of miR-219-5p in SCLC patients remains unclear. This study evaluated the risk factors for SCLC and created a prediction model for them.

Design Retrospective observational cohort study.

Setting The programme has yielded a database of all patients with SCLC in 2 defined geographical regions of China.

Participants We did a real-world study, including data from 133 patients with SCLC between Mar 1, 2010 and June 1, 2015. We collected 86 NSCLC patients in the external validation step.

Primary and secondary outcome measures MiR-219-5p was recorded during the admission. Cox proportional hazard model was applied for survival analyses and for analyzing risk factors for cancer-related mortality and to create a nomogram for prediction. The accuracy of the model was evaluated by C-index and calibration curve. An external data of 86 SCLC patients from Sichuan Cancer Hospital and the First affiliated hospital of Soochow University was conducted.

Results In our data, the mortality in group with high miR-219-5p level (≥ 1.50) was 74.6%. Based on univariate analysis, we put factors ($P < 0.05$) into a multivariate regression model, patients with high miR-219-5p level ($P < 0.001$, HR=0.36), immunotherapy ($P < 0.001$, HR=0.44), PNI score > 47.9 ($P = 0.01$, HR=0.45) remained statistically factors for better OS and regarded as independent protective factors. These independently associated risk factors were used to establish an OS estimation nomogram. Nomogram revealed good accuracy in estimating the risk, with a bootstrap-corrected C index of 0.691. External validation displayed an AUC of 0.749 (0.709-0.788).

Conclusions MiR-219-5p decreased the risk of cancer-related mortality in patients with SCLC. Nomogram based on multivariate analysis demonstrated good accuracy in estimating the risk of overall mortality.

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4 **Keywords:** small cell lung cancer, miR-219-5p, overall survival, nomogram,
5 prediction model
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BACKGROUND

Lung cancer is the leading cause of cancer deaths worldwide, with millions of new cases diagnosed each year[1]. Small cell lung cancer (SCLC) is a kind of neuroendocrine malignant tumor with poor prognosis, accounting for about 15% of lung cancer patients[2]. SCLC is generally divided into limited disease (LD-SCLC) and extensive disease (ED-SCLC). Platinum combined with etoposide is the first-line therapeutic strategy of SCLC, and most patients are easy to receive initial chemotherapy[3]. However, the 5-year survival rates of LD-SCLC and ED-SCLC are only 15% and 3%, respectively[4]. Therefore, improvement in early diagnosis and prognostic prediction of SCLC is vital.

MicroRNAs (miRNAs) are endogenous non-coding RNAs (~ 22 nt), which regulate mRNA activity by hybridization with 3' untranslated region (UTR) of specific genes[5]. Many studies have shown that miRNAs could participate in a variety of cell biological processes, including cell growth, differentiation and apoptosis[6, 7]. In addition, researches have demonstrated that miRNAs are frequently dysregulated in cancers[8, 9], and some miRNAs can serve as diagnostic and prognostic biomarkers for cancers[10]. Recently, several miRNAs have been proved to participate in the occurrence and development of SCLC, but few of them are likely to be a biomarker or therapeutic target for SCLC.

Recently, miR-219-5p has been found to be abnormally expressed and play a significant role in different cancers. Ma et al. found that the expression of miR-219-5p was significantly decreased in esophageal squamous cell carcinoma (ESCC) tissues compared with normal tissues[11]. A study of Gong et al. revealed a tumor suppressive role for miR-219-5p by targeting glypican-3 in hepatocellular carcinoma (HCC)[12]. On the contrary, Yang et al. indicated that miR-219-5p could promote cell growth and metastasis of HCC and serve as a prognostic marker for HCC patients[13]. A research investigated by Wei et al. suggested that miR-219-5p could inhibit proliferation, migration and invasion of epithelial ovarian cancer through downregulation of the Wnt signaling pathway, and it could serve as a diagnostic

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4 biomarker and therapeutic target for epithelial ovarian cancer[14]. However, the
5 biological functions of miR-219-5p and its potential prognostic role for biomarker in
6 SCLC are still unknown.
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10 In this study, we aimed to examine the variation in the expression levels of
11 miR-219-5p in patients with SCLC and explored the potential prognostic role of
12 miR-219-5p for SCLC. We also displayed a nomogram that could provide
13 individualized, evidence-based, highly accurate risk estimates. Nomograms were easy
14 to performed and could facilitate management-related decision making.
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21 **METHODS**

22 **Study Design and Patient Characteristics**

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24 We did a real-world study, including data obtained from 133 patients with SCLC
25 between Mar 2010 and June 2015, in the Suzhou Xiangcheng People's Hospital.
26
27 Those participants who lacked information on complement components data,
28 withdrew from treatment or lacked follow-up information were excluded. Clinical
29 information of patients, including gender, age, BMI, neutrophils count, lymphocytes
30 count, serum CEA level, CRP level, albumin level, hemoglobin level, stage of SCLC,
31 platelet count, PNI score, KPS score, NLR, pathologic type, immunotherapy, therapy
32 of radiation, application of platinum, application of VEGF inhibitor, target therapy,
33 application of TKI, smoking, ACS, diabetes, heart failure and hyperlipemia, were
34 recorded. Diagnosis of SCLC was confirmed by histopathological examination. The
35 median length of follow-up was 23.6 months. The definition and details of all the
36 variables above were provided in Supplemental Materials Part I. Data from 86
37 patients with NSCLC at Sichuan Cancer Hospital and the First affiliated hospital of
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4 Soochow University were applied for external validation. Inform, and consent was
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6 obtained from all patients or their immediate family members. All protocols were in
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8 line with the guidelines with the ethic committee of Suzhou Xiangcheng People's
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10 Hospital, Sichuan Cancer Hospital, the First affiliated hospital of Soochow University
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12 and following the Declaration of Helsinki.
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20 **Assays for Detection of MiR-219-5p Levels**

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22 The quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was
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24 conducted for the detection of miR-219-5p expression levels.
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26 Total RNA from tissues was isolated and extracted using miRcute Extraction and
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28 Separation of miRNAs kit (Tiangen Biotech Co., Ltd., Beijing, China), and then
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30 reversely transcribed into cDNA by PrimeScript™ II 1st strand cDNA synthesis kit
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32 (Takara Biotechnology Co., Ltd., Dalian, China) according to the manufacturer's
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34 protocol. SYBR PrimeScript miRNA RT-PCR kit (Takara Biotechnology Co., Ltd.)
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36 was used for qRT-PCR. The thermocycling conditions were as follows: one cycle at
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38 95°C for 3 min (initial denaturation), 40 cycles at 95°C for 15 s and 60°C for 30 s. U6
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40 small nuclear RNA (U6) served as the respective internal control. The relative
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42 expression of miR-219-5p was quantified by the $2^{-\Delta\Delta Ct}$ methods, and normalized to
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44 the U6. The following primers were used: miR-219-5p forward,
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46 5'-ACACTCCAGCTGGGTGATTGTCCAAACGCAAT-3' and reverse,
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48 5'-CTCAACTGGTGTCTGGA-3'; U6 forward,
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50 5'-GCTTCGGCAGCACATATACTAAAAT-3' and reverse,
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52 5'-CGCTTCACGAATTTGCGTGTCAT-3'. The experiments were repeated at least 3
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54 times.
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57 **Statistical Analysis**

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59 Sample size assessment was performed using NCSS-PASS software version 11.0
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4 (https://www.ncss.com/software/pass/). Power was set as 0.99, and alpha was 0.5. The
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6 mortalities of both miR-219-5p high-level group and miR-219-5p low-level group in
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8 our previous data (2008-2009) (0.750 and 0.950) were entered into the PASS. The
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10 Actual Hazard Ratio was set as 0.50. Then the sample size was calculated using
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12 PASS, and the minimum sample size was 103 (control = 51, experiment = 43). Our
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14 sample size was 133 (66 and 67 for each group), which was suitable. The report of
15
16 sample size assessment was displayed in Supplemental Material Part II. The missing
17
18 data (<5.0%) were estimated by random forest algorithm using the mice package in
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20 RStudio (R version 3.6.1). Categorical variates were presented as percentages and
21
22 compared via the κ^2 test. Continuous variates with skewed and normal distributions
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24 were presented as median with interquartile ranges and mean \pm standard deviation.
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26 The Mann-Whitney U test and the unpaired t-test were applied for comparison
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28 between Groups. Cumulative mortality was showed by the Kaplan-Meier curve and
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30 analyzed by the log-rank test. Univariate and multivariate survival analyses for OS
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32 were conducted using the Cox regression model. The forest plots were used to
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34 visualize the significance of covariates to the prognosis. The restricted cubic spline
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36 analyses were performed with Harrell's Regression Modelling Strategies (rms)
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38 package.
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50 To create a prognostic risk model, the Lasso regression was conducted to
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52 identify risk factors correlated with prognosis. The contribution of each covariate was
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54 quantified and visualized in a prognostic nomogram with internal validation via
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56 1000-times bootstrapping. The consistency of the resulting model was assessed by the
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58 calibration assay. Decision curve analyses were performed to evaluate net clinical
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60 benefits of the model compared with conventional prognostic scores. The scatter plots

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4 were applied for visualization of the consistency of each model. A 1000-time
5 bootstrapping was employed as indicated. The association between miR-219-5p class
6 and survival endpoints was evaluated by Kaplan-Meier curves and log-rank test.
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8 Statistical analysis was performed using the RStudio (R version 3.6.1) with the
9
10 following packages: 'ggplot2', 'rms', 'PredictABLE', 'risk regression', and
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12 'survminer'.
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15 16 17 **Patient and public involvement**

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19 This study was conducted without patient involvement. Patients were not invited to
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21 comment on the study design and were not consulted to develop patient-relevant
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23 outcomes or interpret the results. Moreover, patients were not allowed to contribute to
24
25 the writing or editing of this document for readability or accuracy.
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28 29 **RESULTS**

30 31 **Baseline Characteristics**

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33 A total of 133 SCLC patients who were diagnosed between Mar 2010 and June 2015
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35 were included in this study. A flow chart of the screening process was shown in [figure](#)
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37 [1](#). The median age of these patients was 64 years old (58-70), and it contained 106
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39 (80.0%) males. Median serum CEA and CRP level was 3.43 ng/ml and 7.83 μ mol/L,
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41 respectively. For the stage of SCLC, 51 (38.0%) patients were diagnosed with limited
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43 disease, 82 (61.0%) patients were extensive disease. 25 (19.0%) patients accepted the
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45 immunotherapy therapy. 54 (41.0%) patients got the therapy of radiation. In addition,
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47 platinum was applied for 131 (98.0%) patients, and TKI was used for 15 (12.0%)
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49 patients. KPS score of these patients was examined, and the results revealed that 107
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51 (81.0%) patients got 80 or higher points. The distribution of basic diseases was also
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assessed in our data. Diabetes was found in 17 (13.0%) patients, while hyperlipemia was 13 (10.0%). Cardiovascular diseases such as heart failure and ACS were found in 3 (2.0%) and 4 (3.0%) patients, respectively. 13 (10.0%) patients suffered from hypertension. In addition, 82 (62.0%) patients had a habit of smoking among all the patients. The baseline characteristics of these patients were listed in [table 1](#).

Among all the 133 patients, the overall mortality was 87.2%. The mortality in high miR-219-5p level group was 74.6%. Moreover, in the high miR-219-5p level group, patients with extensive disease were 35 (52.0%), while the low group was 47 (71.0%) ([table 1](#)).

Table 1. Study Participant Characteristics at Enrollment

Variation	Total (n=133)	Cohort, median (IQR)		p.value
		mir-219-5p<1.50, (n=66)	mir-219-5p≥1.50, (n=67)	
Age, (year)	64(58,70)	65(59,70)	63(56.5,68)	0.276
BMI, (kg/m ²)	23.12±3.09	22.99±3.22	23.26±2.98	0.619
Serum CEA level, (ng/ml)	3.43(1.96,9.26)	3.34(1.92,10.05)	3.43(1.99,8.11)	0.87
Serum CRP level, (μmol/L)	7.83(1.68,12.78)	10.68(2.78,13.15)	5.64(1.2,12.16)	0.107
Albumin level, (g/L)	39.46±5.2	38.86±4.68	40.05±5.65	0.188
Neutrophils count, (10 ⁹ /L)	4.55(3.59,5.88)	4.54(3.77,5.75)	4.55(3.48,5.92)	0.975
Lymphocytes count, (10 ⁹ /L)	1.63(1.31,1.95)	1.5(1.21,1.78)	1.73(1.38,2.08)	0.031*
Hemoglobin level, (g/L)	133(125,145)	134(124,145)	132(125,143.5)	0.564
Platelet count, (10 ⁹ /L)	233(184,288)	244.5(180,293.75)	226(184.5,273.5)	0.306
PNI score	47.9(43.95,51.85)	45.98(42.51,50.38)	48.7(44.92,54)	0.026*
NLR	2.66(1.99,4.19)	2.75(2.11,4.57)	2.59(1.92,3.7)	0.232
Gender, (n%)				0.211
Female	27(20)	10(15)	17(25)	
Male	106(80)	56(85)	50(75)	
Metastasis, n(%)				0.299
No	45(34)	19(29)	26(39)	
Yes	88(66)	47(71)	41(61)	
Stage of SCLC				0.038*
Limited disease	51(38)	19(29)	32(48)	
Extensive disease	82(62)	47(71)	35(52)	
Immunotherapy, (n%)				0.197
No	108(81)	57(86)	51(76)	
Yes	25(19)	9(14)	16(24)	
Therapy of radiation, (n%)				0.417
No	79(59)	42(64)	37(55)	

Yes	54(41)	24(36)	30(45)	
Application of platinum, (n%)				0.244
No	2(2)	2(3)	0(0)	
Yes	131(98)	64(97)	67(100)	
Chemotherapy				0.45
AP	28(21)	12(18)	16(24)	
DP	15(11)	6(9)	9(13)	
EP	71(53)	35(53)	36(54)	
GP	3(2)	2(3)	1(1)	
Others	16(12)	11(17)	5(7)	
Target therapy, (n%)				0.627
No	116(87)	59(89)	57(85)	
Yes	17(13)	7(11)	10(15)	
Application of TKI, (n%)				0.449
No	118(89)	60(91)	58(87)	
TKI I	9(7)	4(6)	5(7)	
TKI II	1(1)	1(2)	0(0)	
TKI III	5(4)	1(2)	4(6)	
Application of VEGF inhibitor, n(%)				0.645
No	114(86)	58(88)	56(84)	
Yes	19(14)	8(12)	11(16)	
KPS score, n(%)				0.678
40	2(2)	0(0)	2(3)	
50	5(4)	3(5)	2(3)	
60	7(5)	3(5)	4(6)	
70	12(9)	8(12)	4(6)	
80	29(22)	14(21)	15(22)	
90	56(42)	29(44)	27(40)	
100	22(17)	9(14)	13(19)	
Smoking, n(%)				0.255
No	51(38)	29(44)	22(33)	
Yes	82(62)	37(56)	45(67)	
Hypertension, n(%)				1
No	80(60)	40(61)	40(60)	
Yes	53(40)	26(39)	27(40)	
Diabetes, n(%)				1
No	116(87)	58(88)	58(87)	
Yes	17(13)	8(12)	9(13)	
Hyperlipemia, n(%)				0.579
No	120(90)	61(92)	59(88)	
Yes	13(10)	5(8)	8(12)	
Heart failure, n(%)				1
No	130(98)	65(98)	65(97)	
Yes	3(2)	1(2)	2(3)	

ACS, n(%)				0.619
No	129(97)	65(98)	64(96)	
Yes	4(3)	1(2)	3(4)	

Abbreviation: IQR, interquartile range; CRP, C-reactive protein; PNI, neutrophil lymphocyte ratio; NLR, neutrophil lymphocyte ratio; SCLC, small-cell lung cancer; TKI, Tyrosine Kinase Inhibitor; VEGF, vascular endothelial growth factor; KPS, Karnofsky Performance Status; ACS, acute coronary syndrome. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

MiR-219-5p Expression Level, and Clinical Risk Factors Predict the Development of SCLC Patients

According to the univariate analysis, high miR-219-5p levels (≥ 1.50) was a strong protective predictor of cancer-related mortality (HR 0.36, 95% CI 0.25-0.53, $P < 0.001$) (table 2). Kaplan Meier curve showed that patients in the high miR-219-5p group had a decreased cumulative rate of death than those in the low miR-219-5p group (log-rank $P < 0.001$) (figure 2A). Meanwhile, patients who accepted immunotherapy also showed a low mortality compared to those patients without accepting immunotherapy in the survival curve (HR 0.28, 95% CI 0.15-0.52, $P < 0.001$) (figure 2B).

In addition, gender, age, serum CRP level, albumin level, lymphocytes count, PNI score, immunotherapy, heart failure, and KPS score were also correlated with overall mortality (table 2). When adjusted by age and gender, patients in the miR-219-5p high-level group also displayed a low cumulative rate death compared to those in the low-level group.

Table 2. Cox Regression Analysis of Hazard Ratio on SCLC patients

Variation	Non-adjustment		Model 1	
	Hazard Ratio (95% CI)	p.value	Hazard Ratio (95% CI)	p.value
Gender, male vs. female	1.66 [1.02, 2.69]	0.041*	-	-
Age (year), ≥ 60 vs. < 60	1.52 [1.03, 2.26]	0.036*	-	-
BMI, ≥ 23.12 kg/m ² vs. < 22.86 kg/m ²	0.99 [0.69, 1.43]	0.97	0.95 [0.66, 1.38]	0.806
Serum CEA level, > 3.43 ng/ml vs. ≤ 3.43 ng/ml	1.01 [0.70, 1.46]	0.954	1.00 [0.69, 1.45]	0.999
Serum CRP level, > 7.83 μ mol/L vs. ≤ 7.83 μ mol/L	1.91 [1.31, 2.78]	0.001**	1.92 [1.32, 2.79]	0.001**
Albumin level, > 39.46 g/L vs. ≤ 39.46 g/L	1.66 [1.15, 2.40]	0.007**	1.60 [1.10, 2.31]	0.013*
Neutrophils count, $> 4.55 \times 10^9/L$ vs. $\leq 4.55 \times 10^9/L$	1.18 [0.82, 1.71]	0.367	1.15 [0.79, 1.66]	0.464
Lymphocytes count, $> 1.63 \times 10^9/L$ vs. $\leq 1.63 \times 10^9/L$	0.59 [0.40, 0.85]	0.005**	0.61 [0.42, 0.89]	0.01*

Hemoglobin level, >133 g/L vs. ≤ 133 g/L	0.80 [0.56, 1.16]	0.244	0.72 [0.49, 1.05]	0.089
Platelet count, >233x10 ⁹ /L vs. ≤ 233x10 ⁹ /L	1.23 [0.85, 1.78]	0.275	1.23 [0.85, 1.78]	0.275
PNI score, >47.9 vs. ≤47.9	0.54 [0.37, 0.78]	0.001**	0.53 [0.37, 0.77]	0.001**
NLR, >2.66 vs. ≤2.66	1.18 [0.82, 1.71]	0.367	1.20 [0.83, 1.74]	0.341
Metastasis, Yes vs. No	1.05 [0.70, 1.57]	0.81	1.10 [0.73, 1.64]	0.654
Stage of NSCLC, IV, III vs. II and I	0.94 [0.50, 1.75]	0.838	1.05 [0.56, 1.98]	0.868
Stage of SCLC, Yes vs. No	1.33 [0.90, 1.96]	0.154	1.43 [0.97, 2.12]	0.074
Immunotherapy, Yes vs. No	0.28 [0.15, 0.52]	<0.001***	0.30 [0.16, 0.55]	<0.001***
Therapy of radiation, Yes vs. No	0.88 [0.60, 1.27]	0.488	0.79 [0.54, 1.16]	0.235
Application of platinum, Yes vs. No	0.61 [0.15, 2.47]	0.483	0.66 [0.16, 2.70]	0.562
Target therapy, Yes vs. No	0.75 [0.42, 1.33]	0.323	0.81 [0.45, 1.45]	0.481
Application of TKI, Yes vs. No	0.77 [0.41, 1.44]	0.415	0.82 [0.44, 1.53]	0.534
Application of VEGF inhibitor, Yes vs. No	0.83 [0.47, 1.45]	0.518	0.90 [0.51, 1.59]	0.723
Chemotherapy, AP vs. others	0.61 [0.37, 1.00]	0.052	0.66 [0.40, 1.09]	0.104
Smoking, Yes vs. No	1.23 [0.84, 1.79]	0.292	0.90 [0.56, 1.43]	0.645
Hypertension, Yes vs. No	1.13 [0.78, 1.64]	0.531	1.10 [0.75, 1.60]	0.622
Diabetes, Yes vs. No	0.90 [0.51, 1.61]	0.726	0.97 [0.54, 1.75]	0.927
Hyperlipemia, Yes vs. No	0.79 [0.42, 1.48]	0.461	0.77 [0.40, 1.46]	0.421
Heart failure, Yes vs. No	5.61 [1.71, 18.42]	0.004**	6.43 [1.94, 21.26]	0.002**
ACS, Yes vs. No	0.74 [0.23, 2.35]	0.612	0.69 [0.21, 2.21]	0.53
KPS score, >80 vs. ≤ 80	0.52 [0.35, 0.75]	0.001**	0.51 [0.35, 0.74]	<0.001***
miR-219-5p ≥ 1.50 vs. < 1.50	0.36 [0.25, 0.53]	<0.001***	0.37 [0.25, 0.55]	<0.001***

Abbreviation: HR, hazard risk; BMI, Body Mass Index; CRP, C-reactive protein; PNI, neutrophil lymphocyte ratio; NLR, neutrophil lymphocyte ratio; SCLC, small-cell lung cancer; TKI, Tyrosine Kinase Inhibitor; VEGF, vascular endothelial growth factor; KPS, Karnofsky Performance Status; ACS, acute coronary syndrome. ***p<0.001, **p<0.01, *p<0.05.

Model 1: Adjusted by age and gender

Independent Prognostic Factors for OS of Patients With SCLC

After multivariate adjustment, the miR-219-5p high level (HR 0.39, 95% CI 0.26-0.59, $P < 0.001$) was also associated with a low increase in the risk of death (figure 3). Meanwhile, gender, PNI score, immunotherapy and heart failure were also the independent risk factors for OS.

Development and Validation of an OS-predicting Nomogram

The independently related risk factors derived from the multivariate analysis were used to create an OS estimation nomogram (figure 4). The prognostic model was internally validated according to the bootstrap validation method. With an unadjusted

C index of 0.691 and a bootstrap-corrected C index of 0.691, the nomogram displayed excellent accuracy in estimating the risk of OS. In the validation cohort, the nomogram showed a C index of 0.691 for the estimation of OS. A suitable calibration curve for risk estimation was also displayed ($R^2=0.455$, LR $\chi^2=80.55$) (figure 4B). We collected 86 NSCLC patients from Sichuan Cancer Hospital in the external validation step. The ROC curve showed an AUC of 0.783 (0.743-0.822) for predicting 5-year overall survival, compared with an AUC of 0.749 (0.709-0.788) for the external validation data (figure 5).

Discussion

In this study, we detected the expression of miR-219-5p in a large cohort of SCLC patients at a single institution, between Mar 2010 and June 2015. The results suggested that reduced expression of miR-219-5p was significantly correlated with unfavorable clinical features. Moreover, patients in high miR-219-5p expression group displayed better OS compared with those in low miR-219-5p expression group. The multivariate analysis demonstrated miR-219-5p an independent prognostic factor for OS. In addition, to propose, and retrospectively verify in an independent cohort of patients, these independent risk factors were applied to establish a nomogram for OS estimation. The nomogram revealed good accuracy in estimating the risk of OS.

Carcinogenesis involves multiple biological processes which are related to many key genes[15, 16]. The characteristics of cancer occurrence represent properties that a cell acquire a certain ability to become and maintain itself as a cancer cell[17]. The key genes guide the cellular signaling pathways related to occurrence and progression of cancers[18, 19]. Using miRNA expression to predict the clinical diagnosis and prognosis of cancer has more advantages than mRNAs, as miRNAs are proved to be the vital posttranscriptional regulators of gene expression[20, 21]. In comparison with mRNAs, these vital gene regulators are highly conserved among species[22].

It has been reported that miRNAs were related to the initiation and progression of various cancers, and many miRNAs have been identified as a promising biomarker

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4 for prognostic prediction of cancer[10, 23]. Recently, some miRNAs have been
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6 proved to be a novel prognostic biomarker for SCLC[24, 25]. A study of Yu et al.
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8 indicated that miR-92a-2 was significantly higher in SCLC patients group compared
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10 to healthy control, and detection of miR-92a-2 levels could be a potential biomarker
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12 for patients with SCLC[26]. As a promising biomarker, miR-219-5p has been
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14 identified as a prognostic factor for different cancers. Long et al. found that
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16 miR-219-5p expression level was distinctly decreased in melanoma tissues and cell
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18 lines, and the modulation of miR-219-5p expression could be a prognostic biomarker
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20 and treatment strategy in melanoma[27]. A study from Huang et al. suggested a role
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22 of miR-219-5p for prognostic prediction and therapeutic strategy in colorectal
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24 cancer[28]. However, there is no studies exploring the role of miR-219-5p for
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26 biomarker in patients with SCLC. To the best of our knowledge, this study was the
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28 first attempt ever made to comprehensively evaluate the role for prognostic prediction
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30 based on miR-219-5p expression in patients with SCLC. In the current study, we
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32 initially examined the expression levels of miR-219-5p in SCLC patients. We, for the
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34 first time, demonstrated a correlation of the altered miR-219-5p expression with
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36 available clinical parameters. We found that miR-219-5p was significantly associated
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38 with lymphocytes count, PNI score and stage of SCLC. The univariate analysis
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40 indicated that increased miR-219-5p expression was a protective predictor for
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42 mortality. Kaplan-Meier curve displayed that patients with elevated miR-219-5p
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44 expression levels or accepted immunotherapy had low cumulative incidence of death
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46 compared to those with reduced miR-219-5p expression or unaccepted
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4 immunotherapy, respectively. In addition, gender, age, serum CRP level, albumin
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6 level, lymphocytes count, PNI score, immunotherapy, heart failure, KPS score and
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8 miR-219-5p level were associated with overall mortality. The multivariate analysis
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10 showed that miR-219-5p, gender, PNI score, immunotherapy and heart failure could
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12 predict OS as the independent risk factors.
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17 Nomograms are applied for visualization of statistical models, graphical
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19 evaluation of variable significance and examination of predicted values[29, 30]. They
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21 have been widely performed to predict cancer risks and therapeutic outcomes[31, 32].
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23 Most recently, several studies have successfully established a prognostic nomogram
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25 that combined a miRNA with clinical-related variables for OS estimation in different
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27 cancers[33-35]. Although nomograms are becoming increasingly popular, no studies
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29 have built prognostic models using combination of miR-219-5p and clinical risk
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31 factors in SCLC patients. In this study, based on the combination of miR-219-5p and
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33 independent clinicopathological variables, we created a nomogram model that could
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35 provide an individual prognostic prediction for OS estimation in SCLC patients. The
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37 results indicated excellent accuracy in estimating the risk of OS. There was a suitable
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39 calibration curve for risk estimation, indicating a well-performed nomogram, and
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41 good agreements between observation and prediction. To further verify the accuracy
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43 and efficiency of the model, an external date containing 86 patients from Sichuan
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45 Cancer Hospital was conducted. The results indicated that the prognosic model could
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47 accurately predict the prognosis of SCLC patients. Hence, this was the first prognostic
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49 nomogram for patients with SCLC that considered clinical parameters in addition to
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51 miR-219-5p. This nomogram could provide comprehensive information for patients,
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53 as well as a better guidance for clinical therapy. Based on the model, the potential
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55 high-risk patients with low survival rate could be more accurately selected for a
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57 specific therapeutic strategy.

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59 There are some limitations in this article. Firstly, experimental research
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explaining the biological processes of miR-219-5p is needed. Thus, the molecular

mechanism of miR-219-5p should be investigated in further research. Secondly, the prognostic nomogram needs to be further calculated by a prospective and large-scale multicenter study before it can be applied to clinical practice.

CONCLUSIONS

In conclusion, we found that the miR-219-5p expression levels were significantly correlated with clinical parameters of SCLC patients. Furthermore, miR-219-5p was proved to be an independent factor for prognostic prediction in patients with SCLC. Moreover, nomogram based on multivariate analysis showed excellent accuracy in estimating the risk of OS.

Acknowledgements The authors would like to thank the referees and the associate editor for their constructive advice.

Contributors ZHX and XMW designed the study. XHZ, JGZ and MQX collected and analysed the data. XHZ, JGZ and MQX drafted the initial manuscript. ZHX and XMW reviewed and edited the article. All authors read and approved the final manuscript.

Funding Not applicable.

Competing interests The authors declare that they have no competing interests.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Ethics approval

The study was approved by ethics committee of Suzhou Xiangcheng People's Hospital. The reference number was 20140193. All procedures performed in the present study were in accordance with the principles outlined in the 1964 Helsinki Declaration and its later amendments.

Provenance and peer review Not commissioned; externally peer reviewed.

Data Availability Statement The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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Figure Legends

Figure 1 A flow chart of the screening process.

Figure 2 Overall survival (OS) of SCLC patients in different levels of miR-219-5p and different treatments. (A) OS of SCLC patients with high or low level of miR-219-5p. (B) OS of SCLC patients with different treatments (immunotherapy vs non-immunotherapy).

Figure 3 Multivariate cox regression analysis of 5-year overall survival on data in the SCLC patients.

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6 **Figure 4** Nomogram for overall survival (OS) risk estimation of SCLC patients and
7 its predictive performance. (A) Nomogram to estimate the OS risk of SCLC patients
8 in different variations. To build the nomogram, find the position of each variable on
9 the corresponding axis, draw a line to the points axis for the number of points, add the
10 points from all of the variables, and draw a line from the total points axis to determine
11 the OS probabilities at the lower line of the nomogram. (B) Validity of the predictive
12 performance of the nomogram in estimating the OS risk of SCLC patients.
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21 **Figure 5** External validation of the prognostic model.
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25 **Table 1** Study participant characteristics at enrollment.
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29 **Table 2** Univariate cox regression analysis of overall survival on SCLC patients.
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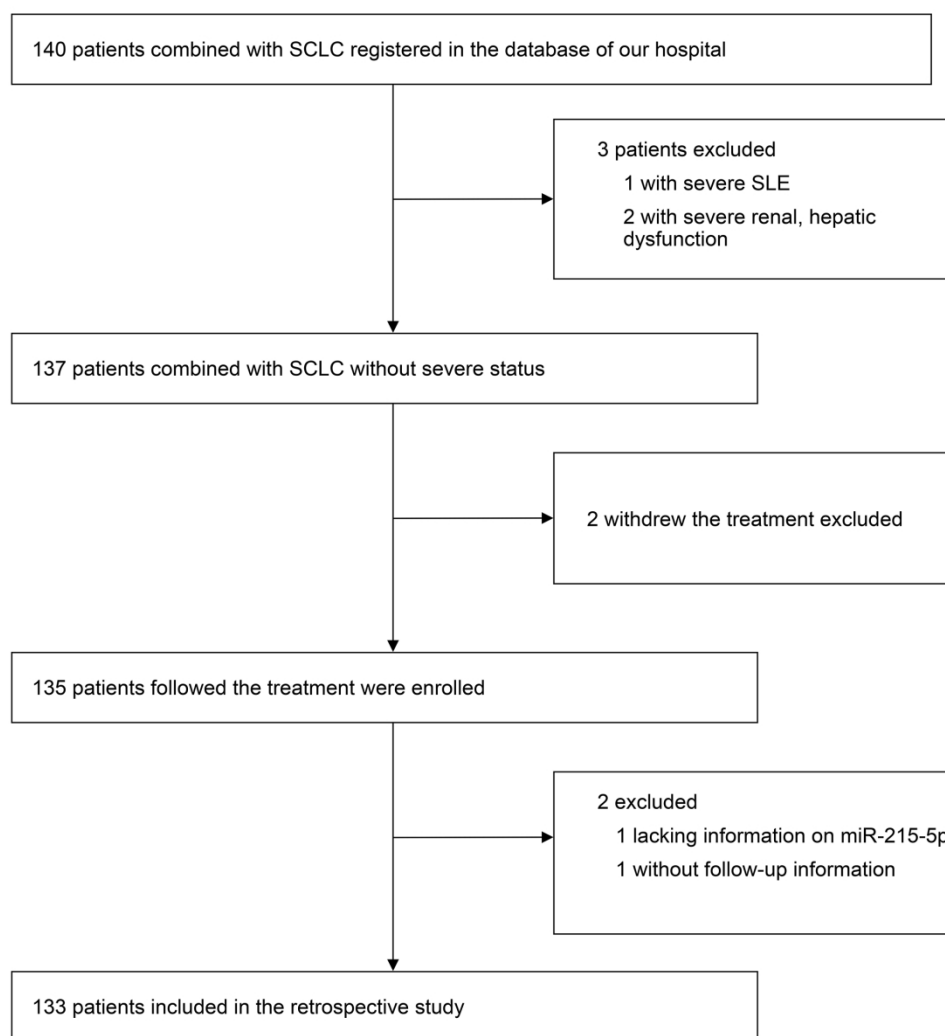


Figure 1 A flow chart of the screening process.

180x195mm (300 x 300 DPI)

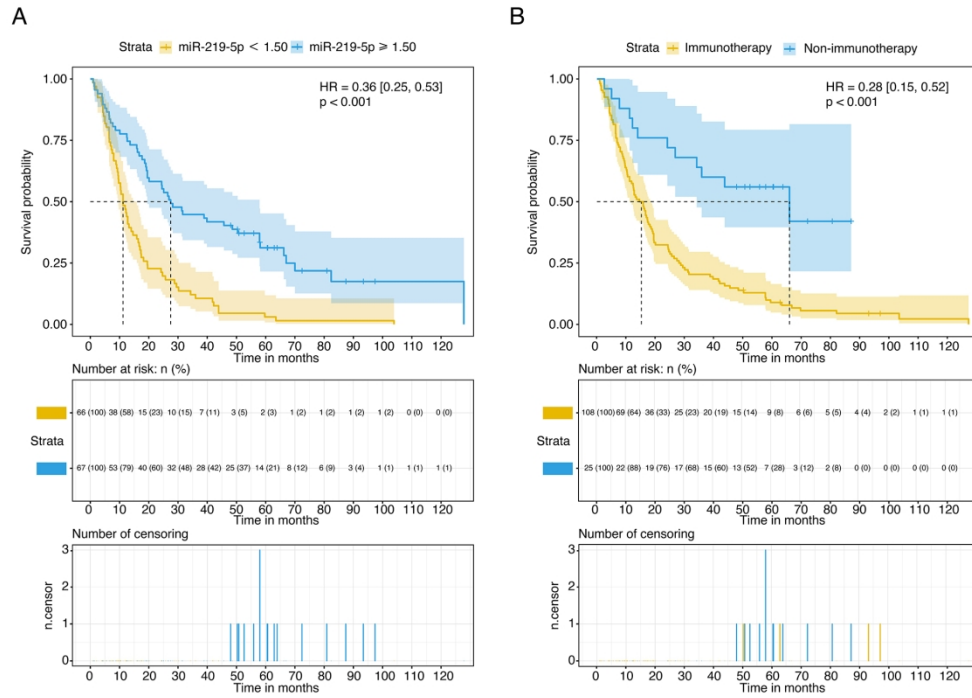


Figure 2 Overall survival (OS) of SCLC patients in different levels of miR-219-5p and different treatments. (A) OS of SCLC patients with high or low level of miR-219-5p. (B) OS of SCLC patients with different treatments (immunotherapy vs non-immunotherapy).

180x126mm (300 x 300 DPI)

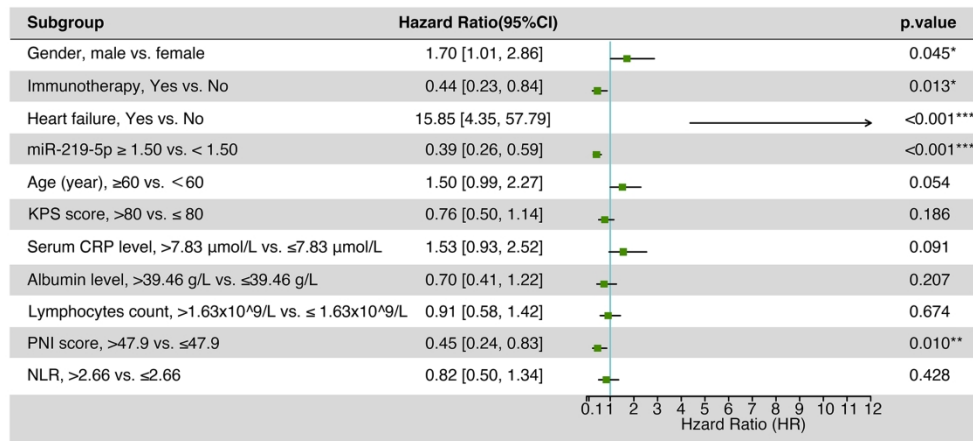
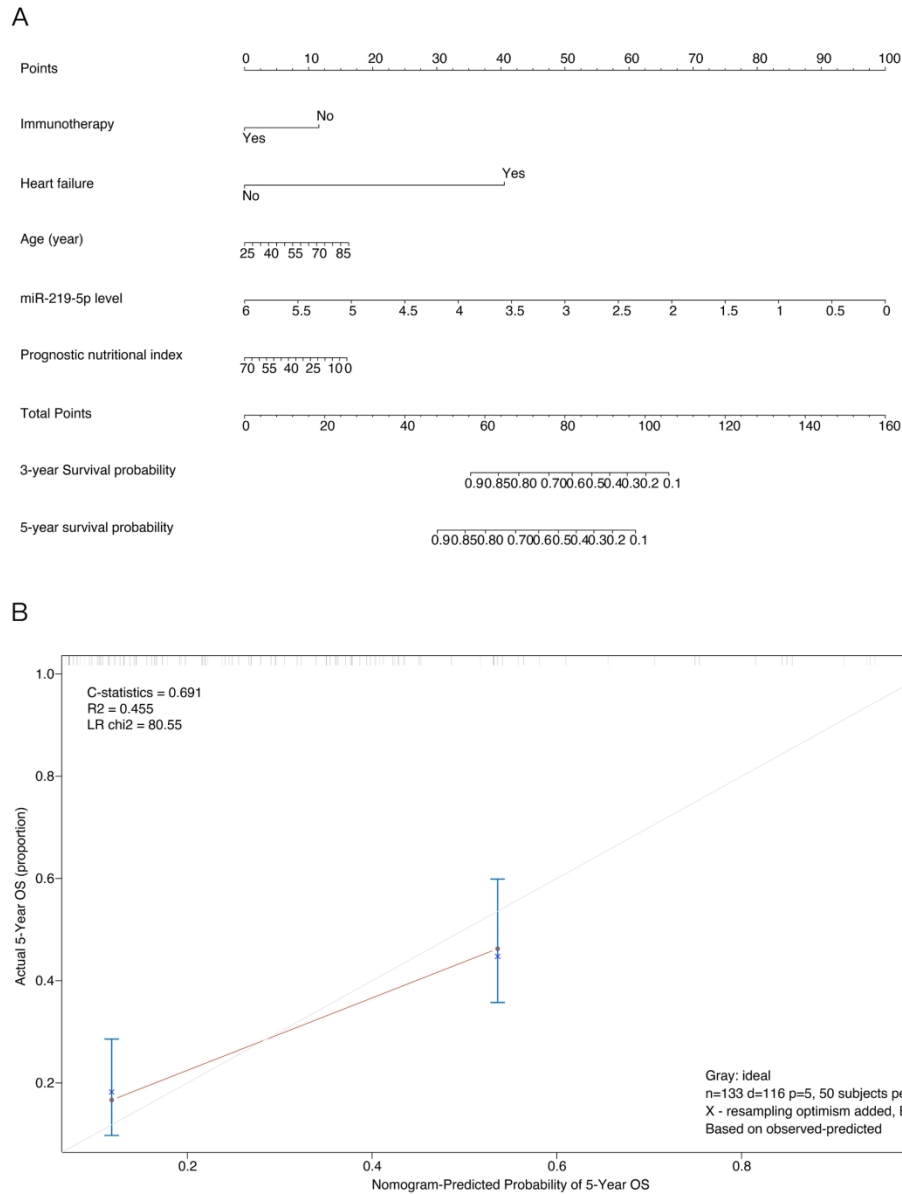


Figure 3 Multivariate cox regression analysis of 5-year overall survival on data in the SCLC patients.

180x83mm (300 x 300 DPI)



45 Figure 4 Nomogram for overall survival (OS) risk estimation of SCLC patients and its predictive performance.
46 (A) Nomogram to estimate the OS risk of SCLC patients in different variations. To build the nomogram, find
47 the position of each variable on the corresponding axis, draw a line to the points axis for the number of
48 points, add the points from all of the variables, and draw a line from the total points axis to determine the
49 OS probabilities at the lower line of the nomogram. (B) Validity of the predictive performance of the
50 nomogram in estimating the OS risk of SCLC patients.

51 180x216mm (300 x 300 DPI)

Figure 5

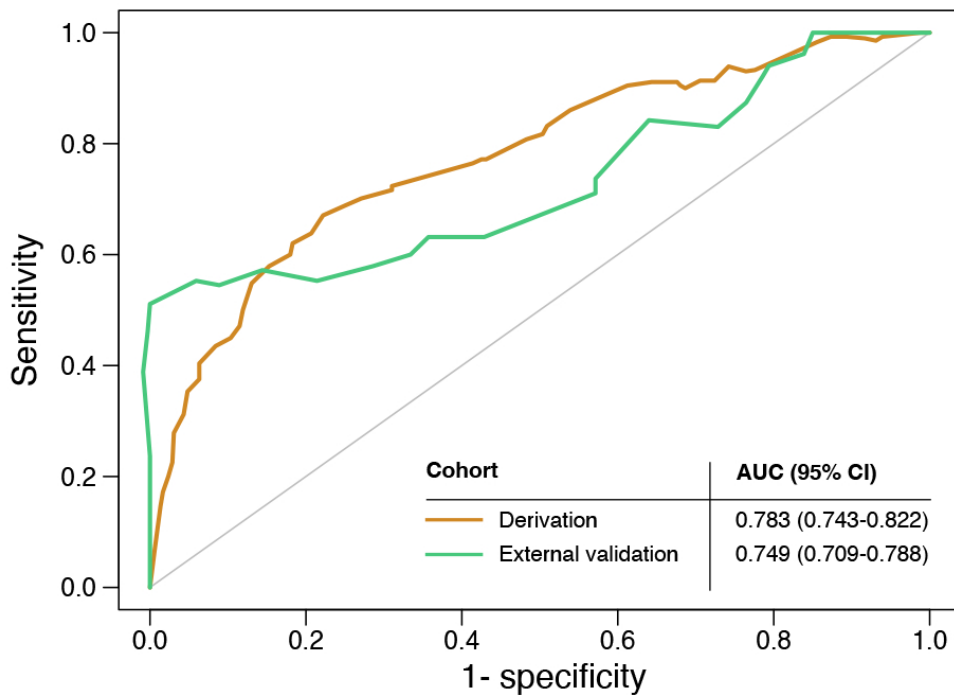


Figure 5 External validation of the prognostic model.

Supplement Materials

Part I. Details of variants

Patients' age: The age of patients was based on the time of diagnosis of NSCLC.

Body mass index: The equation of body mass index (BMI) is calculated as follow:
$$\text{BMI (kg/m}^2\text{)} = \text{weight (kg)} / \text{height (m)}^2$$
. The data of BMI was recorded before clinical treatment.

Serum CEA level: The serum level of carcinoembryonic antigen (CEA) is one of tumor marks, the higher serum CEA level than that of healthy individuals led to its clinical application as a diagnostic biomarker for cancer.

Serum CRP level: C-reactive protein (CRP) is detected by colorimetric determination through fasting blood collection and CRP is one of the inflammation markers.

Albumin level: Serum albumin is detected by colorimetric determination through fasting blood collection. And serum albumin is one of the indicators of nutritional status.

Neutrophils count, lymphocytes count, hemoglobin level, platelet count: All these indexes were extracted from blood routine examination to evaluate preoperative status of patients.

PNI score: Prognostic nutritional index (PNI) is an index to evaluate the nutritional status of surgical patients, to predict the risk of surgery and to help make prognostic judgment. The equation is listed as below: $\text{PNI} = \text{serum albumin (g/L)} + 5 * \text{lymphocytes (*}10^9\text{/L)}$.

NLR: Neutrophil to Lymphocyte Ratio (NLR) is an index of inflammation markers to predict the prognosis of various cancer, including lung cancer.

Pathologic type: Non-small cell lung cancer can be classified as adenocarcinoma, squamous cell carcinoma, large cell lung cancer, or others, depending on surgical or tumor biopsy specimens

Stage of NSCLC: Non-small cell lung cancer was staged according to the seventh edition of international union against cancer (UICC) TNM was published in 2009

Surgery: For early and locally advanced NSCLC, which is generally acceptable, there is a history of surgical resection.

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Therapy of radiation: Radiation therapy is a medical procedure that uses the delivery of high-energy radiation to kill cancer cells and shrink tumors, including external beam radiation therapy, internal radiation therapy and stereotactic body radiotherapy (SBRT). It aims to cure small tumors, treat lung cancer both locally and nearby lymph nodes, treat metastases, relieve symptoms, and also at prevention.

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Application of platinum: Platinum-based chemotherapy may be used as a first or second line therapy for advanced lung cancer, after surgery (adjuvant chemotherapy), or before surgery to reduce the size of a tumor (neoadjuvant therapy). The most commonly used platinum drugs are cisplatin, carboplatin and nedaplatin. Treatment of non-small cell lung cancer begins with either cisplatin or carboplatin combined with another medication, such as docetaxel, pemetrexed, paclitaxel, gemcitabine and gemcitabine.

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Target therapy: Targeted therapies for lung cancer are drugs that are tailored to attack certain mutations of cancer cells. These mutations lead to the production of abnormal proteins which guide the growth and development of a cancer cell. Common driver mutations include EGFR mutations, ALK rearrangements, ROS1 rearrangements, MET amplifications, KRAS mutations, HER2 mutations, BRAF mutations and so on.

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Application of TKI: Tyrosine kinase inhibitors (TKI) act as competitive inhibitors of ATP binding to tyrosine kinases, or as tyrosine analogs that block tyrosine kinase activity and inhibit cell proliferation. Epithelial growth factor receptor (EGFR) is a protein that is present on the surface of both normal cells and lung cancer cells. Mutations in EGFR can occur at different locations on exon 18 to 21. ALK-positive lung cancer refers to people who have lung cancer that tests positive for the EML4-ALK fusion mutation. Several FDA-approved medications available to treat EGFR-positive and ALK-positive lung adenocarcinoma, including:

- 41
- 42 • EGFR inhibitors - Tarceva (erlotinib), Gilotrif (afatinib), Iressa (gefitinib),
- 43 Tagrisso (osimertinib), and Portrazza (necitumumab)
- 44 • ALK inhibitors - Xalkori (crizotinib), Zykadia (ceritinib), and Alecensa
- 45 (alectinib)
- 46 • ROS1 inhibitor - Xalkori (crizotinib)
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Application of VEGF inhibitor: VEGF (Vascular endothelial growth factor) can activate angiogenesis through different signaling pathways. VEGF inhibitors are drugs that block the ability of tumors to form new blood vessels, and hence, grow and spread. Some currently available medications include bevacizumab, ramucirumab, axitinib, endostar and anlotinib.

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KPS score: A performance measure for rating the ability of a person to perform usual activities, evaluating a patient's progress after a therapeutic procedure, and determining a patient's suitability for therapy. It is used most commonly in the

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3 prognosis of cancer therapy, usually after chemotherapy and customarily administered
4 before and after therapy. It was named for Dr. David A. Karnofsky, an American
5 specialist in cancer chemotherapy. Patients with more than 80 scores had better
6 postoperative status and longer survival time. And patients with more than 70 scores
7 can suffer from chemoradiotherapy.
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11 **Smoking:** Smoker refers to continuous or cumulative smoking > 1 cigarette/day over
12 a lifetime of more than 6 months. (1997, WHO)
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15 **Hypertension:** Hypertension is defined as a repeatedly elevated blood pressure
16 exceeding 140 over 90 mmHg.
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19 **Diabetes:** Diabetes is a group of metabolic diseases characterized by hyperglycemia.
20 And fasting blood glucose more than 7.0 mmol/l or blood glucose more than 11.1
21 mmol/l within 2 hours after meal can be diagnosed diabetes.
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24 **Hyperlipemia:** Hyperlipemia means the presence of excess fat or lipids in the blood.
25 And total cholesterol ≥ 6.2 mmol/L, low density lipoprotein cholesterol ≥ 4.1 mmol/L,
26 triglyceride ≥ 2.3 mmol/L, high density lipoprotein cholesterol < 1.0 mmol/L can be
27 diagnosed hyperlipemia.
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30 **Heart failure:** The information was recorded through history taking and verified after
31 hospitalization. Heart failure means inability of the heart to keep up with the demands
32 on it and, specifically, failure of the heart to pump blood with normal efficiency. Heart
33 failure may be due to failure of the right or left or both ventricles. The signs and
34 symptoms depend upon which side of the heart is failing. They can include shortness
35 of breath (dyspnea), asthma due to the heart (cardiac asthma), pooling of blood (stasis)
36 in the general body (systemic) circulation or in the liver's (portal) circulation, swelling
37 (edema), blueness or duskiness (cyanosis), and enlargement (hypertrophy) of the
38 heart.
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43 **ACS:** Acute coronary syndrome is a term for a group of conditions that suddenly stop
44 or severely reduce blood from flowing to the heart. When blood cannot flow to the
45 heart, the heart muscle can become damaged. Heart attack and unstable angina are
46 both acute coronary syndromes (ACS).
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50 **Withdraw treatment:** Reasons for patients withdrew from treatment were listed
51 below: 1) High cost of the treatment: In China, the cost of hormone therapy can be up
52 to 3000 RMB a month if without health insurance. 2) Poor patient compliance: Some
53 patients discontinue treatment because they do not comply with the treatment plan
54 prescribed by their doctor.
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Part II. Report of sample size assessment

PASS 15.0.5

Tests for Two Survival Curves Using Cox's Proportional Hazards Model

Numeric Results with Ha: HR ≠ 1

	Total Sample Size	Control Sample Size	Trtmnt Sample Size	Prop'n Control N1/N	Hazard Ratio h2/h1	Control Prob Event Pev1	Trtmnt Prob Event Pev2	Control Events E1	Trtmnt Events E2	Alpha	Beta
Power	N	N1	N2	P1	HR	Pev1	Pev2	E1	E2	0.050	0.100
0.9005	103	51	52	0.500	0.500	0.750	0.950	38.3	49.4		

References

Chow, S.C., Shao, J., Wang, H. 2008. Sample Size Calculations in Clinical Research, 2nd Edition. Chapman & Hall/CRC.

Schoenfeld, David A. 1983. 'Sample Size Formula for the Proportional-Hazards Regression Model', Biometrics, Volume 39, Pages 499-503.

Report Definitions

Power is the probability of rejecting a false null hypothesis. Power should be close to one.

N is the total sample size.

N1 and N2 are the sample sizes of the control and treatment groups.

P1 is the proportion of the total sample that is in the control group, group 1.

HR is the hazard ratio: h2/h1.

Pev1 and Pev2 are the probabilities of an event in the control and the treatment groups.

E1 and E2 are the number of events required in the control and the treatment groups.

Alpha is the probability of a type one error: rejecting a true null hypothesis.

Beta is the probability of a type two error: failing to reject a false null hypothesis.

Summary Statements

A two-sided test of whether the hazard ratio is one with an overall sample size of 103 subjects (of which 51 are in the control group and 52 are in the treatment group) achieves 90% power at a 0.050 significance level when the hazard ratio is actually 0.500. The number of events required to achieve this power is 87.7. It is anticipated that proportions of subjects having the event during the study is 0.750 for the control group and 0.950 for the treatment group. These results assume that the hazard ratio is constant throughout the study and that Cox proportional hazards regression is used to analyze the data.

Procedure Input Settings

Autosaved Template File

\\Mac\Home\Documents\PASS 15\Procedure Templates\Autosave\Tests for Two Survival Curves Using Cox's

Proportional Hazards Model - Autosaved 2020_1_24-9_57_39.t92

Design Tab

Solve For:	Sample Size
Alternative Hypothesis:	Ha: HR \neq 1
Power:	0.90
Alpha:	0.05
Group Allocation:	Equal (N1 = N2)
Pev1 (Probability of a Control Event):	0.750
Pev2 (Probability of a Treatment Event):	0.950
HR (Actual Hazard Ratio = h2/h1):	0.5

For peer review only

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of cohort studies

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6
		(b) For matched studies, give matching criteria and number of exposed and unexposed	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	7
		(b) Describe any methods used to examine subgroups and interactions	7
		(c) Explain how missing data were addressed	7
		(d) If applicable, explain how loss to follow-up was addressed	7
		(e) Describe any sensitivity analyses	7
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	8
		(c) Consider use of a flow diagram	8
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9
		(b) Indicate number of participants with missing data for each variable of interest	9
		(c) Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	Report numbers of outcome events or summary measures over time	10
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10
		(b) Report category boundaries when continuous variables were categorized	11
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	11
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12
Discussion			
Key results	18	Summarise key results with reference to study objectives	13-14
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

MiR-219-5p decrease the risk of cancer-related mortality in patients with small cell lung cancer

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-064700.R1
Article Type:	Original research
Date Submitted by the Author:	29-Sep-2022
Complete List of Authors:	Cao, Zhijun; Suzhou Ninth People's Hospital, Urology Zhang, Jigang; First Affiliated Hospital of Soochow University Zhang, Xiaohui; First Affiliated Hospital of Soochow University Xiang, Mengqi; Medical School of University of Electronic Science and Technology of China, Department of Medical Oncology Xu, Zhihua; First Affiliated Hospital of Soochow University, General surgery Wu, Xiangmei; Suzhou Xiangcheng People's Hospital
Primary Subject Heading:	Oncology
Secondary Subject Heading:	Surgery
Keywords:	Risk management < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, ONCOLOGY, Gene therapy < ONCOLOGY, Molecular aspects < ONCOLOGY, Oncogenes < ONCOLOGY

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4 1 **MiR-219-5p decrease the risk of cancer-related mortality in**
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6
7 2 **patients with small cell lung cancer**

8
9 3 Zhijun Cao^{2,#}, Jigang Zhang^{4,#}, Xiaohui Zhang^{5,#}, Mengqi Xiang⁶, Zhihua Xu^{3,*},
10
11 4 Xiangmei Wu^{1,*}
12
13 5

14 6 ¹ Department of Endocrinology, Suzhou Xiangcheng People's Hospital, Suzhou,
15
16 7 China

17
18 8 ² Department of Urology, Suzhou Ninth People's Hospital, Soochow University,
19
20 9 Suzhou, China

21
22 10 ³ Department of General Surgery, The First Affiliated Hospital of Soochow
23
24 11 University, Suzhou, China

25
26 12 ⁴ Department of Traumatology Surgery, The First Affiliated Hospital of Soochow
27
28 13 University, Suzhou, China

29
30 14 ⁵ Department of Medicine, Respiratory, Emergency and Intensive Care Medicine, The
31
32 15 First Affiliated Hospital of Soochow University, Suzhou, China

33
34 16 ⁶ Department of Medical Oncology, Sichuan Cancer Hospital, Medical School of
35
36 17 University of Electronic Science and Technology of China, Chengdu, Sichuan
37
38 18

39
40 19 Running title: MiR-219-5p decrease the risk of SCLC patients
41
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43
44 21 # These authors contributed equally to this work

45
46 22 * Correspondence to: dr_xiangmeiwu@163.com (Xiangmei Wu) or
47
48 23 dr_zhihuaxu@163.com (Zhihua Xu)
49
50 24

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3
4 25 **ABSTRACT**

5 26 **Objectives** Small cell lung cancer (SCLC) is a lethal human malignancy, and
6
7 previous studies support the contribution of microRNA (miRNA) to cancer
8
9 progression. The prognostic value of miR-219-5p in SCLC patients remains unclear.
10
11 This study evaluated the risk factors for SCLC and created a prediction model for
12
13 them.
14

15 31 **Design** Retrospective observational cohort study.

16
17 32 **Setting** The programme has yielded a database of all patients with SCLC in 2 defined
18
19 geographical regions of China.
20

21 34 **Participants** We did a real-world study, including data from 133 patients with SCLC
22
23 between Mar 1, 2010 and June 1, 2015. We collected 86 NSCLC patients in the
24
25 external validation step.
26

27 37 **Primary and secondary outcome measures** MiR-219-5p was recorded during the
28
29 admission. Cox proportional hazard model was applied for survival analyses and for
30
31 analyzing risk factors for cancer-related mortality and to create a nomogram for
32
33 prediction. The accuracy of the model was evaluated by C-index and calibration
34
35 curve. An external data of 86 SCLC patients from Sichuan Cancer Hospital and the
36
37 First affiliated hospital of Soochow University was conducted.

38
39 43 **Results** In our data, the mortality in group with high miR-219-5p level (≥ 1.50) was
40
41 74.6%. Based on univariate analysis, we put factors ($P < 0.05$) into a multivariate
42
43 regression model, patients with high miR-219-5p level ($P < 0.001$, HR=0.36),
44
45 immunotherapy ($P < 0.001$, HR=0.44), PNI score > 47.9 ($P = 0.01$, HR=0.45) remained
46
47 statistically factors for better overall survival (OS) and regarded as independent
48
49 protective factors. These independently associated risk factors were used to establish
50
51 an OS estimation nomogram. Nomogram revealed good accuracy in estimating the
52
53 risk, with a bootstrap-corrected C index of 0.691. External validation displayed an
54
55 AUC of 0.749 (0.709-0.788).

56 52 **Conclusions** MiR-219-5p decreased the risk of cancer-related mortality in patients
57
58 with SCLC. Nomogram based on multivariate analysis demonstrated good accuracy
59
60 in estimating the risk of overall mortality.

1
2
3
4 55 **Keywords:** small cell lung cancer, miR-219-5p, overall survival, nomogram,
56 prediction model

57

58 **Strengths and limitations of this study**

59 ▶ The study established the first risk nomogram for predicting the 3-year and 5-year
60 specific mortality probability for SCLC

61 ▶ Based on the retrospective sample, the nomogram can improve the ability of
62 clinicians in predicting survival probabilities in individual patients.

63 ▶ The model is not comprehensive enough because the epidemiology and end results
64 database does not include all prognostic factors for SCLC.

65 ▶ The available data on treatment status are not detailed enough to distinguish the
66 impact of various treatment plans.

67 ▶ The model needs to be prospectively studied to confirm its reliability

68

69 BACKGROUND

70 Lung cancer is the leading cause of cancer deaths worldwide, with millions of new
71 cases diagnosed each year[1]. Small cell lung cancer (SCLC) is a kind of
72 neuroendocrine malignant tumor with poor prognosis, accounting for about 15% of
73 lung cancer patients[2]. SCLC is generally divided into limited disease (LD-SCLC)
74 and extensive disease (ED-SCLC). Platinum combined with etoposide is the first-line
75 therapeutic strategy of SCLC, and most patients are easy to receive initial
76 chemotherapy[3]. However, the 5-year survival rates of LD-SCLC and ED-SCLC are
77 only 15% and 3%, respectively[4]. Therefore, improvement in early diagnosis and
78 prognostic prediction of SCLC is vital.

79 MicroRNAs (miRNAs) are endogenous non-coding RNAs (~ 22 nt), which
80 regulate mRNA activity by hybridization with 3' - untranslated region (UTR) of
81 specific genes[5]. Many studies have shown that miRNAs could participate in a
82 variety of cell biological processes, including cell growth, differentiation and
83 apoptosis[6, 7]. In addition, researches have demonstrated that miRNAs are
84 frequently dysregulated in cancers[8, 9], and some miRNAs can serve as diagnostic
85 and prognostic biomarkers for cancers[10]. Recently, several miRNAs have been
86 proved to participate in the occurrence and development of SCLC, but few of them
87 are likely to be a biomarker or therapeutic target for SCLC.

88 Recently, miR-219-5p has been found to be abnormally expressed and play a
89 significant role in different cancers. Ma et al. found that the expression of miR-219-5p
90 was significantly decreased in esophageal squamous cell carcinoma (ESCC) tissues
91 compared with normal tissues[11]. A study of Gong et al. revealed a tumor
92 suppressive role for miR-219-5p by targeting glypican-3 in hepatocellular carcinoma
93 (HCC)[12]. On the contrary, Yang et al. indicated that miR-219-5p could promote cell
94 growth and metastasis of HCC and serve as a prognostic marker for HCC
95 patients[13]. A research investigated by Wei et al. suggested that miR-219-5p could
96 inhibit proliferation, migration and invasion of epithelial ovarian cancer through
97 downregulation of the Wnt signaling pathway, and it could serve as a diagnostic

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4 98 biomarker and therapeutic target for epithelial ovarian cancer[14]. However, the
5
6 99 biological functions of miR-219-5p and its potential prognostic role for biomarker in
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8 100 SCLC are still unknown.

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10 101 In this study, we aimed to examine the variation in the expression levels of
11
12 102 miR-219-5p in patients with SCLC and explored the potential prognostic role of
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14 103 miR-219-5p for SCLC. We also displayed a nomogram that could provide
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16 104 individualized, evidence-based, highly accurate risk estimates. Nomograms were easy
17
18 105 to performed and could facilitate management-related decision making.

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21 107 **METHODS**

22 108 **Study Design and Patient Characteristics**

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27 109 We did a real-world study, including data obtained from 133 patients with SCLC
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29 110 between Mar 2010 and June 2015, in the Suzhou Xiangcheng People's Hospital.

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31
32 111 Those participants who lacked information on complement components data,
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34 112 withdrew from treatment or lacked follow-up information were excluded. Clinical
35
36
37 113 information of patients, including gender, age, BMI, neutrophils count, lymphocytes
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40 114 count, serum CEA level, CRP level, albumin level, hemoglobin level, stage of SCLC,
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42 115 platelet count, PNI score, KPS score, NLR, pathologic type, immunotherapy, therapy
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44
45 116 of radiation, application of platinum, application of VEGF inhibitor, target therapy,
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47 117 application of TKI, smoking, ACS, diabetes, heart failure and hyperlipemia, were
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49
50 118 recorded. Diagnosis of SCLC was confirmed by histopathological examination. The
51
52
53 119 median length of follow-up was 23.6 months. Median was used as the cut-off value.

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56 120 The definition and details of all the variables above were provided in Supplemental
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58 121 Materials Part I. Data from 86 patients with NSCLC at Sichuan Cancer Hospital and
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4 122 the First affiliated hospital of Soochow University were applied for external
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6 123 validation. Inform, and consent was obtained from all patients or their immediate
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9 124 family members. All protocols were in line with the guidelines with the ethic
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11 125 committee of Suzhou Xiangcheng People's Hospital, Sichuan Cancer Hospital, the
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14 126 First affiliated hospital of Soochow University and following the Declaration of
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17 127 Helsinki.
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21 22 129 **Assays for Detection of MiR-219-5p Levels**

23
24 130 The quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was
25
26 131 conducted for the detection of miR-219-5p expression levels.

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28 132 Total RNA from tissues was isolated and extracted using miRcute Extraction and
29
30 133 Separation of miRNAs kit (Tiangen Biotech Co., Ltd., Beijing, China), and then
31
32 134 reversely transcribed into cDNA by PrimeScript™ II 1st strand cDNA synthesis kit
33
34 135 (Takara Biotechnology Co., Ltd., Dalian, China) according to the manufacturer's
35
36 136 protocol. SYBR PrimeScript miRNA RT-PCR kit (Takara Biotechnology Co., Ltd.)
37
38 137 was used for qRT-PCR. The thermocycling conditions were as follows: one cycle at
39
40 138 95°C for 3 min (initial denaturation), 40 cycles at 95°C for 15 s and 60°C for 30 s. U6
41
42 139 small nuclear RNA (U6) served as the respective internal control. The relative
43
44 140 expression of miR-219-5p was quantified by the $2^{-\Delta\Delta C_t}$ methods, and normalized to
45
46 141 the U6. The following primers were used: miR-219-5p forward,
47
48 142 5'-ACACTCCAGCTGGGTGATTGTCCAAACGCAAT-3' and reverse,
49
50 143 5'-CTCAACTGGTGTCGTGGA-3'; U6 forward,
51
52 144 5'-GCTTCGGCAGCACATATACTAAAAT-3' and reverse,
53
54 145 5'-CGCTTCACGAATTTGCGTGTGCAT-3'. The experiments were repeated at least 3
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56 146 times.

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59 60 148 **Statistical Analysis**

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4 149 Sample size assessment was performed using NCSS-PASS software version 11.0
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6 150 (<https://www.ncss.com/software/pass/>). Power was set as 0.99, and alpha was 0.5. The
7
8
9 151 mortalities of both miR-219-5p high-level group and miR-219-5p low-level group in
10
11 152 our previous data (2008-2009) (0.750 and 0.950) were entered into the PASS. The
12
13
14 153 Actual Hazard Ratio was set as 0.50. Then the sample size was calculated using
15
16
17 154 PASS, and the minimum sample size was 103 (control = 51, experiment = 43). Our
18
19
20 155 sample size was 133 (66 and 67 for each group), which was suitable. The report of
21
22 156 sample size assessment was displayed in Supplemental Material Part II. The missing
23
24
25 157 data (<5.0%) were estimated by random forest algorithm using the mice package in
26
27 158 RStudio (R version 3.6.1). Categorical variates were presented as percentages and
28
29
30 159 compared via the κ^2 test. Continuous variates with skewed and normal distributions
31
32 160 were presented as median with interquartile ranges and mean \pm standard deviation.
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34
35 161 The Mann-Whitney U test and the unpaired t-test were applied for comparison
36
37
38 162 between Groups. Cumulative mortality was showed by the Kaplan-Meier curve and
39
40 163 analyzed by the log-rank test. Univariate and multivariate survival analyses for OS
41
42
43 164 were conducted using the Cox regression model. The forest plots were used to
44
45
46 165 visualize the significance of covariates to the prognosis. The restricted cubic spline
47
48 166 analyses were performed with Harrell's Regression Modelling Strategies (rms)
49
50
51 167 package.

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53 168 We screened multifactor analysis for statistically significant indicators for
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55 169 inclusion in the prediction model. To build the nomogram, find the position of each
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57 170 variable on the corresponding axis, draw a line to the points axis for the number of
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59 171 points, add the points from all the variables, and draw a line from the total points axis
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4 172 to determine the OS probabilities at the lower line of the nomogram. The contribution
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6 173 of each covariate was quantified and visualized in a prognostic nomogram with
7
8 174 internal validation via 1000-times bootstrapping. The consistency of the resulting
9
10 175 model was assessed by the calibration assay. Decision curve analyses were performed
11
12 176 to evaluate net clinical benefits of the model compared with conventional prognostic
13
14 177 scores. The scatter plots were applied for visualization of the consistency of each
15
16 178 model. A 1000-time bootstrapping was employed as indicated. The association
17
18 179 between miR-219-5p class and survival endpoints was evaluated by Kaplan-Meier
19
20 180 curves and log-rank test. Statistical analysis was performed using the RStudio (R
21
22 181 version 3.6.1) with the following packages: 'ggplot2', 'rms', 'PredictABLE', 'risk
23
24 182 regression', and 'survminer'.

25 183

26 27 184 **Patient and public involvement**

28
29 185 This study was conducted without patient involvement. Patients were not invited to
30
31 186 comment on the study design and were not consulted to develop patient-relevant
32
33 187 outcomes or interpret the results. Moreover, patients were not allowed to contribute to
34
35 188 the writing or editing of this document for readability or accuracy.

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38 39 190 **RESULTS**

40 41 191 **Baseline Characteristics**

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43
44 192 A total of 133 SCLC patients who were diagnosed between Mar 2010 and June 2015
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46
47 193 were included in this study. A flow chart of the screening process was shown in [figure](#)
48
49 194 [1](#). The median age of these patients was 64 years old (58-70), and it contained 106
50
51 195 (80.0%) males. Median serum CEA and CRP level was 3.43 ng/ml and 7.83 μ mol/L,
52
53
54 196 respectively. For the stage of SCLC, 51 (38.0%) patients were diagnosed with limited
55
56
57 197 disease, 82 (61.0%) patients were extensive disease. 25 (19.0%) patients accepted the
58
59
60 198 immunotherapy therapy. 54 (41.0%) patients got the therapy of radiation. In addition,

199 platinum was applied for 131 (98.0%) patients, and TKI was used for 15 (12.0%)
 200 patients. KPS score of these patients was examined, and the results revealed that 107
 201 (81.0%) patients got 80 or higher points. The distribution of basic diseases was also
 202 assessed in our data. Diabetes was found in 17 (13.0%) patients, while hyperlipemia
 203 was 13 (10.0%). Cardiovascular diseases such as heart failure and ACS were found in
 204 3 (2.0%) and 4 (3.0%) patients, respectively. 13 (10.0%) patients suffered from
 205 hypertension. In addition, 82 (62.0%) patients had a habit of smoking among all the
 206 patients. The baseline characteristics of these patients were listed in [table 1](#).

207 Among all the 133 patients, the overall mortality was 87.2%. The mortality in
 208 high miR-219-5p level group was 74.6%. Moreover, in the high miR-219-5p level
 209 group, patients with extensive disease were 35 (52.0%), while the low group was 47
 210 (71.0%) ([table 1](#)).

Table 1. Study Participant Characteristics at Enrollment

Variation	Total (n=133)	Cohort, median (IQR)		p.value
		mir-219-5p<1.50, (n=66)	mir-219-5p≥1.50, (n=67)	
Age, (year)	64(58,70)	65(59,70)	63(56.5,68)	0.276
BMI, (kg/m ²)	23.12±3.09	22.99±3.22	23.26±2.98	0.619
Serum CEA level, (ng/ml)	3.43(1.96,9.26)	3.34(1.92,10.05)	3.43(1.99,8.11)	0.87
Serum CRP level, (μmol/L)	7.83(1.68,12.78)	10.68(2.78,13.15)	5.64(1.2,12.16)	0.107
Albumin level, (g/L)	39.46±5.2	38.86±4.68	40.05±5.65	0.188
Neutrophils count, (10 ⁹ /L)	4.55(3.59,5.88)	4.54(3.77,5.75)	4.55(3.48,5.92)	0.975
Lymphocytes count, (10 ⁹ /L)	1.63(1.31,1.95)	1.5(1.21,1.78)	1.73(1.38,2.08)	0.031*
Hemoglobin level, (g/L)	133(125,145)	134(124,145)	132(125,143.5)	0.564
Platelet count, (10 ⁹ /L)	233(184,288)	244.5(180,293.75)	226(184.5,273.5)	0.306
PNI score	47.9(43.95,51.85)	45.98(42.51,50.38)	48.7(44.92,54)	0.026*
NLR	2.66(1.99,4.19)	2.75(2.11,4.57)	2.59(1.92,3.7)	0.232
Gender, (n%)				0.211
Female	27(20)	10(15)	17(25)	
Male	106(80)	56(85)	50(75)	
Metastasis, n(%)				0.299
No	45(34)	19(29)	26(39)	
Yes	88(66)	47(71)	41(61)	
Stage of SCLC				0.038*
Limited disease	51(38)	19(29)	32(48)	

Extensive disease	82(62)	47(71)	35(52)	
Immunotherapy, (n%)				0.197
No	108(81)	57(86)	51(76)	
Yes	25(19)	9(14)	16(24)	
Therapy of radiation, (n%)				0.417
No	79(59)	42(64)	37(55)	
Yes	54(41)	24(36)	30(45)	
Application of platinum, (n%)				0.244
No	2(2)	2(3)	0(0)	
Yes	131(98)	64(97)	67(100)	
Chemotherapy				0.45
AP	28(21)	12(18)	16(24)	
DP	15(11)	6(9)	9(13)	
EP	71(53)	35(53)	36(54)	
GP	3(2)	2(3)	1(1)	
Others	16(12)	11(17)	5(7)	
Target therapy, (n%)				0.627
No	116(87)	59(89)	57(85)	
Yes	17(13)	7(11)	10(15)	
Application of TKI, (n%)				0.449
No	118(89)	60(91)	58(87)	
TKI I	9(7)	4(6)	5(7)	
TKI II	1(1)	1(2)	0(0)	
TKI III	5(4)	1(2)	4(6)	
Application of VEGF inhibitor, n(%)				0.645
No	114(86)	58(88)	56(84)	
Yes	19(14)	8(12)	11(16)	
KPS score, n(%)				0.678
40	2(2)	0(0)	2(3)	
50	5(4)	3(5)	2(3)	
60	7(5)	3(5)	4(6)	
70	12(9)	8(12)	4(6)	
80	29(22)	14(21)	15(22)	
90	56(42)	29(44)	27(40)	
100	22(17)	9(14)	13(19)	
Smoking, n(%)				0.255
No	51(38)	29(44)	22(33)	
Yes	82(62)	37(56)	45(67)	
Hypertension, n(%)				1
No	80(60)	40(61)	40(60)	
Yes	53(40)	26(39)	27(40)	
Diabetes, n(%)				1
No	116(87)	58(88)	58(87)	
Yes	17(13)	8(12)	9(13)	

Hyperlipemia, n(%)				0.579
No	120(90)	61(92)	59(88)	
Yes	13(10)	5(8)	8(12)	
Heart failure, n(%)				1
No	130(98)	65(98)	65(97)	
Yes	3(2)	1(2)	2(3)	
ACS, n(%)				0.619
No	129(97)	65(98)	64(96)	
Yes	4(3)	1(2)	3(4)	

Abbreviation: IQR, interquartile range; CRP, C-reactive protein; PNI, neutrophil lymphocyte ratio; NLR, neutrophil lymphocyte ratio; SCLC, small-cell lung cancer; TKI, Tyrosine Kinase Inhibitor; VEGF, vascular endothelial growth factor; KPS, Karnofsky Performance Status; ACS, acute coronary syndrome. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

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213 MiR-219-5p Expression Level, and Clinical Risk Factors Predict the 214 Development of SCLC Patients

215 According to the univariate analysis, high miR-219-5p levels (≥ 1.50) was a strong
216 protective predictor of cancer-related mortality (HR 0.36, 95% CI 0.25-0.53, P
217 < 0.001) (table 2). Kaplan Meier curve showed that patients in the high miR-219-5p
218 group had a decreased cumulative rate of death than those in the low miR-219-5p
219 group (log-rank $P < 0.001$) (figure 2A). Meanwhile, patients who accepted
220 immunotherapy also showed a low mortality compared to those patients without
221 accepting immunotherapy in the survival curve (HR 0.28, 95% CI 0.15-0.52, P
222 < 0.001) (figure 2B).

223 In addition, gender, age, serum CRP level, albumin level, lymphocytes count,
224 PNI score, immunotherapy, heart failure, and KPS score were also correlated with
225 overall mortality (table 2). When adjusted by age and gender, patients in the
226 miR-219-5p high-level group also displayed a low cumulative rate death compared to
227 those in the low-level group.

Table 2. Cox Regression Analysis of Hazard Ratio on SCLC patients

Variation	Non-adjustment		Model 1	
	Hazard Ratio (95% CI)	p.value	Hazard Ratio (95% CI)	p.value
Gender, male vs. female	1.66 [1.02, 2.69]	0.041*	-	-
Age (year), ≥ 60 vs. < 60	1.52 [1.03, 2.26]	0.036*	-	-

BMI, ≥ 23.12 kg/m ² vs. < 22.86 kg/m ²	0.99 [0.69, 1.43]	0.97	0.95 [0.66, 1.38]	0.806
Serum CEA level, > 3.43 ng/ml vs. ≤ 3.43 ng/ml	1.01 [0.70, 1.46]	0.954	1.00 [0.69, 1.45]	0.999
Serum CRP level, > 7.83 μ mol/L vs. ≤ 7.83 μ mol/L	1.91 [1.31, 2.78]	0.001**	1.92 [1.32, 2.79]	0.001**
Albumin level, > 39.46 g/L vs. ≤ 39.46 g/L	1.66 [1.15, 2.40]	0.007**	1.60 [1.10, 2.31]	0.013*
Neutrophils count, $> 4.55 \times 10^9$ /L vs. $\leq 4.55 \times 10^9$ /L	1.18 [0.82, 1.71]	0.367	1.15 [0.79, 1.66]	0.464
Lymphocytes count, $> 1.63 \times 10^9$ /L vs. $\leq 1.63 \times 10^9$ /L	0.59 [0.40, 0.85]	0.005**	0.61 [0.42, 0.89]	0.01*
Hemoglobin level, > 133 g/L vs. ≤ 133 g/L	0.80 [0.56, 1.16]	0.244	0.72 [0.49, 1.05]	0.089
Platelet count, $> 233 \times 10^9$ /L vs. $\leq 233 \times 10^9$ /L	1.23 [0.85, 1.78]	0.275	1.23 [0.85, 1.78]	0.275
PNI score, > 47.9 vs. ≤ 47.9	0.54 [0.37, 0.78]	0.001**	0.53 [0.37, 0.77]	0.001**
NLR, > 2.66 vs. ≤ 2.66	1.18 [0.82, 1.71]	0.367	1.20 [0.83, 1.74]	0.341
Metastasis, Yes vs. No	1.05 [0.70, 1.57]	0.81	1.10 [0.73, 1.64]	0.654
Stage of NSCLC, IV, III vs. II and I	0.94 [0.50, 1.75]	0.838	1.05 [0.56, 1.98]	0.868
Stage of SCLC, Yes vs. No	1.33 [0.90, 1.96]	0.154	1.43 [0.97, 2.12]	0.074
Immunotherapy, Yes vs. No	0.28 [0.15, 0.52]	< 0.001 ***	0.30 [0.16, 0.55]	< 0.001 ***
Therapy of radiation, Yes vs. No	0.88 [0.60, 1.27]	0.488	0.79 [0.54, 1.16]	0.235
Application of platinum, Yes vs. No	0.61 [0.15, 2.47]	0.483	0.66 [0.16, 2.70]	0.562
Target therapy, Yes vs. No	0.75 [0.42, 1.33]	0.323	0.81 [0.45, 1.45]	0.481
Application of TKI, Yes vs. No	0.77 [0.41, 1.44]	0.415	0.82 [0.44, 1.53]	0.534
Application of VEGF inhibitor, Yes vs. No	0.83 [0.47, 1.45]	0.518	0.90 [0.51, 1.59]	0.723
Chemotherapy, AP vs. others	0.61 [0.37, 1.00]	0.052	0.66 [0.40, 1.09]	0.104
Smoking, Yes vs. No	1.23 [0.84, 1.79]	0.292	0.90 [0.56, 1.43]	0.645
Hypertension, Yes vs. No	1.13 [0.78, 1.64]	0.531	1.10 [0.75, 1.60]	0.622
Diabetes, Yes vs. No	0.90 [0.51, 1.61]	0.726	0.97 [0.54, 1.75]	0.927
Hyperlipemia, Yes vs. No	0.79 [0.42, 1.48]	0.461	0.77 [0.40, 1.46]	0.421
Heart failure, Yes vs. No	5.61 [1.71, 18.42]	0.004**	6.43 [1.94, 21.26]	0.002**
ACS, Yes vs. No	0.74 [0.23, 2.35]	0.612	0.69 [0.21, 2.21]	0.53
KPS score, > 80 vs. ≤ 80	0.52 [0.35, 0.75]	0.001**	0.51 [0.35, 0.74]	< 0.001 ***
miR-219-5p ≥ 1.50 vs. < 1.50	0.36 [0.25, 0.53]	< 0.001 ***	0.37 [0.25, 0.55]	< 0.001 ***

Abbreviation: HR, hazard risk; BMI, Body Mass Index; CRP, C-reactive protein; PNI, neutrophil lymphocyte ratio; NLR, neutrophil lymphocyte ratio; SCLC, small-cell lung cancer; TKI, Tyrosine Kinase Inhibitor; VEGF, vascular endothelial growth factor; KPS, Karnofsky Performance Status; ACS, acute coronary syndrome. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

Model 1: Adjusted by age and gender

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229 Independent Prognostic Factors for OS of Patients With SCLC

230 After multivariate adjustment, the miR-219-5p high level (HR 0.39, 95% CI
 231 0.26-0.59, $P < 0.001$) was also associated with a low increase in the risk of death
 232 (figure 3). Meanwhile, gender, PNI score, immunotherapy and heart failure were also
 233 the independent risk factors for OS.

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235 **Development and Validation of an OS-predicting Nomogram**

236 The independently related risk factors derived from the multivariate analysis were
237 used to create an OS estimation nomogram (figure 4). The prognostic model was
238 internally validated according to the bootstrap validation method. With an unadjusted
239 C index of 0.691 and a bootstrap-corrected C index of 0.691, the nomogram displayed
240 excellent accuracy in estimating the risk of OS. In the validation cohort, the
241 nomogram showed a C index of 0.691 for the estimation of OS. A suitable calibration
242 curve for risk estimation was also displayed ($R^2=0.455$, LR $\chi^2=80.55$) (figure 4B).
243 We collected 86 NSCLC patients from Sichuan Cancer Hospital in the external
244 validation step. The ROC curve showed an AUC of 0.783 (0.743-0.822) for predicting
245 5-year overall survival, compared with an AUC of 0.749 (0.709-0.788) for the
246 external validation data (figure 5). We calculated the total score using Nomogram for
247 patients in the training and validation sets, respectively, and divided them into four
248 groups according to 40-60,61-80,81-100,101-120, and performed Kaplan-Meier
249 analysis and plotted survival curves, which were found to have good separation and
250 were statistically significant (Figure S1a, S1b).

252 **Discussion**

253 In this study, we detected the expression of miR-219-5p in a large cohort of SCLC
254 patients at a single institution, between Mar 2010 and June 2015. The results
255 suggested that reduced expression of miR-219-5p was significantly correlated with
256 unfavorable clinical features. Moreover, patients in high miR-219-5p expression
257 group displayed better OS compared with those in low miR-219-5p expression group.
258 The multivariate analysis demonstrated miR-219-5p an independent prognostic factor
259 for OS. In addition, to propose, and retrospectively verify in an independent cohort of
260 patients, these independent risk factors were applied to establish a nomogram for OS
261 estimation. The nomogram revealed good accuracy in estimating the risk of OS.

262 Carcinogenesis involves multiple biological processes which are related to many
263 key genes[15, 16]. The characteristics of cancer occurrence represent properties that a

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4 264 cell acquire a certain ability to become and maintain itself as a cancer cell[17]. The
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6 265 key genes guide the cellular signaling pathways related to occurrence and progression
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8 266 of cancers[18, 19]. Using miRNA expression to predict the clinical diagnosis and
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10 267 prognosis of cancer has more advantages than mRNAs, as miRNAs are proved to be
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12 268 the vital posttranscriptional regulators of gene expression[20, 21]. In comparison with
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14 269 mRNAs, these vital gene regulators are highly conserved among species[22].

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16 270 It has been reported that miRNAs were related to the initiation and progression
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18 271 of various cancers, and many miRNAs have been identified as a promising biomarker
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20 272 for prognostic prediction of cancer[10, 23]. Recently, some miRNAs have been
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22 273 proved to be a novel prognostic biomarker for SCLC[24, 25]. A study of Yu et al.
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24 274 indicated that miR-92a-2 was significantly higher in SCLC patients group compared
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26 275 to healthy control, and detection of miR-92a-2 levels could be a potential biomarker
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28 276 for patients with SCLC[26]. As a promising biomarker, miR-219-5p has been
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30 277 identified as a prognostic factor for different cancers. Long et al. found that
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32 278 miR-219-5p expression level was distinctly decreased in melanoma tissues and cell
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34 279 lines, and the modulation of miR-219-5p expression could be a prognostic biomarker
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36 280 and treatment strategy in melanoma[27]. A study from Huang et al. suggested a role
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38 281 of miR-219-5p for prognostic prediction and therapeutic strategy in colorectal
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40 282 cancer[28]. However, there is no studies exploring the role of miR-219-5p for
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42 283 biomarker in patients with SCLC. To the best of our knowledge, this study was the
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44 284 first attempt ever made to comprehensively evaluate the role for prognostic prediction
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46 285 based on miR-219-5p expression in patients with SCLC. In the current study, we
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48 286 initially examined the expression levels of miR-219-5p in SCLC patients. We, for the
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50 287 first time, demonstrated a correlation of the altered miR-219-5p expression with
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4 288 available clinical parameters. We found that miR-219-5p was significantly associated
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6 289 with lymphocytes count, PNI score and stage of SCLC. The univariate analysis
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9 290 indicated that increased miR-219-5p expression was a protective predictor for
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11 291 mortality. Kaplan-Meier curve displayed that patients with elevated miR-219-5p
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14 292 expression levels or accepted immunotherapy had low cumulative incidence of death
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17 293 compared to those with reduced miR-219-5p expression or unaccepted
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19 294 immunotherapy, respectively. In addition, gender, age, serum CRP level, albumin
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22 295 level, lymphocytes count, PNI score, immunotherapy, heart failure, KPS score and
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25 296 miR-219-5p level were associated with overall mortality. The multivariate analysis
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27 297 showed that miR-219-5p, gender, PNI score, immunotherapy and heart failure could
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30 298 predict OS as the independent risk factors.

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32 299 Nomograms are applied for visualization of statistical models, graphical
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34 300 evaluation of variable significance and examination of predicted values[29, 30]. They
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36 301 have been widely performed to predict cancer risks and therapeutic outcomes[31, 32].
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38 302 Most recently, several studies have successfully established a prognostic nomogram
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40 303 that combined a miRNA with clinical-related variables for OS estimation in different
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42 304 cancers[33-35]. Although nomogram is becoming increasingly popular, no studies
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44 305 have built prognostic models using combination of miR-219-5p and clinical risk
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46 306 factors in SCLC patients. In this study, based on the combination of miR-219-5p and
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48 307 independent clinicopathological variables, we created a nomogram model that could
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50 308 provide an individual prognostic prediction for OS estimation in SCLC patients. The
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52 309 results indicated excellent accuracy in estimating the risk of OS. There was a suitable
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54 310 calibration curve for risk estimation, indicating a well-performed nomogram, and
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56 311 good agreements between observation and prediction. To further verify the accuracy
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58 312 and efficiency of the model, an external date containing 86 patients from Sichuan
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60 313 Cancer Hospital was conducted. The results indicated that the prognostic model could

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4 314 accurately predict the prognosis of SCLC patients. Hence, this was the first prognostic
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6 315 nomogram for patients with SCLC that considered clinical parameters in addition to
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8 316 miR-219-5p. This nomogram could provide comprehensive information for patients,
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10 317 as well as a better guidance for clinical therapy. Based on the model, the potential
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12 318 high-risk patients with low survival rate could be more accurately selected for a
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14 319 specific therapeutic strategy.

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16 320 There are some limitations in this article. Firstly, experimental research
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18 321 explaining the biological processes of miR-219-5p is needed. Thus, the molecular
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20 322 mechanism of miR-219-5p should be investigated in further research. Secondly, the
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22 323 prognostic nomogram needs to be further calculated by a prospective and large-scale
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24 324 multicenter study before it can be applied to clinical practice.

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27 326 **CONCLUSIONS**

28
29 327 In conclusion, we found that the miR-219-5p expression levels were significantly
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31 328 correlated with clinical parameters of SCLC patients. Furthermore, miR-219-5p was
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33 329 proved to be an independent factor for prognostic prediction in patients with SCLC.
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35 330 Moreover, nomogram based on multivariate analysis showed excellent accuracy in
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37 331 estimating the risk of OS.

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41 333 **Acknowledgements** The authors would like to thank the referees and the associate
42
43 334 editor for their constructive advice.

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46
47 336 **Contributors** ZHX and XMW designed the study. ZJC, XHZ, JGZ and MQX
48
49 337 collected and analysed the data. ZJC, JGZ and XHZ drafted the initial manuscript.
50
51 338 ZHX and XMW reviewed and edited the article. All authors read and approved the
52
53 339 final manuscript.

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57 341 **Funding** Not applicable.

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4 343 **Competing interests** The authors declare that they have no competing interests.

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8 345 **Patient consent for publication** Not required.

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12 347 **Ethics approval**

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14 348 The study was approved by ethics committee of Suzhou Xiangcheng People's
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16 349 Hospital. The reference number was 20140193. All procedures performed in the
17
18 350 present study were in accordance with the principles outlined in the 1964 Helsinki
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20 351 Declaration and its later amendments.

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24 353 **Provenance and peer review** Not commissioned; externally peer reviewed.

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28 355 **Data Availability Statement** The datasets used and analyzed during the current study
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30 356 are available from the corresponding author on reasonable request.

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33 358 **REFERENCES**

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458

459 **Figure Legends**460 **Figure 1** A flow chart of the screening process.

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462 **Figure 2** Overall survival (OS) of SCLC patients in different levels of miR-219-5p
463 and different treatments. (A) OS of SCLC patients with high or low level of

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4 464 miR-219-5p. (B) OS of SCLC patients with different treatments (immunotherapy vs
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9 467 **Figure 3** Multivariate cox regression analysis of 5-year overall survival on data in the
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11 468 SCLC patients.

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15 470 **Figure 4** Nomogram for overall survival (OS) risk estimation of SCLC patients and
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17 471 its predictive performance. (A) Nomogram to estimate the OS risk of SCLC patients
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19 472 in different variations. (B) Validity of the predictive performance of the nomogram in
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21 473 estimating the OS risk of SCLC patients.

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25 475 **Figure 5** External validation of the prognostic model.

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29 477 **Table 1** Study participant characteristics at enrollment.

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33 479 **Table 2** Univariate cox regression analysis of overall survival on SCLC patients.

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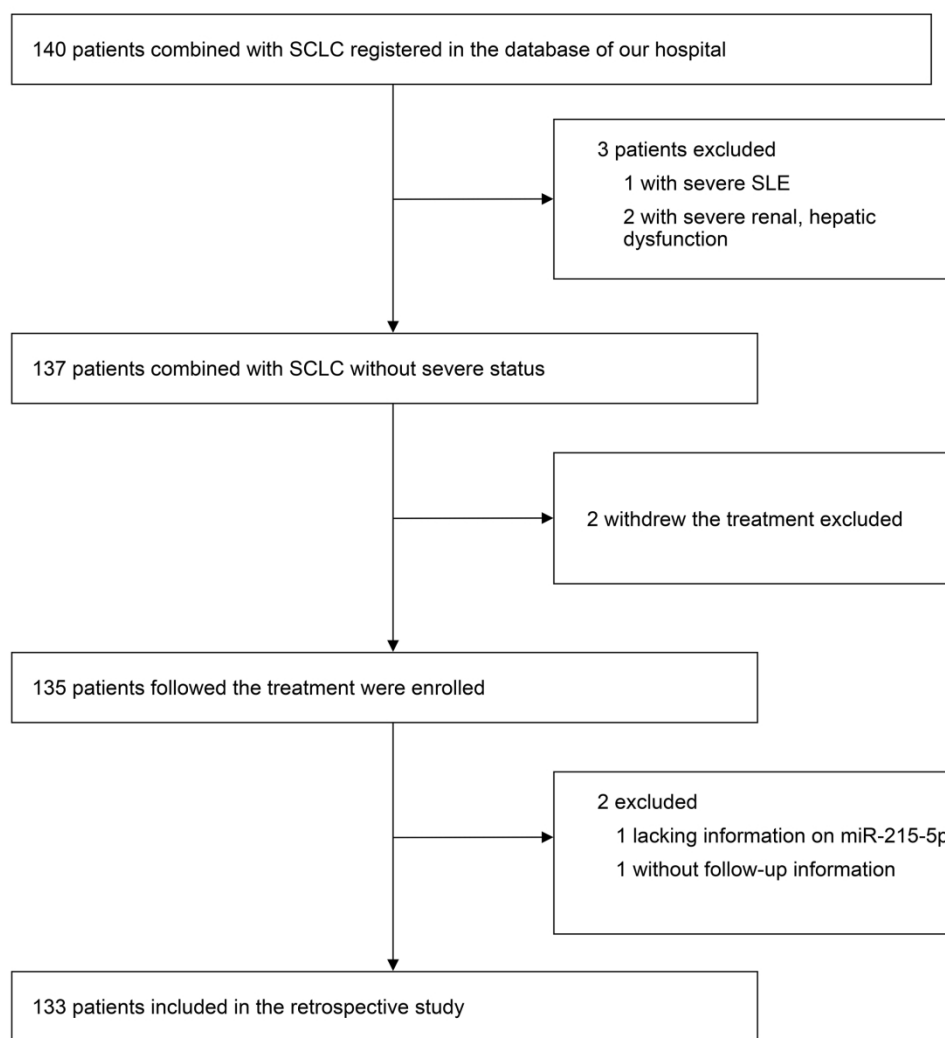


Figure 1 A flow chart of the screening process.

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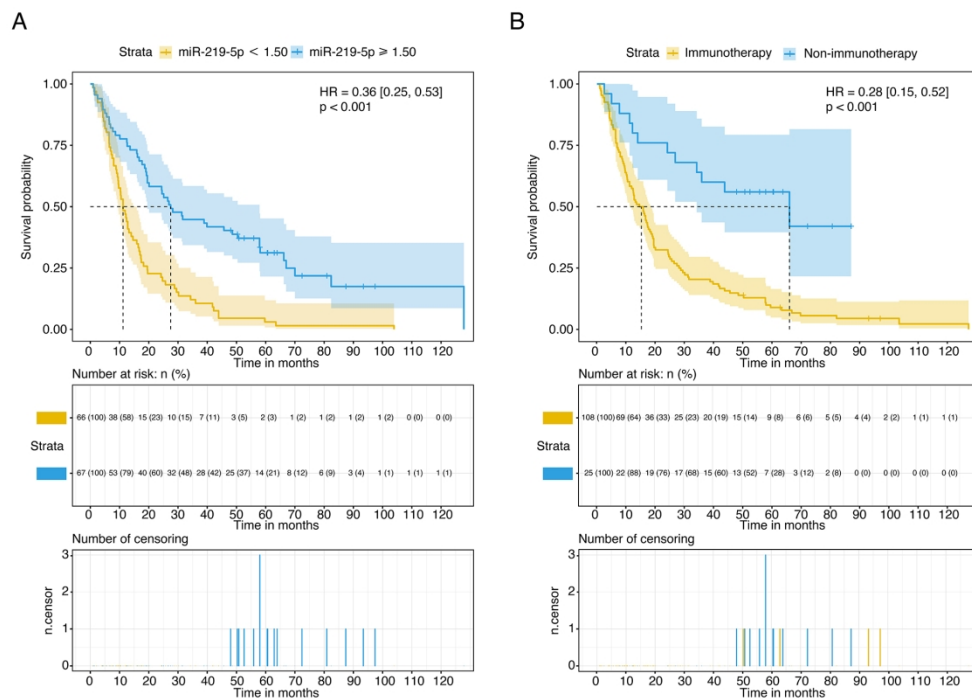


Figure 2 Overall survival (OS) of SCLC patients in different levels of miR-219-5p and different treatments. (A) OS of SCLC patients with high or low level of miR-219-5p. (B) OS of SCLC patients with different treatments (immunotherapy vs non-immunotherapy).

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Figure 3

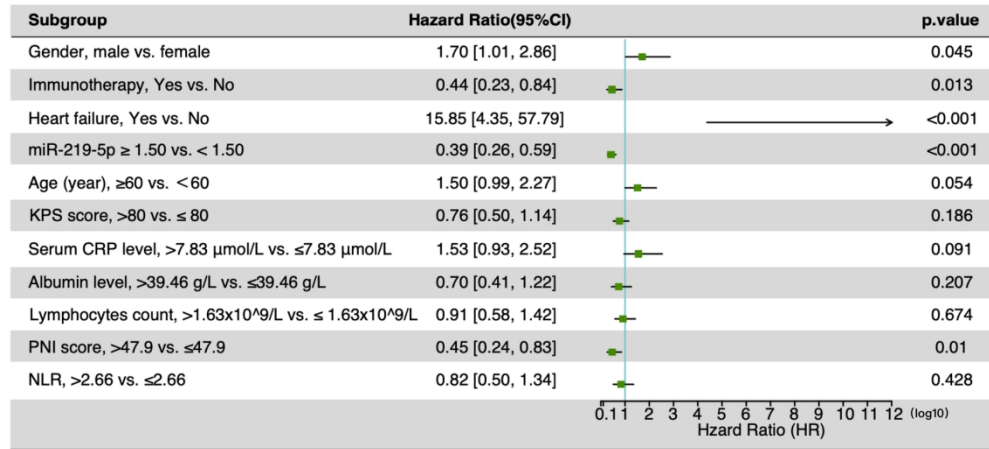


Figure 3 Multivariate cox regression analysis of 5-year overall survival on data in the SCLC patients.

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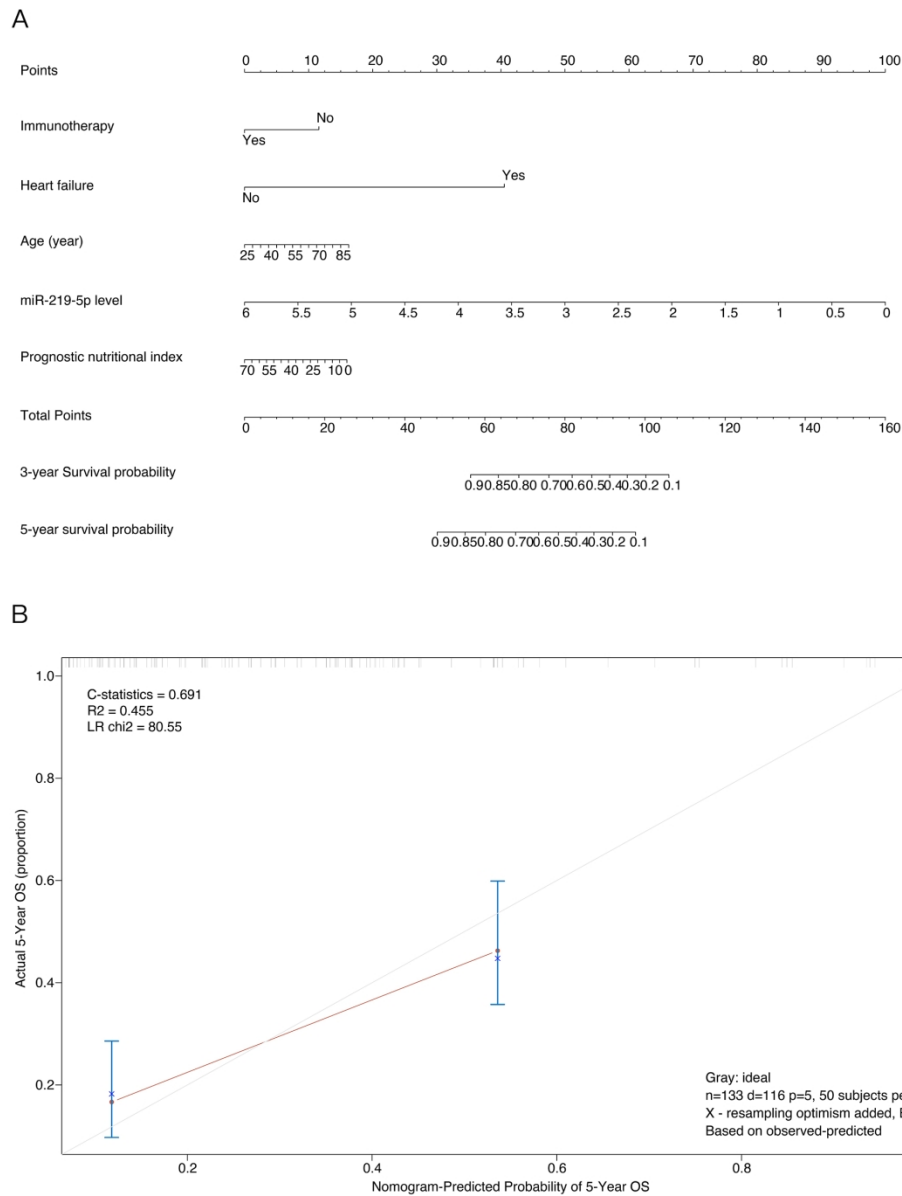


Figure 4 Nomogram for overall survival (OS) risk estimation of SCLC patients and its predictive performance. (A) Nomogram to estimate the OS risk of SCLC patients in different variations. To build the nomogram, find the position of each variable on the corresponding axis, draw a line to the points axis for the number of points, add the points from all of the variables, and draw a line from the total points axis to determine the OS probabilities at the lower line of the nomogram. (B) Validity of the predictive performance of the nomogram in estimating the OS risk of SCLC patients.

180x216mm (300 x 300 DPI)

Figure 5

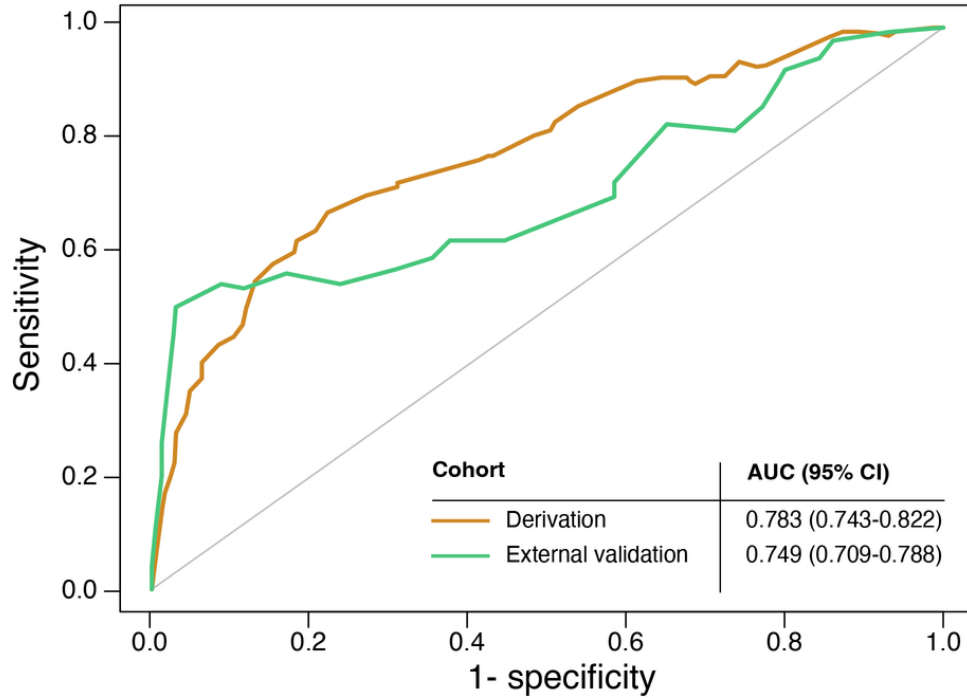


Figure 5 External validation of the prognostic model.

80x63mm (300 x 300 DPI)

Supplement Materials

Part I. Details of variants

Patients' age: The age of patients was based on the time of diagnosis of NSCLC.

Body mass index: The equation of body mass index (BMI) is calculated as follow:
$$\text{BMI (kg/m}^2\text{)} = \text{weight (kg)} / \text{height (m)}^2$$
. The data of BMI was recorded before clinical treatment.

Serum CEA level: The serum level of carcinoembryonic antigen (CEA) is one of tumor marks, the higher serum *CEA* level than that of healthy individuals led to its clinical application as a diagnostic biomarker for cancer.

Serum CRP level: C-reactive protein (CRP) is detected by colorimetric determination through fasting blood collection and CRP is one of the inflammation markers.

Albumin level: Serum albumin is detected by colorimetric determination through fasting blood collection. And serum albumin is one of the indicators of nutritional status.

Neutrophils count, lymphocytes count, hemoglobin level, platelet count: All these indexes were extracted from blood routine examination to evaluate preoperative status of patients.

PNI score: Prognostic nutritional index (PNI) is an index to evaluate the nutritional status of surgical patients, to predict the risk of surgery and to help make prognostic judgment. The equation is listed as below: $\text{PNI} = \text{serum albumin (g/L)} + 5 * \text{lymphocytes (*}10^9\text{/L)}$.

NLR: Neutrophil to Lymphocyte Ratio (NLR) is an index of inflammation markers to predict the prognosis of various cancer, including lung cancer.

Pathologic type: Non-small cell lung cancer can be classified as adenocarcinoma, squamous cell carcinoma, large cell lung cancer, or others, depending on surgical or tumor biopsy specimens

Stage of NSCLC: Non-small cell lung cancer was staged according to the seventh edition of international union against cancer (UICC) TNM was published in 2009

Surgery: For early and locally advanced NSCLC, which is generally acceptable, there is a history of surgical resection.

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Therapy of radiation: Radiation therapy is a medical procedure that uses the delivery of high-energy radiation to kill cancer cells and shrink tumors, including external beam radiation therapy, internal radiation therapy and stereotactic body radiotherapy (SBRT). It aims to cure small tumors, treat lung cancer both locally and nearby lymph nodes, treat metastases, relieve symptoms, and also at prevention.

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Application of platinum: Platinum-based chemotherapy may be used as a first or second line therapy for advanced lung cancer, after surgery (adjuvant chemotherapy), or before surgery to reduce the size of a tumor (neoadjuvant therapy). The most commonly used platinum drugs are cisplatin, carboplatin and nedaplatin. Treatment of non-small cell lung cancer begins with either cisplatin or carboplatin combined with another medication, such as docetaxel, pemetrexed, paclitaxel, gemcitabine and gemcitabine.

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Target therapy: Targeted therapies for lung cancer are drugs that are tailored to attack certain mutations of cancer cells. These mutations lead to the production of abnormal proteins which guide the growth and development of a cancer cell. Common driver mutations include EGFR mutations, ALK rearrangements, ROS1 rearrangements, MET amplifications, KRAS mutations, HER2 mutations, BRAF mutations and so on.

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Application of TKI: Tyrosine kinase inhibitors (TKI) act as competitive inhibitors of ATP binding to tyrosine kinases, or as tyrosine analogs that block tyrosine kinase activity and inhibit cell proliferation. Epithelial growth factor receptor (EGFR) is a protein that is present on the surface of both normal cells and lung cancer cells. Mutations in EGFR can occur at different locations on exon 18 to 21. ALK-positive lung cancer refers to people who have lung cancer that tests positive for the EML4-ALK fusion mutation. Several FDA-approved medications available to treat EGFR-positive and ALK-positive lung adenocarcinoma, including:

- 41
- 42 • EGFR inhibitors - Tarceva (erlotinib), Gilotrif (afatinib), Iressa (gefitinib),
- 43 Tagrisso (osimertinib), and Portrazza (necitumumab)
- 44 • ALK inhibitors - Xalkori (crizotinib), Zykadia (ceritinib), and Alecensa
- 45 (alectinib)
- 46 • ROS1 inhibitor - Xalkori (crizotinib)
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Application of VEGF inhibitor: VEGF (Vascular endothelial growth factor) can activate angiogenesis through different signaling pathways. VEGF inhibitors are drugs that block the ability of tumors to form new blood vessels, and hence, grow and spread. Some currently available medications include bevacizumab, ramucirumab, axitinib, endostar and anlotinib.

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KPS score: A performance measure for rating the ability of a person to perform usual activities, evaluating a patient's progress after a therapeutic procedure, and determining a patient's suitability for therapy. It is used most commonly in the

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3 prognosis of cancer therapy, usually after chemotherapy and customarily administered
4 before and after therapy. It was named for Dr. David A. Karnofsky, an American
5 specialist in cancer chemotherapy. Patients with more than 80 scores had better
6 postoperative status and longer survival time. And patients with more than 70 scores
7 can suffer from chemoradiotherapy.
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11 **Smoking:** Smoker refers to continuous or cumulative smoking > 1 cigarette/day over
12 a lifetime of more than 6 months. (1997, WHO)
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15 **Hypertension:** Hypertension is defined as a repeatedly elevated blood pressure
16 exceeding 140 over 90 mmHg.
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19 **Diabetes:** Diabetes is a group of metabolic diseases characterized by hyperglycemia.
20 And fasting blood glucose more than 7.0 mmol/l or blood glucose more than 11.1
21 mmol/l within 2 hours after meal can be diagnosed diabetes.
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24 **Hyperlipemia:** Hyperlipemia means the presence of excess fat or lipids in the blood.
25 And total cholesterol ≥ 6.2 mmol/L, low density lipoprotein cholesterol ≥ 4.1 mmol/L,
26 triglyceride ≥ 2.3 mmol/L, high density lipoprotein cholesterol < 1.0 mmol/L can be
27 diagnosed hyperlipemia.
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30 **Heart failure:** The information was recorded through history taking and verified after
31 hospitalization. Heart failure means inability of the heart to keep up with the demands
32 on it and, specifically, failure of the heart to pump blood with normal efficiency. Heart
33 failure may be due to failure of the right or left or both ventricles. The signs and
34 symptoms depend upon which side of the heart is failing. They can include shortness
35 of breath (dyspnea), asthma due to the heart (cardiac asthma), pooling of blood (stasis)
36 in the general body (systemic) circulation or in the liver's (portal) circulation, swelling
37 (edema), blueness or duskiness (cyanosis), and enlargement (hypertrophy) of the
38 heart.
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44 **ACS:** Acute coronary syndrome is a term for a group of conditions that suddenly stop
45 or severely reduce blood from flowing to the heart. When blood cannot flow to the
46 heart, the heart muscle can become damaged. Heart attack and unstable angina are
47 both acute coronary syndromes (ACS).
48

49
50 **Withdraw treatment:** Reasons for patients withdrew from treatment were listed
51 below: 1) High cost of the treatment: In China, the cost of hormone therapy can be up
52 to 3000 RMB a month if without health insurance. 2) Poor patient compliance: Some
53 patients discontinue treatment because they do not comply with the treatment plan
54 prescribed by their doctor.
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Part II. Report of sample size assessment

PASS 15.0.5

Tests for Two Survival Curves Using Cox's Proportional Hazards Model

Numeric Results with $H_a: HR \neq 1$

	Total Sample Size N	Control Sample Size N1	Trtmnt Sample Size N2	Prop'n Control N1/N P1	Hazard Ratio h2/h1 HR	Control Prob Event Pev1	Trtmnt Prob Event Pev2	Control Events E1	Trtmnt Events E2	Alpha	Beta
Power	103	51	52	0.500	0.500	0.750	0.950	38.3	49.4	0.050	0.100

References

- Chow, S.C., Shao, J., Wang, H. 2008. Sample Size Calculations in Clinical Research, 2nd Edition. Chapman & Hall/CRC.
- Schoenfeld, David A. 1983. 'Sample Size Formula for the Proportional-Hazards Regression Model', Biometrics, Volume 39, Pages 499-503.

Report Definitions

Power is the probability of rejecting a false null hypothesis. Power should be close to one.

N is the total sample size.

N1 and N2 are the sample sizes of the control and treatment groups.

P1 is the proportion of the total sample that is in the control group, group 1.

HR is the hazard ratio: h_2/h_1 .

Pev1 and Pev2 are the probabilities of an event in the control and the treatment groups.

E1 and E2 are the number of events required in the control and the treatment groups.

Alpha is the probability of a type one error: rejecting a true null hypothesis.

Beta is the probability of a type two error: failing to reject a false null hypothesis.

Summary Statements

A two-sided test of whether the hazard ratio is one with an overall sample size of 103 subjects (of which 51 are in the control group and 52 are in the treatment group) achieves 90% power at a 0.050 significance level when the hazard ratio is actually 0.500. The number of events required to achieve this power is 87.7. It is anticipated that proportions of subjects having the event during the study is 0.750 for the control group and 0.950 for the treatment group. These results assume that the hazard ratio is constant throughout the study and that Cox proportional hazards regression is used to analyze the data.

Procedure Input Settings

Autosaved Template File

\\Mac\Home\Documents\PASS 15\Procedure Templates\Autosave\Tests for Two Survival Curves Using Cox's

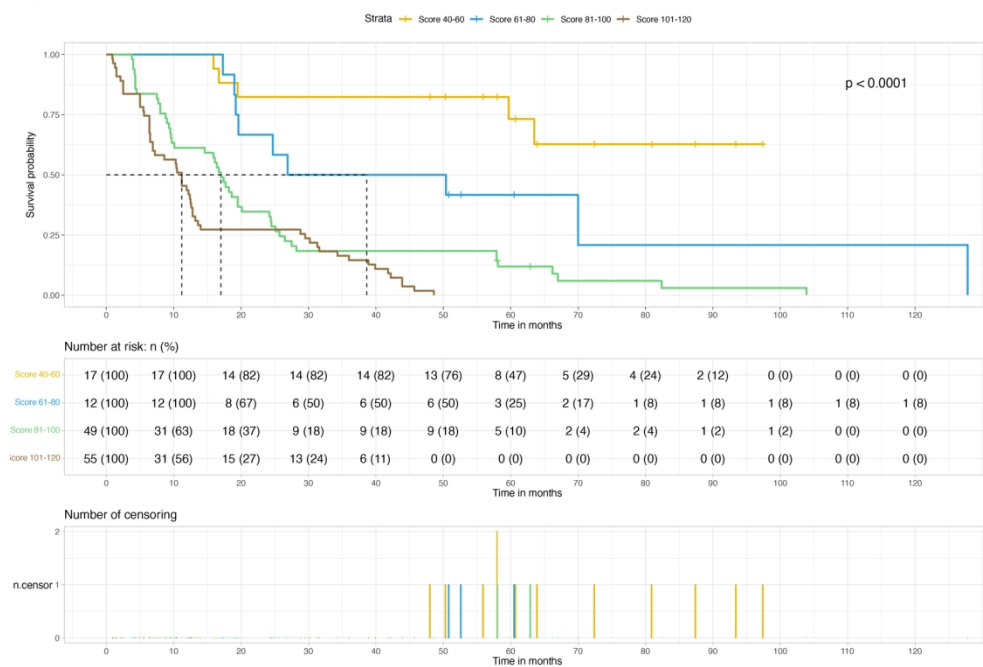
Proportional Hazards Model - Autosaved 2020_1_24-9_57_39.t92

Design Tab

Solve For:	Sample Size
Alternative Hypothesis:	Ha: HR \neq 1
Power:	0.90
Alpha:	0.05
Group Allocation:	Equal (N1 = N2)
Pev1 (Probability of a Control Event):	0.750
Pev2 (Probability of a Treatment Event):	0.950
HR (Actual Hazard Ratio = h2/h1):	0.5

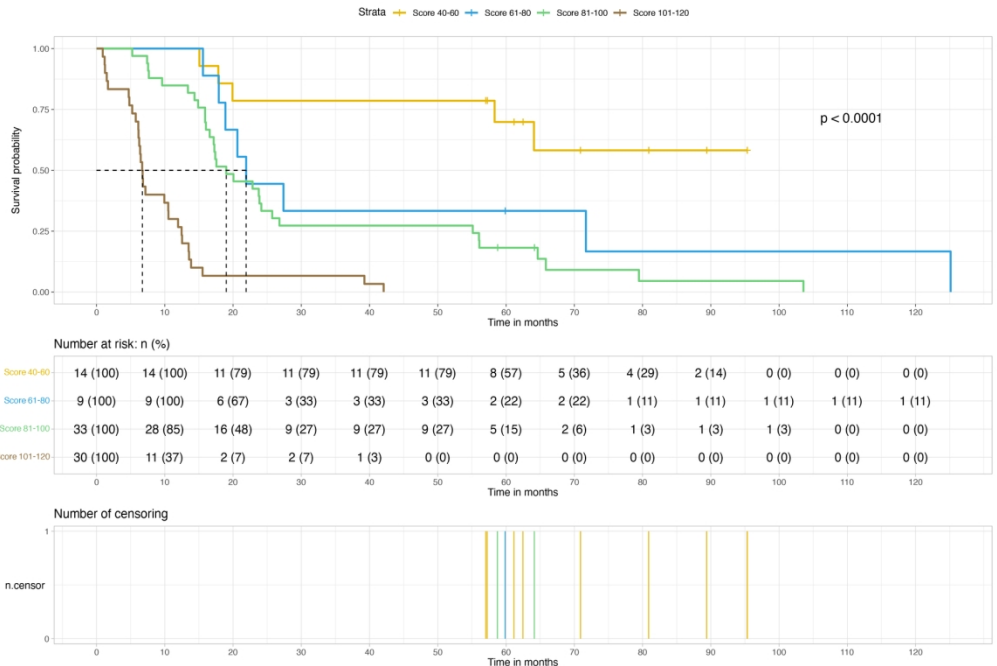
For peer review only

Figure S1a



160x109mm (300 x 300 DPI)

Figure S1b



159x110mm (300 x 300 DPI)

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of cohort studies

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			5
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6
		(b) For matched studies, give matching criteria and number of exposed and unexposed	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	7
		(b) Describe any methods used to examine subgroups and interactions	7
		(c) Explain how missing data were addressed	7
		(d) If applicable, explain how loss to follow-up was addressed	7
		(e) Describe any sensitivity analyses	7
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	8
		(c) Consider use of a flow diagram	8
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9
		(b) Indicate number of participants with missing data for each variable of interest	9
		(c) Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	Report numbers of outcome events or summary measures over time	10
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10
		(b) Report category boundaries when continuous variables were categorized	11
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	11
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12
Discussion			
Key results	18	Summarise key results with reference to study objectives	13-14
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Prognostic value of MiR-219-5p in relation to cancer-related mortality in patients with small cell lung cancer: a retrospective observational cohort study in China

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-064700.R2
Article Type:	Original research
Date Submitted by the Author:	25-Oct-2022
Complete List of Authors:	Cao, Zhijun; Suzhou Ninth People's Hospital, Urology Zhang, Jigang; First Affiliated Hospital of Soochow University Zhang, Xiaohui; First Affiliated Hospital of Soochow University Xiang, Mengqi; Medical School of University of Electronic Science and Technology of China, Department of Medical Oncology Xu, Zhihua; First Affiliated Hospital of Soochow University, General surgery Wu, Xiangmei; Suzhou Xiangcheng People's Hospital
Primary Subject Heading:	Oncology
Secondary Subject Heading:	Surgery
Keywords:	Risk management < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, ONCOLOGY, Gene therapy < ONCOLOGY, Molecular aspects < ONCOLOGY, Oncogenes < ONCOLOGY

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4 1 **Prognostic value of MiR-219-5p in relation to cancer-related**
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6 2 **mortality in patients with small cell lung cancer: a retrospective**
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8 3 **observational cohort study in China**
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13 5 Zhijun Cao^{2,#}, Jigang Zhang^{4,#}, Xiaohui Zhang^{5,#}, Mengqi Xiang⁶, Zhihua Xu^{3,*},
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15 6 Xiangmei Wu^{1,*}
16
17 7

18
19 8 ¹ Department of Endocrinology, Suzhou Xiangcheng People's Hospital, Suzhou,
20
21 9 China
22

23 10 ² Department of Urology, Suzhou Ninth People's Hospital, Soochow University,
24
25 11 Suzhou, China
26

27 12 ³ Department of General Surgery, The First Affiliated Hospital of Soochow
28
29 13 University, Suzhou, China
30

31 14 ⁴ Department of Traumatology Surgery, The First Affiliated Hospital of Soochow
32
33 15 University, Suzhou, China
34

35 16 ⁵ Department of Medicine, Respiratory, Emergency and Intensive Care Medicine, The
36
37 17 First Affiliated Hospital of Soochow University, Suzhou, China
38

39 18 ⁶ Department of Medical Oncology, Sichuan Cancer Hospital, Medical School of
40
41 19 University of Electronic Science and Technology of China, Chengdu, Sichuan
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45 21 Running title: MiR-219-5p decrease the risk of SCLC patients
46
47 22

48 23 # These authors contributed equally to this work
49

50 24 * Correspondence to: dr_xiangmeiwu@163.com (Xiangmei Wu) or
51
52 25 dr_zhihuaxu@163.com (Zhihua Xu)
53
54 26

27 ABSTRACT

28 **Objectives** Small cell lung cancer (SCLC) is a lethal human malignancy, and
29 previous studies support the contribution of microRNA (miRNA) to cancer
30 progression. The prognostic value of miR-219-5p in SCLC patients remains unclear.
31 This study evaluated the role of MiR-219-5p for SCLC and created a prediction
32 model for them.

33 **Design** Retrospective observational cohort study.

34 **Setting and participants** The program has yielded a database of all patients with
35 SCLC in 2 defined geographical regions of China. We did a real-world study,
36 including data from 133 patients with SCLC between Mar 1, 2010, and June 1, 2015
37 in the Suzhou Xiangcheng People's Hospital. External data from 86 SCLC patients
38 from Sichuan Cancer Hospital and the First affiliated hospital of Soochow University
39 was conducted.

40 **Primary and secondary outcome measures** MiR-219-5p was recorded during the
41 admission. Cox proportional hazard model was applied for survival analyses and for
42 analyzing risk factors for cancer-related mortality and to create a nomogram for
43 prediction. The accuracy of the model was evaluated by C-index and calibration
44 curve.

45 **Results** In our data, the mortality in the group with a high miR-219-5p level (≥ 1.50)
46 ($n=67$) was 74.6%, while the mortality in the low group ($n=66$) was 100.0%. Based
47 on univariate analysis, we put factors ($P < 0.05$) into a multivariate regression model,
48 patients with high miR-219-5p level (HR=0.39, 95%CI=[0.26-0.59], $P < 0.001$),
49 immunotherapy (HR=0.44, 95%CI=[0.23-0.84], $P < 0.001$), PNI score >47.9
50 (HR=0.45, 95%CI=[0.24-0.83], $P = 0.01$) remained statistically factors for better
51 overall survival (OS). Nomogram revealed good accuracy in estimating the risk, with
52 a bootstrap-corrected C index of 0.691. External validation displayed an AUC of
53 0.749 (0.709-0.788).

54 **Conclusions** MiR-219-5p was associated with a reduced risk of cancer-related
55 mortality. Nomogram demonstrated good accuracy in estimating the risk of overall
56 mortality. Prospective validation of the prognostic nomogram is needed in the future.

1
2
3
4 57 **Keywords:** small cell lung cancer, miR-219-5p, overall survival, nomogram,
58 prediction model

59

60 **Strengths and limitations of this study**

61 ▶ The study established the first risk nomogram for predicting the 3-year and 5-year
62 specific mortality probability for SCLC

63 ▶ Based on the retrospective sample, the nomogram can improve the ability of
64 clinicians in predicting survival probabilities in individual patients.

65 ▶ The model is not comprehensive enough because the epidemiology and end results
66 database does not include all prognostic factors for SCLC.

67 ▶ The available data on treatment status are not detailed enough to distinguish the
68 impact of various treatment plans.

69 ▶ The model needs to be prospectively studied to confirm its reliability

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72 INTRODUCTION

73 Lung cancer is the leading cause of cancer deaths worldwide, with millions of new
74 cases diagnosed each year[1]. Small cell lung cancer (SCLC) is a kind of
75 neuroendocrine malignant tumor with poor prognosis, accounting for about 15% of
76 lung cancer patients[2]. SCLC is generally divided into limited disease (LD-SCLC)
77 and extensive disease (ED-SCLC). Platinum combined with etoposide is the first-line
78 therapeutic strategy of SCLC, and most patients are easy to receive initial
79 chemotherapy[3]. However, the 5-year survival rates of LD-SCLC and ED-SCLC are
80 only 15% and 3%, respectively[4]. Therefore, improvement in early diagnosis and
81 prognostic prediction of SCLC is vital.

82 MicroRNAs (miRNAs) are endogenous non-coding RNAs (~ 22 nt), which
83 regulate mRNA activity by hybridization with 3' untranslated region (UTR) of
84 specific genes[5]. Many studies have shown that miRNAs could participate in a
85 variety of cell biological processes, including cell growth, differentiation and
86 apoptosis[6, 7]. In addition, researches have demonstrated that miRNAs are
87 frequently dysregulated in cancers[8, 9], and some miRNAs can serve as diagnostic
88 and prognostic biomarkers for cancers[10]. Recently, several miRNAs have been
89 proved to participate in the occurrence and development of SCLC, but few of them
90 are likely to be a biomarker or therapeutic target for SCLC.

91 Recently, miR-219-5p has been found to be abnormally expressed and play a
92 significant role in different cancers. Ma et al. found that the expression of miR-219-5p
93 was significantly decreased in esophageal squamous cell carcinoma (ESCC) tissues
94 compared with normal tissues[11]. A study of Gong et al. revealed a tumor
95 suppressive role for miR-219-5p by targeting glypican-3 in hepatocellular carcinoma
96 (HCC)[12]. On the contrary, Yang et al. indicated that miR-219-5p could promote cell
97 growth and metastasis of HCC and serve as a prognostic marker for HCC
98 patients[13]. A research investigated by Wei et al. suggested that miR-219-5p could
99 inhibit proliferation, migration and invasion of epithelial ovarian cancer through
100 downregulation of the Wnt signaling pathway, and it could serve as a diagnostic

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4 101 biomarker and therapeutic target for epithelial ovarian cancer[14]. However, the
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6 102 biological functions of miR-219-5p and its potential prognostic role for biomarker in
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8 103 SCLC are still unknown.

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10 104 In this study, we aimed to examine the variation in the expression levels of
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12 105 miR-219-5p in patients with SCLC and explored the potential prognostic role of
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14 106 miR-219-5p for SCLC. We also displayed a nomogram that could provide
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16 107 individualized, evidence-based, highly accurate risk estimates. Nomograms were easy
17
18 108 to performed and could facilitate management-related decision making.

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21 110 **METHODS**

22 111 **Study Design and Patient Characteristics**

23
24 112 We did a real-world study, including data obtained from 133 patients with SCLC
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26 113 between Mar 2010 and June 2015, in the Suzhou Xiangcheng People's Hospital.
27
28 114 Those participants who lacked information on complement components data,
29
30 115 withdrew from treatment or lacked follow-up information were excluded. Clinical
31
32 116 information of patients, including gender, age, BMI, neutrophils count, lymphocytes
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34 117 count, serum CEA level, CRP level, albumin level, hemoglobin level, stage of SCLC,
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36 118 platelet count, PNI score, KPS score, NLR, pathologic type, immunotherapy, therapy
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38 119 of radiation, application of platinum, application of VEGF inhibitor, target therapy,
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40 120 application of TKI, smoking, ACS, diabetes, heart failure and hyperlipemia, were
41
42 121 recorded. Diagnosis of SCLC was confirmed by histopathological examination. The
43
44 122 median length of follow-up was 23.6 months. Median was used as the cut-off value.
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46 123 The definition and details of all the variables above were provided in Supplemental
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48 124 Materials Part I. Data from 86 patients with NSCLC at Sichuan Cancer Hospital and
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4 125 the First affiliated hospital of Soochow University were applied for external
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6 126 validation. Inform, and consent was obtained from all patients or their immediate
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9 127 family members. All protocols were in line with the guidelines with the ethic
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11 128 committee of Suzhou Xiangcheng People's Hospital, Sichuan Cancer Hospital, the
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14 129 First affiliated hospital of Soochow University and following the Declaration of
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17 130 Helsinki.

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21 22 132 **Assays for Detection of MiR-219-5p Levels**

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24 133 The quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was
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26 134 conducted for the detection of miR-219-5p expression levels.

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28 135 Total RNA from tissues was isolated and extracted using miRcute Extraction and
29
30 136 Separation of miRNAs kit (Tiangen Biotech Co., Ltd., Beijing, China), and then
31
32 137 reversely transcribed into cDNA by PrimeScript™ II 1st strand cDNA synthesis kit
33
34 138 (Takara Biotechnology Co., Ltd., Dalian, China) according to the manufacturer's
35
36 139 protocol. SYBR PrimeScript miRNA RT-PCR kit (Takara Biotechnology Co., Ltd.)
37
38 140 was used for qRT-PCR. The thermocycling conditions were as follows: one cycle at
39
40 141 95°C for 3 min (initial denaturation), 40 cycles at 95°C for 15 s and 60°C for 30 s. U6
41
42 142 small nuclear RNA (U6) served as the respective internal control. The relative
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44 143 expression of miR-219-5p was quantified by the $2^{-\Delta\Delta Ct}$ methods, and normalized to
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46 144 the U6. The following primers were used: miR-219-5p forward,
47
48 145 5'-ACACTCCAGCTGGGTGATTGTCCAAACGCAAT-3' and reverse,
49
50 146 5'-CTCAACTGGTGTCGTGGA-3'; U6 forward,
51
52 147 5'-GCTTCGGCAGCACATATACTAAAAT-3' and reverse,
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54 148 5'-CGCTTCACGAATTTGCGTGTCAT-3'. The experiments were repeated at least 3
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56 149 times.

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59 60 151 **Statistical Analysis**

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4 152 Sample size assessment was performed using NCSS-PASS software version 11.0
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6 153 (<https://www.ncss.com/software/pass/>). Power was set as 0.99, and alpha was 0.5. The
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9 154 mortalities of both miR-219-5p high-level group and miR-219-5p low-level group in
10
11 155 our previous data (2008-2009) (0.750 and 0.950) were entered into the PASS. The
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13
14 156 Actual Hazard Ratio was set as 0.50. Then the sample size was calculated using
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16
17 157 PASS, and the minimum sample size was 103 (control = 51, experiment = 43). Our
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19
20 158 sample size was 133 (66 and 67 for each group), which was suitable. The report of
21
22 159 sample size assessment was displayed in Supplemental Material Part II. The missing
23
24
25 160 data (<5.0%) were estimated by random forest algorithm using the mice package in
26
27 161 RStudio (R version 3.6.1). Categorical variates were presented as percentages and
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29
30 162 compared via the κ^2 test. Continuous variates with skewed and normal distributions
31
32 163 were presented as median with interquartile ranges and mean \pm standard deviation.
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34
35 164 The Mann-Whitney U test and the unpaired t-test were applied for comparison
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37
38 165 between Groups. Cumulative mortality was showed by the Kaplan-Meier curve and
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40 166 analyzed by the log-rank test. Univariate and multivariate survival analyses for OS
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42
43 167 were conducted using the Cox regression model. The forest plots were used to
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45
46 168 visualize the significance of covariates to the prognosis. The restricted cubic spline
47
48 169 analyses were performed with Harrell's Regression Modelling Strategies (rms)
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51 170 package.

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53 171 We screened multifactor analysis for statistically significant indicators for
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55 172 inclusion in the prediction model. To build the nomogram, find the position of each
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57 173 variable on the corresponding axis, draw a line to the points axis for the number of
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59 174 points, add the points from all the variables, and draw a line from the total points axis
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4 175 to determine the OS probabilities at the lower line of the nomogram. The contribution
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6 176 of each covariate was quantified and visualized in a prognostic nomogram with
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8 177 internal validation via 1000-times bootstrapping. The consistency of the resulting
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10 178 model was assessed by the calibration assay. Decision curve analyses were performed
11
12 179 to evaluate net clinical benefits of the model compared with conventional prognostic
13
14 180 scores. The scatter plots were applied for visualization of the consistency of each
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16 181 model. A 1000-time bootstrapping was employed as indicated. The association
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18 182 between miR-219-5p class and survival endpoints was evaluated by Kaplan-Meier
19
20 183 curves and log-rank test. Statistical analysis was performed using the RStudio (R
21
22 184 version 3.6.1) with the following packages: 'ggplot2', 'rms', 'PredictABLE', 'risk
23
24 185 regression', and 'survminer'.
25

26 186

27 187 **Patient and public involvement**

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29 188 This study was conducted without patient involvement. Patients were not invited to
30
31 189 comment on the study design and were not consulted to develop patient-relevant
32
33 190 outcomes or interpret the results. Moreover, patients were not allowed to contribute to
34
35 191 the writing or editing of this document for readability or accuracy.
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37 192

38 39 193 **RESULTS**

40 41 42 194 **Baseline Characteristics**

43
44 195 A total of 133 SCLC patients who were diagnosed between Mar 2010 and June 2015
45
46 196 were included in this study. A flow chart of the screening process was shown in [figure](#)
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48 197 [1](#). The median age of these patients was 64 years old (58-70), and it contained 106
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50 198 (80.0%) males. Median serum CEA and CRP level was 3.43 ng/ml and 7.83 μ mol/L,
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52 199 respectively. For the stage of SCLC, 51 (38.0%) patients were diagnosed with limited
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54 200 disease, 82 (61.0%) patients were extensive disease. 25 (19.0%) patients accepted the
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56 201 immunotherapy therapy. 54 (41.0%) patients got the therapy of radiation. In addition,
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202 platinum was applied for 131 (98.0%) patients, and TKI was used for 15 (12.0%)
 203 patients. KPS score of these patients was examined, and the results revealed that 107
 204 (81.0%) patients got 80 or higher points. The distribution of basic diseases was also
 205 assessed in our data. Diabetes was found in 17 (13.0%) patients, while hyperlipemia
 206 was 13 (10.0%). Cardiovascular diseases such as heart failure and ACS were found in
 207 3 (2.0%) and 4 (3.0%) patients, respectively. 13 (10.0%) patients suffered from
 208 hypertension. In addition, 82 (62.0%) patients had a habit of smoking among all the
 209 patients. The baseline characteristics of these patients were listed in [table 1](#).

210 Among all the 133 patients, the overall mortality was 87.2%. The mortality in
 211 high miR-219-5p level group (n=67) was 74.6%, , while the mortality in the low
 212 group (n=66) was 100.0%. Moreover, in the high miR-219-5p level group, patients
 213 with extensive disease were 35 (52.0%), while the low group was 47 (71.0%) ([table](#)
 214 [1](#)).

Table 1. Study Participant Characteristics at Enrollment

Variation	Total (n=133)	Cohort, median (IQR)		p.value
		mir-219-5p<1.50, (n=66)	mir-219-5p≥1.50, (n=67)	
Age, (year)	64(58,70)	65(59,70)	63(56.5,68)	0.276
BMI, (kg/m ²)	23.12±3.09	22.99±3.22	23.26±2.98	0.619
Serum CEA level, (ng/ml)	3.43(1.96,9.26)	3.34(1.92,10.05)	3.43(1.99,8.11)	0.87
Serum CRP level, (μmol/L)	7.83(1.68,12.78)	10.68(2.78,13.15)	5.64(1.2,12.16)	0.107
Albumin level, (g/L)	39.46±5.2	38.86±4.68	40.05±5.65	0.188
Neutrophils count, (10 ⁹ /L)	4.55(3.59,5.88)	4.54(3.77,5.75)	4.55(3.48,5.92)	0.975
Lymphocytes count, (10 ⁹ /L)	1.63(1.31,1.95)	1.5(1.21,1.78)	1.73(1.38,2.08)	0.031*
Hemoglobin level, (g/L)	133(125,145)	134(124,145)	132(125,143.5)	0.564
Platelet count, (10 ⁹ /L)	233(184,288)	244.5(180,293.75)	226(184.5,273.5)	0.306
PNI score	47.9(43.95,51.85)	45.98(42.51,50.38)	48.7(44.92,54)	0.026*
NLR	2.66(1.99,4.19)	2.75(2.11,4.57)	2.59(1.92,3.7)	0.232
Gender, (n%)				0.211
Female	27(20)	10(15)	17(25)	
Male	106(80)	56(85)	50(75)	
Metastasis, n(%)				0.299
No	45(34)	19(29)	26(39)	
Yes	88(66)	47(71)	41(61)	
Stage of SCLC				0.038*

Limited disease	51(38)	19(29)	32(48)	
Extensive disease	82(62)	47(71)	35(52)	
Immunotherapy, (n%)				0.197
No	108(81)	57(86)	51(76)	
Yes	25(19)	9(14)	16(24)	
Therapy of radiation, (n%)				0.417
No	79(59)	42(64)	37(55)	
Yes	54(41)	24(36)	30(45)	
Application of platinum, (n%)				0.244
No	2(2)	2(3)	0(0)	
Yes	131(98)	64(97)	67(100)	
Chemotherapy				0.45
AP	28(21)	12(18)	16(24)	
DP	15(11)	6(9)	9(13)	
EP	71(53)	35(53)	36(54)	
GP	3(2)	2(3)	1(1)	
Others	16(12)	11(17)	5(7)	
Target therapy, (n%)				0.627
No	116(87)	59(89)	57(85)	
Yes	17(13)	7(11)	10(15)	
Application of TKI, (n%)				0.449
No	118(89)	60(91)	58(87)	
TKI I	9(7)	4(6)	5(7)	
TKI II	1(1)	1(2)	0(0)	
TKI III	5(4)	1(2)	4(6)	
Application of VEGF inhibitor, n(%)				0.645
No	114(86)	58(88)	56(84)	
Yes	19(14)	8(12)	11(16)	
KPS score, n(%)				0.678
40	2(2)	0(0)	2(3)	
50	5(4)	3(5)	2(3)	
60	7(5)	3(5)	4(6)	
70	12(9)	8(12)	4(6)	
80	29(22)	14(21)	15(22)	
90	56(42)	29(44)	27(40)	
100	22(17)	9(14)	13(19)	
Smoking, n(%)				0.255
No	51(38)	29(44)	22(33)	
Yes	82(62)	37(56)	45(67)	
Hypertension, n(%)				1
No	80(60)	40(61)	40(60)	
Yes	53(40)	26(39)	27(40)	
Diabetes, n(%)				1
No	116(87)	58(88)	58(87)	

Yes	17(13)	8(12)	9(13)	
Hyperlipemia, n(%)				0.579
No	120(90)	61(92)	59(88)	
Yes	13(10)	5(8)	8(12)	
Heart failure, n(%)				1
No	130(98)	65(98)	65(97)	
Yes	3(2)	1(2)	2(3)	
ACS, n(%)				0.619
No	129(97)	65(98)	64(96)	
Yes	4(3)	1(2)	3(4)	

Abbreviation: IQR, interquartile range; CRP, C-reactive protein; PNI, neutrophil lymphocyte ratio; NLR, neutrophil lymphocyte ratio; SCLC, small-cell lung cancer; TKI, Tyrosine Kinase Inhibitor; VEGF, vascular endothelial growth factor; KPS, Karnofsky Performance Status; ACS, acute coronary syndrome. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

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217 MiR-219-5p Expression Level, and Clinical Risk Factors Predict the 218 Development of SCLC Patients

219 According to the univariate analysis, high miR-219-5p levels (≥ 1.50) was a strong
220 protective predictor of cancer-related mortality (HR 0.36, 95% CI 0.25-0.53, P
221 < 0.001) (table 2). Kaplan Meier curve showed that patients in the high miR-219-5p
222 group had a decreased cumulative rate of death than those in the low miR-219-5p
223 group (log-rank $P < 0.001$) (figure 2A). Meanwhile, patients who accepted
224 immunotherapy also showed a low mortality compared to those patients without
225 accepting immunotherapy in the survival curve (HR 0.28, 95% CI 0.15-0.52, P
226 < 0.001) (figure 2B).

227 In addition, gender, age, serum CRP level, albumin level, lymphocytes count,
228 PNI score, immunotherapy, heart failure, and KPS score were also correlated with
229 overall mortality (table 2). When adjusted by age and gender, patients in the
230 miR-219-5p high-level group also displayed a low cumulative rate death compared to
231 those in the low-level group.

Table 2. Cox Regression Analysis of Hazard Ratio on SCLC patients

Variation	Non-adjustment		Model 1	
	Hazard Ratio (95% CI)	p.value	Hazard Ratio (95% CI)	p.value
Gender, male vs. female	1.66 [1.02, 2.69]	0.041*	-	-

Age (year), ≥60 vs. < 60	1.52 [1.03, 2.26]	0.036*	-	-
BMI, ≥23.12 kg/m ² vs. < 22.86kg/m ²	0.99 [0.69, 1.43]	0.97	0.95 [0.66, 1.38]	0.806
Serum CEA level, >3.43 ng/ml vs. ≤3.43 ng/ml	1.01 [0.70, 1.46]	0.954	1.00 [0.69, 1.45]	0.999
Serum CRP level, >7.83 μmol/L vs. ≤7.83 μmol/L	1.91 [1.31, 2.78]	0.001**	1.92 [1.32, 2.79]	0.001**
Albumin level, >39.46 g/L vs. ≤39.46 g/L	1.66 [1.15, 2.40]	0.007**	1.60 [1.10, 2.31]	0.013*
Neutrophils count, >4.55x10 ⁹ /L vs. ≤ 4.55x10 ⁹ /L	1.18 [0.82, 1.71]	0.367	1.15 [0.79, 1.66]	0.464
Lymphocytes count, >1.63x10 ⁹ /L vs. ≤ 1.63x10 ⁹ /L	0.59 [0.40, 0.85]	0.005**	0.61 [0.42, 0.89]	0.01*
Hemoglobin level, >133 g/L vs. ≤ 133 g/L	0.80 [0.56, 1.16]	0.244	0.72 [0.49, 1.05]	0.089
Platelet count, >233x10 ⁹ /L vs. ≤ 233x10 ⁹ /L	1.23 [0.85, 1.78]	0.275	1.23 [0.85, 1.78]	0.275
PNI score, >47.9 vs. ≤47.9	0.54 [0.37, 0.78]	0.001**	0.53 [0.37, 0.77]	0.001**
NLR, >2.66 vs. ≤2.66	1.18 [0.82, 1.71]	0.367	1.20 [0.83, 1.74]	0.341
Metastasis, Yes vs. No	1.05 [0.70, 1.57]	0.81	1.10 [0.73, 1.64]	0.654
Stage of NSCLC, IV, III vs. II and I	0.94 [0.50, 1.75]	0.838	1.05 [0.56, 1.98]	0.868
Stage of SCLC, Yes vs. No	1.33 [0.90, 1.96]	0.154	1.43 [0.97, 2.12]	0.074
Immunotherapy, Yes vs. No	0.28 [0.15, 0.52]	<0.001***	0.30 [0.16, 0.55]	<0.001***
Therapy of radiation, Yes vs. No	0.88 [0.60, 1.27]	0.488	0.79 [0.54, 1.16]	0.235
Application of platinum, Yes vs. No	0.61 [0.15, 2.47]	0.483	0.66 [0.16, 2.70]	0.562
Target therapy, Yes vs. No	0.75 [0.42, 1.33]	0.323	0.81 [0.45, 1.45]	0.481
Application of TKI, Yes vs. No	0.77 [0.41, 1.44]	0.415	0.82 [0.44, 1.53]	0.534
Application of VEGF inhibitor, Yes vs. No	0.83 [0.47, 1.45]	0.518	0.90 [0.51, 1.59]	0.723
Chemotherapy, AP vs. others	0.61 [0.37, 1.00]	0.052	0.66 [0.40, 1.09]	0.104
Smoking, Yes vs. No	1.23 [0.84, 1.79]	0.292	0.90 [0.56, 1.43]	0.645
Hypertension, Yes vs. No	1.13 [0.78, 1.64]	0.531	1.10 [0.75, 1.60]	0.622
Diabetes, Yes vs. No	0.90 [0.51, 1.61]	0.726	0.97 [0.54, 1.75]	0.927
Hyperlipemia, Yes vs. No	0.79 [0.42, 1.48]	0.461	0.77 [0.40, 1.46]	0.421
Heart failure, Yes vs. No	5.61 [1.71, 18.42]	0.004**	6.43 [1.94, 21.26]	0.002**
ACS, Yes vs. No	0.74 [0.23, 2.35]	0.612	0.69 [0.21, 2.21]	0.53
KPS score, >80 vs. ≤ 80	0.52 [0.35, 0.75]	0.001**	0.51 [0.35, 0.74]	<0.001***
miR-219-5p ≥ 1.50 vs. < 1.50	0.36 [0.25, 0.53]	<0.001***	0.37 [0.25, 0.55]	<0.001***

Abbreviation: HR, hazard risk; BMI, Body Mass Index; CRP, C-reactive protein; PNI, neutrophil lymphocyte ratio; NLR, neutrophil lymphocyte ratio; SCLC, small-cell lung cancer; TKI, Tyrosine Kinase Inhibitor; VEGF, vascular endothelial growth factor; KPS, Karnofsky Performance Status; ACS, acute coronary syndrome. ***p<0.001, **p<0.01, *p<0.05.

Model 1: Adjusted by age and gender

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233 Independent Prognostic Factors for OS of Patients With SCLC

234 After multivariate adjustment, the miR-219-5p high level (HR 0.39, 95% CI
235 0.26-0.59, $P < 0.001$) was also associated with a low increase in the risk of death
236 (figure 3). Meanwhile, gender, PNI score, immunotherapy and heart failure were also
237 the independent risk factors for OS.

238

239 **Development and Validation of an OS-predicting Nomogram**

240 The independently related risk factors derived from the multivariate analysis were
241 used to create an OS estimation nomogram (figure 4). The prognostic model was
242 internally validated according to the bootstrap validation method. With an unadjusted
243 C index of 0.691 and a bootstrap-corrected C index of 0.691, the nomogram displayed
244 excellent accuracy in estimating the risk of OS. In the validation cohort, the
245 nomogram showed a C index of 0.691 for the estimation of OS. A suitable calibration
246 curve for risk estimation was also displayed ($R^2=0.455$, LR $\chi^2=80.55$) (figure 4B).
247 We collected 86 NSCLC patients from Sichuan Cancer Hospital in the external
248 validation step. The ROC curve showed an AUC of 0.783 (0.743-0.822) for predicting
249 5-year overall survival, compared with an AUC of 0.749 (0.709-0.788) for the
250 external validation data (figure 5). We calculated the total score using Nomogram for
251 patients in the training and validation sets, respectively, and divided them into four
252 groups according to 40-60,61-80,81-100,101-120, and performed Kaplan-Meier
253 analysis and plotted survival curves, which were found to have good separation and
254 were statistically significant (Figure S1a, S1b).

256 **Discussion**

257 In this study, we detected the expression of miR-219-5p in a large cohort of SCLC
258 patients at a single institution, between Mar 2010 and June 2015. The results
259 suggested that reduced expression of miR-219-5p was significantly correlated with
260 unfavorable clinical features. Moreover, patients in high miR-219-5p expression
261 group displayed better OS compared with those in low miR-219-5p expression group.
262 The multivariate analysis demonstrated miR-219-5p an independent prognostic factor
263 for OS. In addition, to propose, and retrospectively verify in an independent cohort of
264 patients, these independent risk factors were applied to establish a nomogram for OS
265 estimation. The nomogram revealed good accuracy in estimating the risk of OS.

266 Carcinogenesis involves multiple biological processes which are related to many
267 key genes[15, 16]. The characteristics of cancer occurrence represent properties that a

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4 268 cell acquire a certain ability to become and maintain itself as a cancer cell[17]. The
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6 269 key genes guide the cellular signaling pathways related to occurrence and progression
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8 270 of cancers[18, 19]. Using miRNA expression to predict the clinical diagnosis and
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10 271 prognosis of cancer has more advantages than mRNAs, as miRNAs are proved to be
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12 272 the vital posttranscriptional regulators of gene expression[20, 21]. In comparison with
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14 273 mRNAs, these vital gene regulators are highly conserved among species[22].

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16 274 It has been reported that miRNAs were related to the initiation and progression
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18 275 of various cancers, and many miRNAs have been identified as a promising biomarker
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20 276 for prognostic prediction of cancer[10, 23]. Recently, some miRNAs have been
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22 277 proved to be a novel prognostic biomarker for SCLC[24, 25]. A study of Yu et al.
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24 278 indicated that miR-92a-2 was significantly higher in SCLC patients group compared
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26 279 to healthy control, and detection of miR-92a-2 levels could be a potential biomarker
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28 280 for patients with SCLC[26]. As a promising biomarker, miR-219-5p has been
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30 281 identified as a prognostic factor for different cancers. Long et al. found that
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32 282 miR-219-5p expression level was distinctly decreased in melanoma tissues and cell
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34 283 lines, and the modulation of miR-219-5p expression could be a prognostic biomarker
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36 284 and treatment strategy in melanoma[27]. A study from Huang et al. suggested a role
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38 285 of miR-219-5p for prognostic prediction and therapeutic strategy in colorectal
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40 286 cancer[28]. However, there is no studies exploring the role of miR-219-5p for
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42 287 biomarker in patients with SCLC. To the best of our knowledge, this study was the
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44 288 first attempt ever made to comprehensively evaluate the role for prognostic prediction
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46 289 based on miR-219-5p expression in patients with SCLC. In the current study, we
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48 290 initially examined the expression levels of miR-219-5p in SCLC patients. We, for the
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50 291 first time, demonstrated a correlation of the altered miR-219-5p expression with
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4 292 available clinical parameters. We found that miR-219-5p was significantly associated
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6 293 with lymphocytes count, PNI score and stage of SCLC. The univariate analysis
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9 294 indicated that increased miR-219-5p expression was a protective predictor for
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11 295 mortality. Kaplan-Meier curve displayed that patients with elevated miR-219-5p
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13 296 expression levels or accepted immunotherapy had low cumulative incidence of death
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17 297 compared to those with reduced miR-219-5p expression or unaccepted
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19 298 immunotherapy, respectively. In addition, gender, age, serum CRP level, albumin
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21 299 level, lymphocytes count, PNI score, immunotherapy, heart failure, KPS score and
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23 300 miR-219-5p level were associated with overall mortality. The multivariate analysis
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26 301 showed that miR-219-5p, gender, PNI score, immunotherapy and heart failure could
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29 302 predict OS as the independent risk factors.

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32 303 Nomograms are applied for visualization of statistical models, graphical
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34 304 evaluation of variable significance and examination of predicted values[29, 30]. They
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36 305 have been widely performed to predict cancer risks and therapeutic outcomes[31, 32].
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38 306 Most recently, several studies have successfully established a prognostic nomogram
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40 307 that combined a miRNA with clinical-related variables for OS estimation in different
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42 308 cancers[33-35]. Although nomogram is becoming increasingly popular, no studies
43
44 309 have built prognostic models using combination of miR-219-5p and clinical risk
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46 310 factors in SCLC patients. In this study, based on the combination of miR-219-5p and
47
48 311 independent clinicopathological variables, we created a nomogram model that could
49
50 312 provide an individual prognostic prediction for OS estimation in SCLC patients. The
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52 313 results indicated excellent accuracy in estimating the risk of OS. There was a suitable
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54 314 calibration curve for risk estimation, indicating a well-performed nomogram, and
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56 315 good agreements between observation and prediction. To further verify the accuracy
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58 316 and efficiency of the model, an external date containing 86 patients from Sichuan
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60 317 Cancer Hospital was conducted. The results indicated that the prognostic model could

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4 318 accurately predict the prognosis of SCLC patients. Hence, this was the first prognostic
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6 319 nomogram for patients with SCLC that considered clinical parameters in addition to
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8 320 miR-219-5p. This nomogram could provide comprehensive information for patients,
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10 321 as well as a better guidance for clinical therapy. Based on the model, the potential
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12 322 high-risk patients with low survival rate could be more accurately selected for a
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14 323 specific therapeutic strategy.

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17 325 **CONCLUSIONS**

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20 326 In conclusion, we found that the miR-219-5p expression levels were significantly
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22 327 correlated with clinical parameters of SCLC patients. Furthermore, miR-219-5p was
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24 328 proved to be an independent factor for prognostic prediction in patients with SCLC.
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26 329 Moreover, nomogram based on multivariate analysis showed excellent accuracy in
27
28 330 estimating the risk of OS. However, the prospective validation of the prognostic
29
30 331 nomogram is needed in the future.

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32 332

33 333 **Acknowledgements** The authors would like to thank the referees and the associate
34
35 334 editor for their constructive advice.

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39 336 **Contributors** ZHX and XMW designed the study. ZJC, XHZ, JGZ and MQX
40
41 337 collected and analysed the data. ZJC, JGZ and XHZ drafted the initial manuscript.
42
43 338 ZHX and XMW reviewed and edited the article. All authors read and approved the
44
45 339 final manuscript.

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48
49 341 **Funding** Not applicable.

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53 343 **Competing interests** The authors declare that they have no competing interests.

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57 345 **Patient consent for publication** Not required.

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4 347 **Ethics approval**

5 348 The study was approved by ethics committee of Suzhou Xiangcheng People's
6 349 Hospital. The reference number was 20140193. All procedures performed in the
7
8 350 present study were in accordance with the principles outlined in the 1964 Helsinki
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11 351 Declaration and its later amendments.

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15 353 **Provenance and peer review** Not commissioned; externally peer reviewed.

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19 355 **Data Availability Statement** The datasets used and analyzed during the current study
20
21 356 are available from the corresponding author on reasonable request.

22
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25 358 **REFERENCES**

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459 Figure Legends

460 **Figure 1** A flow chart of the screening process.

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462 **Figure 2** Overall survival (OS) of SCLC patients in different levels of miR-219-5p
463 and different treatments. (A) OS of SCLC patients with high or low level of
464 miR-219-5p. (B) OS of SCLC patients with different treatments (immunotherapy vs
465 non-immunotherapy).

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467 **Figure 3** Multivariate cox regression analysis of 5-year overall survival on data in the

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4 468 SCLC patients.

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8 470 **Figure 4** Nomogram for overall survival (OS) risk estimation of SCLC patients and
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10 471 its predictive performance. (A) Nomogram to estimate the OS risk of SCLC patients
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12 472 in different variations. (B) Validity of the predictive performance of the nomogram in
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14 473 estimating the OS risk of SCLC patients.

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17 475 **Figure 5** External validation of the prognostic model.

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21 477 **Table 1** Study participant characteristics at enrollment.

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25 479 **Table 2** Univariate cox regression analysis of overall survival on SCLC patients.

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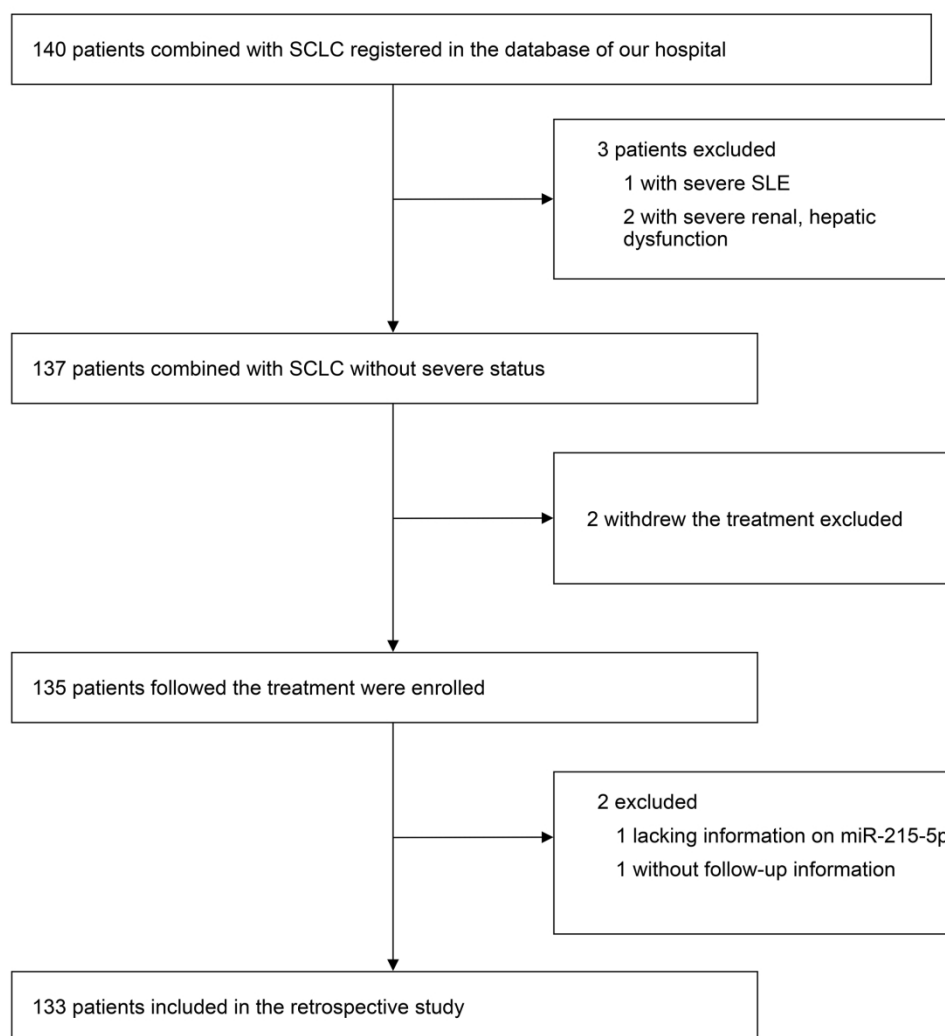


Figure 1 A flow chart of the screening process.

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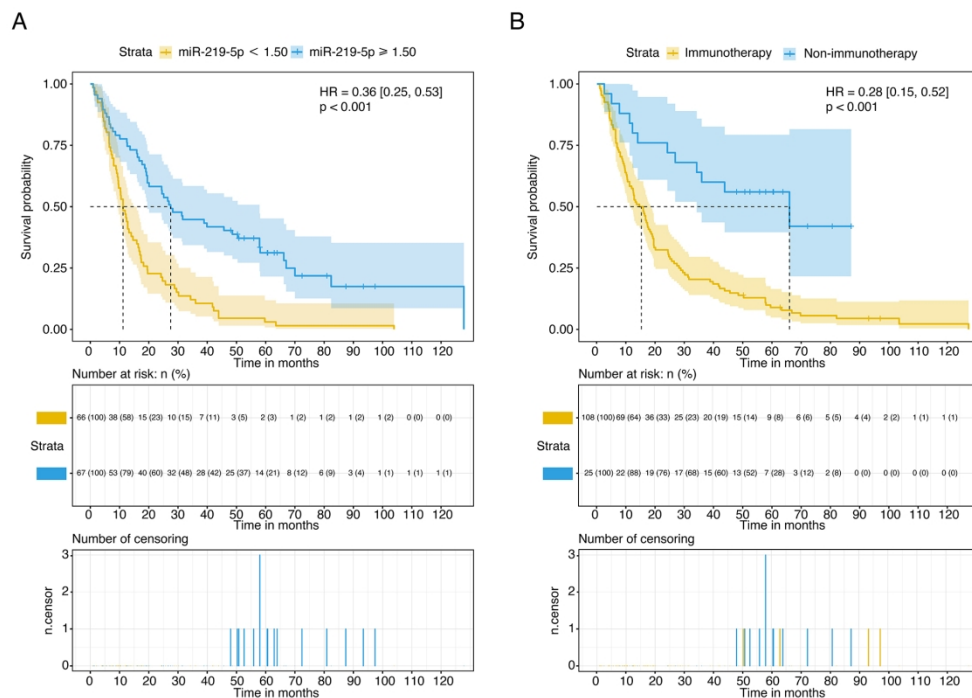


Figure 2 Overall survival (OS) of SCLC patients in different levels of miR-219-5p and different treatments. (A) OS of SCLC patients with high or low level of miR-219-5p. (B) OS of SCLC patients with different treatments (immunotherapy vs non-immunotherapy).

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Figure 3

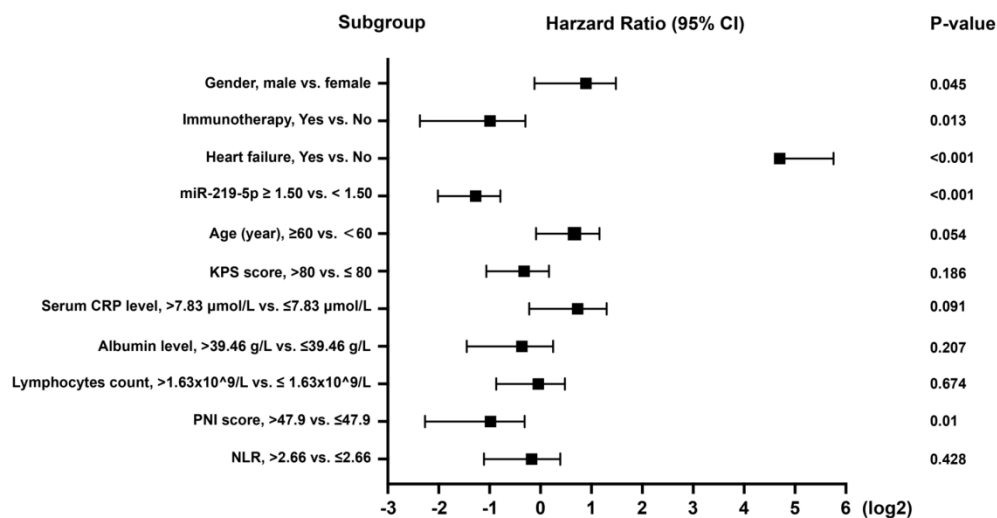


Figure 3 Multivariate cox regression analysis of 5-year overall survival on data in the SCLC patients.

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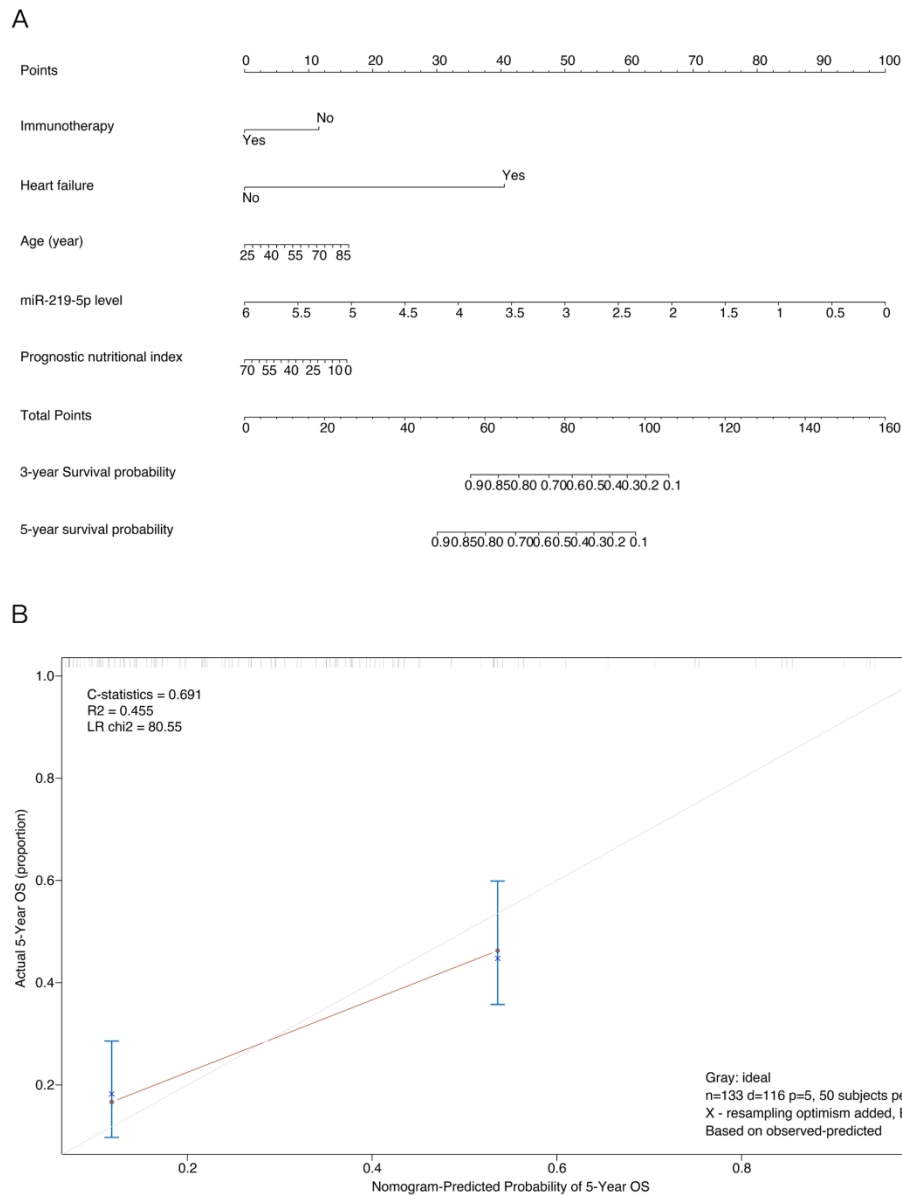


Figure 4 Nomogram for overall survival (OS) risk estimation of SCLC patients and its predictive performance. (A) Nomogram to estimate the OS risk of SCLC patients in different variations. To build the nomogram, find the position of each variable on the corresponding axis, draw a line to the points axis for the number of points, add the points from all of the variables, and draw a line from the total points axis to determine the OS probabilities at the lower line of the nomogram. (B) Validity of the predictive performance of the nomogram in estimating the OS risk of SCLC patients.

180x216mm (300 x 300 DPI)

Figure 5

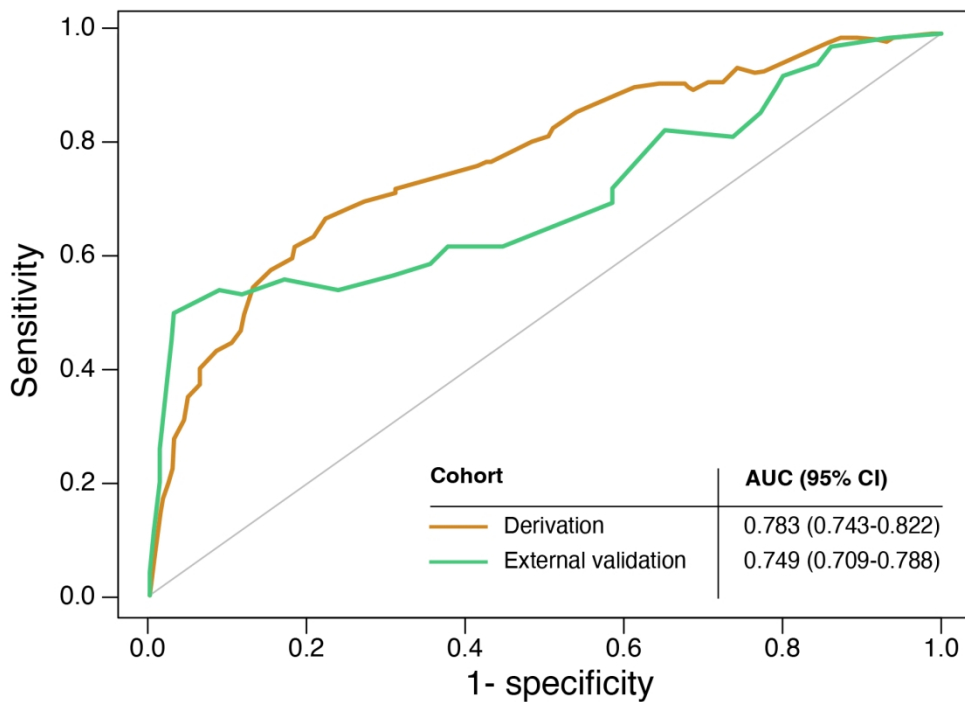


Figure 5 External validation of the prognostic model.

Supplement Materials

Part I. Details of variants

Patients' age: The age of patients was based on the time of diagnosis of NSCLC.

Body mass index: The equation of body mass index (BMI) is calculated as follow:
$$\text{BMI (kg/m}^2\text{)} = \text{weight (kg)} / \text{height (m)}^2$$
. The data of BMI was recorded before clinical treatment.

Serum CEA level: The serum level of carcinoembryonic antigen (CEA) is one of tumor marks, the higher serum *CEA* level than that of healthy individuals led to its clinical application as a diagnostic biomarker for cancer.

Serum CRP level: C-reactive protein (CRP) is detected by colorimetric determination through fasting blood collection and CRP is one of the inflammation markers.

Albumin level: Serum albumin is detected by colorimetric determination through fasting blood collection. And serum albumin is one of the indicators of nutritional status.

Neutrophils count, lymphocytes count, hemoglobin level, platelet count: All these indexes were extracted from blood routine examination to evaluate preoperative status of patients.

PNI score: Prognostic nutritional index (PNI) is an index to evaluate the nutritional status of surgical patients, to predict the risk of surgery and to help make prognostic judgment. The equation is listed as below: $\text{PNI} = \text{serum albumin (g/L)} + 5 * \text{lymphocytes (*}10^9\text{/L)}$.

NLR: Neutrophil to Lymphocyte Ratio (NLR) is an index of inflammation markers to predict the prognosis of various cancer, including lung cancer.

Pathologic type: Non-small cell lung cancer can be classified as adenocarcinoma, squamous cell carcinoma, large cell lung cancer, or others, depending on surgical or tumor biopsy specimens

Stage of NSCLC: Non-small cell lung cancer was staged according to the seventh edition of international union against cancer (UICC) TNM was published in 2009

Surgery: For early and locally advanced NSCLC, which is generally acceptable, there is a history of surgical resection.

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Therapy of radiation: Radiation therapy is a medical procedure that uses the delivery of high-energy radiation to kill cancer cells and shrink tumors, including external beam radiation therapy, internal radiation therapy and stereotactic body radiotherapy (SBRT). It aims to cure small tumors, treat lung cancer both locally and nearby lymph nodes, treat metastases, relieve symptoms, and also at prevention.

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Application of platinum: Platinum-based chemotherapy may be used as a first or second line therapy for advanced lung cancer, after surgery (adjuvant chemotherapy), or before surgery to reduce the size of a tumor (neoadjuvant therapy). The most commonly used platinum drugs are cisplatin, carboplatin and nedaplatin. Treatment of non-small cell lung cancer begins with either cisplatin or carboplatin combined with another medication, such as docetaxel, pemetrexed, paclitaxel, gemcitabine and gemcitabine.

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Target therapy: Targeted therapies for lung cancer are drugs that are tailored to attack certain mutations of cancer cells. These mutations lead to the production of abnormal proteins which guide the growth and development of a cancer cell. Common driver mutations include EGFR mutations, ALK rearrangements, ROS1 rearrangements, MET amplifications, KRAS mutations, HER2 mutations, BRAF mutations and so on.

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Application of TKI: Tyrosine kinase inhibitors (TKI) act as competitive inhibitors of ATP binding to tyrosine kinases, or as tyrosine analogs that block tyrosine kinase activity and inhibit cell proliferation. Epithelial growth factor receptor (EGFR) is a protein that is present on the surface of both normal cells and lung cancer cells. Mutations in EGFR can occur at different locations on exon 18 to 21. ALK-positive lung cancer refers to people who have lung cancer that tests positive for the EML4-ALK fusion mutation. Several FDA-approved medications available to treat EGFR-positive and ALK-positive lung adenocarcinoma, including:

- 42 • EGFR inhibitors - Tarceva (erlotinib), Gilotrif (afatinib), Iressa (gefitinib), Tagrisso (osimertinib), and Portrazza (necitumumab)
- 43 • ALK inhibitors - Xalkori (crizotinib), Zykadia (ceritinib), and Alecensa (alectinib)
- 44 • ROS1 inhibitor - Xalkori (crizotinib)

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Application of VEGF inhibitor: VEGF (Vascular endothelial growth factor) can activate angiogenesis through different signaling pathways. VEGF inhibitors are drugs that block the ability of tumors to form new blood vessels, and hence, grow and spread. Some currently available medications include bevacizumab, ramucirumab, axitinib, endostar and anlotinib.

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KPS score: A performance measure for rating the ability of a person to perform usual activities, evaluating a patient's progress after a therapeutic procedure, and determining a patient's suitability for therapy. It is used most commonly in the

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3 prognosis of cancer therapy, usually after chemotherapy and customarily administered
4 before and after therapy. It was named for Dr. David A. Karnofsky, an American
5 specialist in cancer chemotherapy. Patients with more than 80 scores had better
6 postoperative status and longer survival time. And patients with more than 70 scores
7 can suffer from chemoradiotherapy.
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11 **Smoking:** Smoker refers to continuous or cumulative smoking > 1 cigarette/day over
12 a lifetime of more than 6 months. (1997, WHO)
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15 **Hypertension:** Hypertension is defined as a repeatedly elevated blood pressure
16 exceeding 140 over 90 mmHg.
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19 **Diabetes:** Diabetes is a group of metabolic diseases characterized by hyperglycemia.
20 And fasting blood glucose more than 7.0 mmol/l or blood glucose more than 11.1
21 mmol/l within 2 hours after meal can be diagnosed diabetes.
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24 **Hyperlipemia:** Hyperlipemia means the presence of excess fat or lipids in the blood.
25 And total cholesterol ≥ 6.2 mmol/L, low density lipoprotein cholesterol ≥ 4.1 mmol/L,
26 triglyceride ≥ 2.3 mmol/L, high density lipoprotein cholesterol < 1.0 mmol/L can be
27 diagnosed hyperlipemia.
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30 **Heart failure:** The information was recorded through history taking and verified after
31 hospitalization. Heart failure means inability of the heart to keep up with the demands
32 on it and, specifically, failure of the heart to pump blood with normal efficiency. Heart
33 failure may be due to failure of the right or left or both ventricles. The signs and
34 symptoms depend upon which side of the heart is failing. They can include shortness
35 of breath (dyspnea), asthma due to the heart (cardiac asthma), pooling of blood (stasis)
36 in the general body (systemic) circulation or in the liver's (portal) circulation, swelling
37 (edema), blueness or duskiness (cyanosis), and enlargement (hypertrophy) of the
38 heart.
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44 **ACS:** Acute coronary syndrome is a term for a group of conditions that suddenly stop
45 or severely reduce blood from flowing to the heart. When blood cannot flow to the
46 heart, the heart muscle can become damaged. Heart attack and unstable angina are
47 both acute coronary syndromes (ACS).
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50 **Withdraw treatment:** Reasons for patients withdrew from treatment were listed
51 below: 1) High cost of the treatment: In China, the cost of hormone therapy can be up
52 to 3000 RMB a month if without health insurance. 2) Poor patient compliance: Some
53 patients discontinue treatment because they do not comply with the treatment plan
54 prescribed by their doctor.
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Part II. Report of sample size assessment

PASS 15.0.5

Tests for Two Survival Curves Using Cox's Proportional Hazards Model

Numeric Results with $H_a: HR \neq 1$

	Total Sample Size N	Control Sample Size N1	Trtmnt Sample Size N2	Prop'n Control N1/N P1	Hazard Ratio h2/h1 HR	Control Prob Event Pev1	Trtmnt Prob Event Pev2	Control Events E1	Trtmnt Events E2	Alpha	Beta
Power	103	51	52	0.500	0.500	0.750	0.950	38.3	49.4	0.050	0.100

References

- Chow, S.C., Shao, J., Wang, H. 2008. Sample Size Calculations in Clinical Research, 2nd Edition. Chapman & Hall/CRC.
- Schoenfeld, David A. 1983. 'Sample Size Formula for the Proportional-Hazards Regression Model', Biometrics, Volume 39, Pages 499-503.

Report Definitions

Power is the probability of rejecting a false null hypothesis. Power should be close to one.

N is the total sample size.

N1 and N2 are the sample sizes of the control and treatment groups.

P1 is the proportion of the total sample that is in the control group, group 1.

HR is the hazard ratio: h_2/h_1 .

Pev1 and Pev2 are the probabilities of an event in the control and the treatment groups.

E1 and E2 are the number of events required in the control and the treatment groups.

Alpha is the probability of a type one error: rejecting a true null hypothesis.

Beta is the probability of a type two error: failing to reject a false null hypothesis.

Summary Statements

A two-sided test of whether the hazard ratio is one with an overall sample size of 103 subjects (of which 51 are in the control group and 52 are in the treatment group) achieves 90% power at a 0.050 significance level when the hazard ratio is actually 0.500. The number of events required to achieve this power is 87.7. It is anticipated that proportions of subjects having the event during the study is 0.750 for the control group and 0.950 for the treatment group. These results assume that the hazard ratio is constant throughout the study and that Cox proportional hazards regression is used to analyze the data.

Procedure Input Settings

Autosaved Template File

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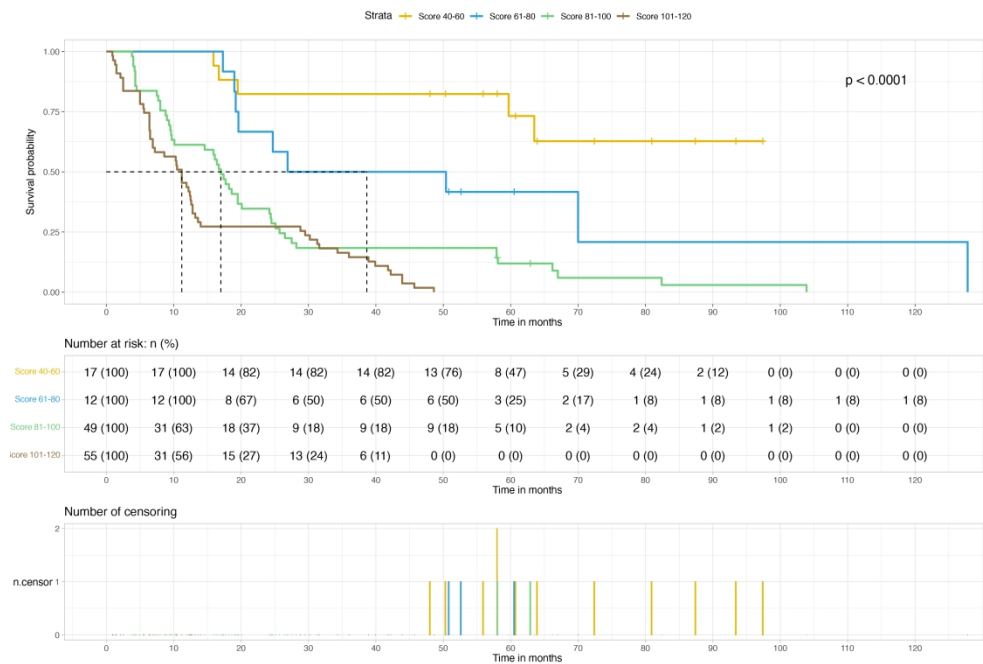
Proportional Hazards Model - Autosaved 2020_1_24-9_57_39.t92

Design Tab

Solve For:	Sample Size
Alternative Hypothesis:	Ha: HR \neq 1
Power:	0.90
Alpha:	0.05
Group Allocation:	Equal (N1 = N2)
Pev1 (Probability of a Control Event):	0.750
Pev2 (Probability of a Treatment Event):	0.950
HR (Actual Hazard Ratio = h2/h1):	0.5

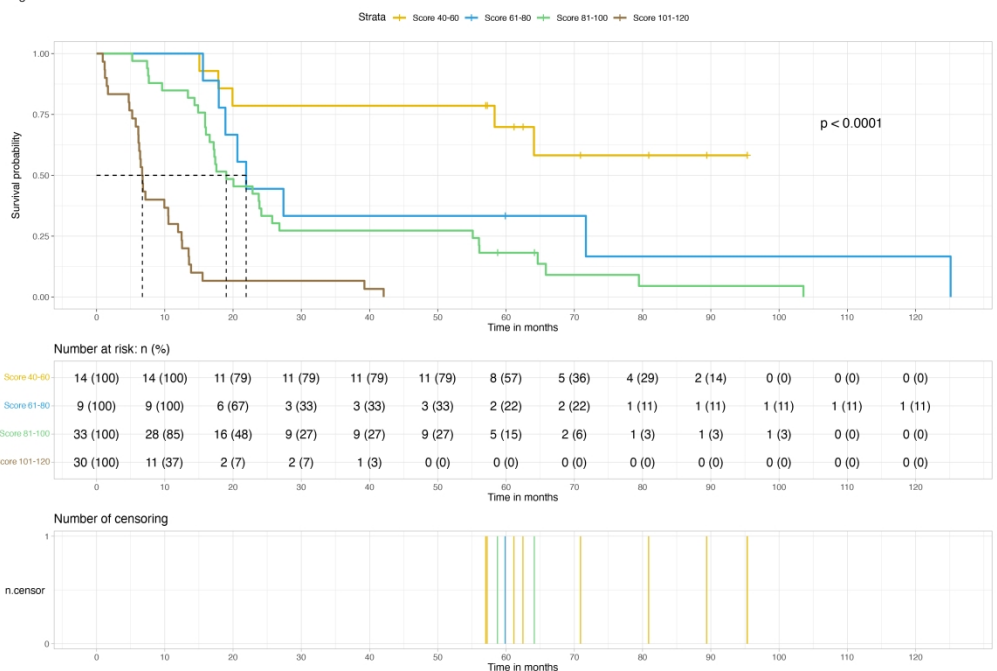
For peer review only

Figure S1a



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Figure S1b



STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of cohort studies

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			5
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6
		(b) For matched studies, give matching criteria and number of exposed and unexposed	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	7
		(b) Describe any methods used to examine subgroups and interactions	7
		(c) Explain how missing data were addressed	7
		(d) If applicable, explain how loss to follow-up was addressed	7
		(e) Describe any sensitivity analyses	7
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	8
		(c) Consider use of a flow diagram	8
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9
		(b) Indicate number of participants with missing data for each variable of interest	9
		(c) Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	Report numbers of outcome events or summary measures over time	10
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10
		(b) Report category boundaries when continuous variables were categorized	11
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	11
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12
Discussion			
Key results	18	Summarise key results with reference to study objectives	13-14
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Prognostic value of MiR-219-5p in relation to mortality in patients with small cell lung cancer: a retrospective, observational cohort study in China

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-064700.R3
Article Type:	Original research
Date Submitted by the Author:	29-Nov-2022
Complete List of Authors:	Cao, Zhijun; Suzhou Ninth People's Hospital, Urology Zhang, Jigang; First Affiliated Hospital of Soochow University Zhang, Xiaohui; First Affiliated Hospital of Soochow University Xiang, Mengqi; Medical School of University of Electronic Science and Technology of China, Department of Medical Oncology Xu, Zhihua; First Affiliated Hospital of Soochow University, General surgery Wu, Xiangmei; Suzhou Xiangcheng People's Hospital
Primary Subject Heading:	Oncology
Secondary Subject Heading:	Surgery
Keywords:	Risk management < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, ONCOLOGY, Gene therapy < ONCOLOGY, Molecular aspects < ONCOLOGY, Oncogenes < ONCOLOGY

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4 1 **Prognostic value of MiR-219-5p in relation to mortality in**
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6 2 **patients with small cell lung cancer: a retrospective,**
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8 3 **observational cohort study in China**
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13 5 Zhijun Cao^{2#}, Jigang Zhang^{4,#}, Xiaohui Zhang^{5,#}, Mengqi Xiang⁶, Zhihua Xu^{3*},
14
15 6 Xiangmei Wu^{1*}
16
17 7

18
19 8 ¹ Department of Endocrinology, Suzhou Xiangcheng People's Hospital, Suzhou, China

20
21 9 ² Department of Urology, Suzhou Ninth People's Hospital, Soochow University,
22
23 10 Suzhou, China

24
25 11 ³ Department of General Surgery, The First Affiliated Hospital of Soochow University,
26
27 12 Suzhou, China

28
29 13 ⁴ Department of Traumatology Surgery, The First Affiliated Hospital of Soochow
30
31 14 University, Suzhou, China

32
33 15 ⁵ Department of Medicine, Respiratory, Emergency and Intensive Care Medicine, The
34
35 16 First Affiliated Hospital of Soochow University, Suzhou, China

36
37 17 ⁶ Department of Medical Oncology, Sichuan Cancer Hospital, Medical School of
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39 18 University of Electronic Science and Technology of China, Chengdu, Sichuan, China
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43 20 # These authors contributed equally to this work
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47 22 *Correspondence to:

48
49 23 Xiangmei Wu

50
51 24 dr_xiangmeiwu@163.com

52
53 25 or

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55 26 Zhihua Xu

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57 27 dr_zhihuaxu@163.com
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29 ABSTRACT

30 **Objectives** Small cell lung cancer (SCLC) is a lethal human malignancy, and previous
31 studies support the contribution of microRNA (miRNA) to cancer progression. The
32 prognostic value of miR-219-5p in SCLC patients remains unclear. This study aimed
33 to evaluate the predictive value of miR-219-5p with respect to mortality in patients with
34 SCLC and to incorporate miR-219-5p level into a prediction model and nomogram for
35 mortality.

36 **Design** Retrospective observational cohort study.

37 **Setting and participants** Our main cohort included data from 133 patients with SCLC
38 between Mar 1, 2010, and June 1, 2015 from the Suzhou Xiangcheng People's Hospital.
39 Data from 86 patients with NSCLC at Sichuan Cancer Hospital and the First Affiliated
40 Hospital of Soochow University were used for external validation.

41 **Outcome measures** Tissue samples were taken during admission and stored and miR-
42 219-5p levels were measured at a later date. A Cox proportional hazard model was used
43 for survival analyses and for analyzing risk factors to create a nomogram for mortality
44 prediction. The accuracy of the model was evaluated by C-index and calibration curve.

45 **Results** Mortality in patients with a high miR-219-5p level (≥ 1.50) ($n=67$) was 74.6%,
46 while mortality in the low-level group ($n=66$) was 100.0%. Based on univariate analysis,
47 we included significant factors ($P < 0.05$) in a multivariate regression model: patients
48 with high miR-219-5p level (HR 0.39, 95%CI 0.26-0.59, $P < 0.001$), immunotherapy
49 (HR 0.44, 95%CI 0.23-0.84, $P < 0.001$), PNI score >47.9 (HR=0.45, 95%CI 0.24-0.83,
50 $P = 0.01$) remained statistically significant factors for improved overall survival (OS).
51 The nomogram had good accuracy in estimating the risk, with a bootstrap-corrected C
52 index of 0.691. External validation indicated an AUC of 0.749 (0.709-0.788).

53 **Conclusions** MiR-219-5p level was associated with a reduced risk of mortality in
54 patients with SCLC. A nomogram incorporating MiR-219-5p level and clinical factors
55 demonstrated good accuracy in estimating the risk of overall mortality. Prospective
56 validation of the prognostic nomogram is needed.

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58 **Keywords:** small cell lung cancer, miR-219-5p, overall survival, nomogram,

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4 59 prediction model

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8 61 **Strengths and limitations of this study**

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10 62 ▶ The study utilizes databases of all patients with small cell lung cancer (SCLC) in
11 63 two defined geographical regions of China.

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13 64 ▶ The study included the creation of a nomogram for predicting survival probabilities
14 65 in individual patients.

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16 66 ▶ However, the model is not comprehensive since the database does not include all
17 67 prognostic factors for SCLC.

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19 68 ▶ Additionally, the available data on treatment status are not adequately detailed to
20 69 distinguish the impact of various treatment plans.

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22 70 ▶ The model needs to be prospectively assessed to determine its reliability.

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73 INTRODUCTION

74 Lung cancer is the leading cause of cancer deaths worldwide, with millions of new
75 cases diagnosed each year[1]. Small cell lung cancer (SCLC) is a kind of
76 neuroendocrine malignant tumor with poor prognosis, accounting for about 15% of
77 lung cancer patients[2]. SCLC is generally divided into limited disease (LD-SCLC) and
78 extensive disease (ED-SCLC). Platinum combined with etoposide is the first-line
79 therapeutic strategy of SCLC, and most patients are easy to receive initial
80 chemotherapy[3]. However, the 5-year survival rates of LD-SCLC and ED-SCLC are
81 only 15% and 3%, respectively[4]. Therefore, improvement in early diagnosis and
82 prognostic prediction of SCLC is vital.

83 MicroRNAs (miRNAs) are endogenous non-coding RNAs (~ 22 nt), which
84 regulate mRNA activity by hybridization with 3' - untranslated region (UTR) of specific
85 genes[5]. Many studies have shown that miRNAs could participate in a variety of cell
86 biological processes, including cell growth, differentiation and apoptosis[6, 7]. In
87 addition, researches have demonstrated that miRNAs are frequently dysregulated in
88 cancers[8, 9], and some miRNAs can serve as diagnostic and prognostic biomarkers for
89 cancers[10]. Recently, several miRNAs have been proved to participate in the
90 occurrence and development of SCLC, but few of them are likely to be a biomarker or
91 therapeutic target for SCLC.

92 Recently, miR-219-5p has been found to be abnormally expressed and play a
93 significant role in different cancers. Ma et al. found that the expression of miR-219-5p
94 was significantly decreased in esophageal squamous cell carcinoma (ESCC) tissues
95 compared with normal tissues[11]. A study of Gong et al. revealed a tumor suppressive
96 role for miR-219-5p by targeting glypican-3 in hepatocellular carcinoma (HCC)[12].
97 On the contrary, Yang et al. indicated that miR-219-5p could promote cell growth and
98 metastasis of HCC and serve as a prognostic marker for HCC patients[13]. A research
99 investigated by Wei et al. suggested that miR-219-5p could inhibit proliferation,
100 migration and invasion of epithelial ovarian cancer through downregulation of the Wnt
101 signaling pathway, and it could serve as a diagnostic biomarker and therapeutic target

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4 102 for epithelial ovarian cancer[14]. However, the biological functions of miR-219-5p and
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6 103 its potential prognostic role for biomarker in SCLC are still unknown.

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8 104 In this study, we aimed to examine the variation in the expression levels of miR-
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10 105 219-5p in patients with SCLC, to evaluate the predictive value of miR-219-5p with
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12 106 respect to mortality in patients with SCLC, and to incorporate miR-219-5p level into a
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14 107 prediction model and nomogram for mortality.

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17 109 **METHODS**

18 109 **Study design and patients**

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22 111 The study utilizes databases of all patients with small cell lung cancer (SCLC) in two
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24 112 defined geographical regions of China. Our main cohort included data obtained from
25
26 113 133 patients with SCLC between Mar 2010 and June 2015, in the Suzhou Xiangcheng
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28 114 People's Hospital. Tissue samples were taken during admission and stored and the miR-
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30 115 219-5p levels were measured at a later date. Those participants who lacked information
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32 116 on complement components data, withdrew from treatment or lacked follow-up
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34 117 information were excluded. Clinical information of patients, including gender, age,
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36 118 BMI, neutrophils count, lymphocytes count, serum CEA level, CRP level, albumin
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38 119 level, hemoglobin level, stage of SCLC, platelet count, PNI score, KPS score, NLR,
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40 120 pathologic type, immunotherapy, therapy of radiation, application of platinum,
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42 121 application of VEGF inhibitor, target therapy, application of TKI, smoking, ACS,
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44 122 diabetes, heart failure and hyperlipemia, were recorded. Diagnosis of SCLC was
45
46 123 confirmed by histopathological examination. The median length of follow-up was 23.6
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48 124 months. Median was used as the cut-off value. The definition and details of all the
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50 125 variables above were provided in Supplemental Materials Part I. Data from 86 patients
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4 126 with NSCLC at Sichuan Cancer Hospital and the First Affiliated Hospital of Soochow
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6 127 University were used for external validation. For both the main cohort and the
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9 128 validation cohort, informed consent was obtained from all patients or their immediate
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12 129 family members for the collection and storage of samples and their use for future
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14 130 scientific research. All procedures were in line with the guidelines of the ethics
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17 131 committee of Suzhou Xiangcheng People's Hospital, Sichuan Cancer Hospital, and the
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20 132 First Affiliated Hospital of Soochow University and the study was performed in
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22 133 accordance with the Declaration of Helsinki.
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135 **Assays for detection of MiR-219-5p levels**

136 The quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was
137 conducted for the detection of miR-219-5p expression levels.

138 Total RNA from tissues was isolated and extracted using miRcute Extraction and
139 Separation of miRNAs kit (Tiangen Biotech Co., Ltd., Beijing, China), and then
140 reversely transcribed into cDNA by PrimeScript™ II 1st strand cDNA synthesis kit
141 (Takara Biotechnology Co., Ltd., Dalian, China) according to the manufacturer's
142 protocol. SYBR PrimeScript miRNA RT-PCR kit (Takara Biotechnology Co., Ltd.)
143 was used for qRT-PCR. The thermocycling conditions were as follows: one cycle at
144 95°C for 3 min (initial denaturation), 40 cycles at 95°C for 15 s and 60°C for 30 s. U6
145 small nuclear RNA (U6) served as the respective internal control. The relative
146 expression of miR-219-5p was quantified by the $2^{-\Delta\Delta C_t}$ methods, and normalized to the
147 U6. The following primers were used: miR-219-5p forward, 5'-
148 ACACTCCAGCTGGGTGATTGTCCAAACGCAAT-3' and reverse, 5'-
149 CTCAACTGGTGTCGTGGA-3'; U6 forward, 5'-
150 GCTTCGGCAGCACATATACTAAAAT-3' and reverse, 5'-
151 CGCTTCACGAATTTGCGTGTTCAT-3'. The experiments were repeated at least 3

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8 154 **Statistical analysis**

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10 155 Sample size assessment was performed using NCSS-PASS software version 11.0

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12 156 (<https://www.ncss.com/software/pass/>). Power was set as 0.99, and alpha was 0.5. The

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14 157 mortality in both the miR-219-5p high-level group and miR-219-5p low-level group in

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16 158 our previous data (2008-2009) (0.750 and 0.950) were entered into the PASS. The

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18 159 Actual Hazard Ratio was set as 0.50. Then the sample size was calculated using PASS,

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20 160 and the minimum sample size was 103 (control = 51, experiment = 43). Our sample

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22 161 size was 133 (66 and 67 for each group), which was suitable. The report of sample size

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24 162 assessment was displayed in Supplemental Material Part II. The missing data (<5.0%)

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26 163 were estimated by random forest algorithm using the mice package in RStudio (R

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28 164 version 3.6.1). Categorical variates were presented as percentages and compared via

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30 165 the κ^2 test. Continuous variates with skewed and normal distributions were presented

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32 166 as median with interquartile ranges and mean \pm standard deviation. The Mann-Whitney

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34 167 U test and the unpaired t-test were applied for comparison between Groups. Cumulative

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36 168 mortality was showed by the Kaplan-Meier curve and analyzed by the log-rank test.

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38 169 Univariate and multivariate survival analyses for OS were conducted using the Cox

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40 170 regression model. The forest plots were used to visualize the significance of covariates

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42 171 to the prognosis. The restricted cubic spline analyses were performed with Harrell's

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44 172 Regression Modelling Strategies (rms) package.

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46 173 We screened multifactor analysis for statistically significant indicators for

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48 174 inclusion in the prediction model. To build the nomogram, find the position of each

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4 175 variable on the corresponding axis, draw a line to the points axis for the number of
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6 176 points, add the points from all the variables, and draw a line from the total points axis
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8 177 to determine the OS probabilities at the lower line of the nomogram. The contribution
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10 178 of each covariate was quantified and visualized in a prognostic nomogram with internal
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12 179 validation via 1000-times bootstrapping. The consistency of the resulting model was
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14 180 assessed by the calibration assay. Decision curve analyses were performed to evaluate
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16 181 net clinical benefits of the model compared with conventional prognostic scores. The
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18 182 scatter plots were applied for visualization of the consistency of each model. A 1000-
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20 183 time bootstrapping was employed as indicated. The association between miR-219-5p
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22 184 class and survival endpoints was evaluated by Kaplan-Meier curves and log-rank test.
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24 185 Statistical analysis was performed using the RStudio (R version 3.6.1) with the
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26 186 following packages: 'ggplot2', 'rms', 'PredictABLE', 'risk regression', and
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28 187 'survminer'.

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30 189 **Patient and public involvement**

31 190 None.

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33 192 **RESULTS**

34 193 **Baseline characteristics**

35 194 A total of 133 SCLC patients who were diagnosed between Mar 2010 and June 2015
36 195 were included in the main cohort. A flowchart of the screening process was shown in
37 196 figure 1. The median age of these patients was 64 years old (58-70), and it contained
38 197 106 (80.0%) males. Median serum CEA and CRP level was 3.43 ng/ml and 7.83 μ mol/L,
39 198 respectively. For the stage of SCLC, 51 (38.0%) patients were diagnosed with limited
40 199 disease, 82 (61.0%) patients were extensive disease. 25 (19.0%) patients accepted the
41 200 immunotherapy therapy. 54 (41.0%) patients got the therapy of radiation. In addition,

201 platinum was applied for 131 (98.0%) patients, and TKI was used for 15 (12.0%)
 202 patients. KPS score of these patients was examined, and the results revealed that 107
 203 (81.0%) patients got 80 or higher points. The distribution of basic diseases was also
 204 assessed in our data. Diabetes was found in 17 (13.0%) patients, while hyperlipemia
 205 was 13 (10.0%). Cardiovascular diseases such as heart failure and ACS were found in
 206 3 (2.0%) and 4 (3.0%) patients, respectively. 13 (10.0%) patients suffered from
 207 hypertension. In addition, 82 (62.0%) patients had a habit of smoking among all the
 208 patients. The baseline characteristics of these patients were listed in table 1.

209 Among all the 133 patients, overall mortality was 87.2%. The mortality in high
 210 miR-219-5p level group (n=67) was 74.6%, while the mortality in the low-level group
 211 (n=66) was 100.0%. Moreover, in the high miR-219-5p level group, patients with
 212 extensive disease were 35 (52.0%), while the low group was 47 (71.0%) (table 1).

Table 1. Study participant characteristics at enrollment

Variation	Total (n=133)	Cohort, median (IQR)		p.value
		mir-219-5p<1.50, (n=66)	mir-219-5p≥1.50, (n=67)	
Age, (year)	64(58,70)	65(59,70)	63(56.5,68)	0.276
BMI, (kg/m ²)	23.12±3.09	22.99±3.22	23.26±2.98	0.619
Serum CEA level, (ng/ml)	3.43(1.96,9.26)	3.34(1.92,10.05)	3.43(1.99,8.11)	0.87
Serum CRP level, (μmol/L)	7.83(1.68,12.78)	10.68(2.78,13.15)	5.64(1.2,12.16)	0.107
Albumin level, (g/L)	39.46±5.2	38.86±4.68	40.05±5.65	0.188
Neutrophils count, (10 ⁹ /L)	4.55(3.59,5.88)	4.54(3.77,5.75)	4.55(3.48,5.92)	0.975
Lymphocytes count, (10 ⁹ /L)	1.63(1.31,1.95)	1.5(1.21,1.78)	1.73(1.38,2.08)	0.031*
Hemoglobin level, (g/L)	133(125,145)	134(124,145)	132(125,143.5)	0.564
Platelet count, (10 ⁹ /L)	233(184,288)	244.5(180,293.75)	226(184.5,273.5)	0.306
PNI score	47.9(43.95,51.85)	45.98(42.51,50.38)	48.7(44.92,54)	0.026*
NLR	2.66(1.99,4.19)	2.75(2.11,4.57)	2.59(1.92,3.7)	0.232
Gender, (n%)				0.211
Female	27(20)	10(15)	17(25)	
Male	106(80)	56(85)	50(75)	
Metastasis, n(%)				0.299
No	45(34)	19(29)	26(39)	
Yes	88(66)	47(71)	41(61)	
Stage of SCLC				0.038*
Limited disease	51(38)	19(29)	32(48)	

Extensive disease	82(62)	47(71)	35(52)	
Immunotherapy, (n%)				0.197
No	108(81)	57(86)	51(76)	
Yes	25(19)	9(14)	16(24)	
Therapy of radiation, (n%)				0.417
No	79(59)	42(64)	37(55)	
Yes	54(41)	24(36)	30(45)	
Application of platinum, (n%)				0.244
No	2(2)	2(3)	0(0)	
Yes	131(98)	64(97)	67(100)	
Chemotherapy				0.45
AP	28(21)	12(18)	16(24)	
DP	15(11)	6(9)	9(13)	
EP	71(53)	35(53)	36(54)	
GP	3(2)	2(3)	1(1)	
Others	16(12)	11(17)	5(7)	
Target therapy, (n%)				0.627
No	116(87)	59(89)	57(85)	
Yes	17(13)	7(11)	10(15)	
Application of TKI, (n%)				0.449
No	118(89)	60(91)	58(87)	
TKI I	9(7)	4(6)	5(7)	
TKI II	1(1)	1(2)	0(0)	
TKI III	5(4)	1(2)	4(6)	
Application of VEGF inhibitor, n(%)				0.645
No	114(86)	58(88)	56(84)	
Yes	19(14)	8(12)	11(16)	
KPS score, n(%)				0.678
40	2(2)	0(0)	2(3)	
50	5(4)	3(5)	2(3)	
60	7(5)	3(5)	4(6)	
70	12(9)	8(12)	4(6)	
80	29(22)	14(21)	15(22)	
90	56(42)	29(44)	27(40)	
100	22(17)	9(14)	13(19)	
Smoking, n(%)				0.255
No	51(38)	29(44)	22(33)	
Yes	82(62)	37(56)	45(67)	
Hypertension, n(%)				1
No	80(60)	40(61)	40(60)	
Yes	53(40)	26(39)	27(40)	
Diabetes, n(%)				1
No	116(87)	58(88)	58(87)	
Yes	17(13)	8(12)	9(13)	

Hyperlipemia, n(%)				0.579
No	120(90)	61(92)	59(88)	
Yes	13(10)	5(8)	8(12)	
Heart failure, n(%)				1
No	130(98)	65(98)	65(97)	
Yes	3(2)	1(2)	2(3)	
ACS, n(%)				0.619
No	129(97)	65(98)	64(96)	
Yes	4(3)	1(2)	3(4)	

Abbreviation: IQR, interquartile range; CRP, C-reactive protein; PNI, neutrophil lymphocyte ratio; NLR, neutrophil lymphocyte ratio; SCLC, small cell lung cancer; TKI, Tyrosine Kinase Inhibitor; VEGF, vascular endothelial growth factor; KPS, Karnofsky Performance Status; ACS, acute coronary syndrome. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

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214 MiR-219-5p expression level and clinical risk factors

215 According to the univariate analysis, high miR-219-5p levels (≥ 1.50) was a strong
 216 protective predictor of mortality (HR 0.36, 95% CI 0.25-0.53, $P < 0.001$) (table 2).
 217 Kaplan Meier curve showed that patients in the high miR-219-5p group had a decreased
 218 cumulative rate of death than those in the low miR-219-5p group (log-rank $P < 0.001$)
 219 (figure 2A). Meanwhile, patients who accepted immunotherapy also showed a low
 220 morality compared to those patients without accepting immunotherapy in the survival
 221 curve (HR 0.28, 95% CI 0.15-0.52, $P < 0.001$) (figure 2B).

222 In addition, gender, age, serum CRP level, albumin level, lymphocytes count, PNI
 223 score, immunotherapy, heart failure, and KPS score were also correlated with overall
 224 mortality (table 2). When adjusted by age and gender, patients in the miR-219-5p high-
 225 level group also displayed a low cumulative rate death compared to those in the low-
 226 level group.

Table 2. Results of univariate Cox regression analysis for overall mortality

Variation	Non-adjustment		Model 1	
	Hazard Ratio (95% CI)	p.value	Hazard Ratio (95% CI)	p.value
Gender, male vs. female	1.66 [1.02, 2.69]	0.041*	-	-
Age (year), ≥ 60 vs. < 60	1.52 [1.03, 2.26]	0.036*	-	-
BMI, ≥ 23.12 kg/m ² vs. < 22.86 kg/m ²	0.99 [0.69, 1.43]	0.97	0.95 [0.66, 1.38]	0.806
Serum CEA level, > 3.43 ng/ml vs. ≤ 3.43 ng/ml	1.01 [0.70, 1.46]	0.954	1.00 [0.69, 1.45]	0.999
Serum CRP level, > 7.83 μ mol/L vs. ≤ 7.83 μ mol/L	1.91 [1.31, 2.78]	0.001**	1.92 [1.32, 2.79]	0.001**
Albumin level, > 39.46 g/L vs. ≤ 39.46 g/L	1.66 [1.15, 2.40]	0.007**	1.60 [1.10, 2.31]	0.013*

Neutrophils count, >4.55x10 ⁹ /L vs. ≤ 4.55x10 ⁹ /L	1.18 [0.82, 1.71]	0.367	1.15 [0.79, 1.66]	0.464
Lymphocytes count, >1.63x10 ⁹ /L vs. ≤ 1.63x10 ⁹ /L	0.59 [0.40, 0.85]	0.005**	0.61 [0.42, 0.89]	0.01*
Hemoglobin level, >133 g/L vs. ≤ 133 g/L	0.80 [0.56, 1.16]	0.244	0.72 [0.49, 1.05]	0.089
Platelet count, >233x10 ⁹ /L vs. ≤ 233x10 ⁹ /L	1.23 [0.85, 1.78]	0.275	1.23 [0.85, 1.78]	0.275
PNI score, >47.9 vs. ≤47.9	0.54 [0.37, 0.78]	0.001**	0.53 [0.37, 0.77]	0.001**
NLR, >2.66 vs. ≤2.66	1.18 [0.82, 1.71]	0.367	1.20 [0.83, 1.74]	0.341
Metastasis, Yes vs. No	1.05 [0.70, 1.57]	0.81	1.10 [0.73, 1.64]	0.654
Stage of NSCLC, IV, III vs. II and I	0.94 [0.50, 1.75]	0.838	1.05 [0.56, 1.98]	0.868
Stage of SCLC, Yes vs. No	1.33 [0.90, 1.96]	0.154	1.43 [0.97, 2.12]	0.074
Immunotherapy, Yes vs. No	0.28 [0.15, 0.52]	<0.001***	0.30 [0.16, 0.55]	<0.001***
Therapy of radiation, Yes vs. No	0.88 [0.60, 1.27]	0.488	0.79 [0.54, 1.16]	0.235
Application of platinum, Yes vs. No	0.61 [0.15, 2.47]	0.483	0.66 [0.16, 2.70]	0.562
Target therapy, Yes vs. No	0.75 [0.42, 1.33]	0.323	0.81 [0.45, 1.45]	0.481
Application of TKI, Yes vs. No	0.77 [0.41, 1.44]	0.415	0.82 [0.44, 1.53]	0.534
Application of VEGF inhibitor, Yes vs. No	0.83 [0.47, 1.45]	0.518	0.90 [0.51, 1.59]	0.723
Chemotherapy, AP vs. others	0.61 [0.37, 1.00]	0.052	0.66 [0.40, 1.09]	0.104
Smoking, Yes vs. No	1.23 [0.84, 1.79]	0.292	0.90 [0.56, 1.43]	0.645
Hypertension, Yes vs. No	1.13 [0.78, 1.64]	0.531	1.10 [0.75, 1.60]	0.622
Diabetes, Yes vs. No	0.90 [0.51, 1.61]	0.726	0.97 [0.54, 1.75]	0.927
Hyperlipemia, Yes vs. No	0.79 [0.42, 1.48]	0.461	0.77 [0.40, 1.46]	0.421
Heart failure, Yes vs. No	5.61 [1.71, 18.42]	0.004**	6.43 [1.94, 21.26]	0.002**
ACS, Yes vs. No	0.74 [0.23, 2.35]	0.612	0.69 [0.21, 2.21]	0.53
KPS score, >80 vs. ≤ 80	0.52 [0.35, 0.75]	0.001**	0.51 [0.35, 0.74]	<0.001***
miR-219-5p ≥ 1.50 vs. < 1.50	0.36 [0.25, 0.53]	<0.001***	0.37 [0.25, 0.55]	<0.001***

Abbreviation: HR, hazard risk; BMI, Body Mass Index; CRP, C-reactive protein; PNI, neutrophil lymphocyte ratio; NLR, neutrophil lymphocyte ratio; SCLC, small cell lung cancer; TKI, Tyrosine Kinase Inhibitor; VEGF, vascular endothelial growth factor; KPS, Karnofsky Performance Status; ACS, acute coronary syndrome. ***p<0.001, **p<0.01, *p<0.05.

Model 1: Adjusted by age and gender

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228 Independent prognostic factors for OS

229 After multivariate adjustment, the miR-219-5p high level (HR 0.39, 95% CI 0.26-0.59,
230 $P < 0.001$) was also associated with a low increase in the risk of death (figure 3).
231 Meanwhile, gender, PNI score, immunotherapy and heart failure were also the
232 independent risk factors for OS.

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234 Development and validation of an OS-prediction nomogram

235 The independently related risk factors derived from the multivariate analysis were used
236 to create an OS estimation nomogram (figure 4A). The prognostic model was internally

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4 237 validated according to the bootstrap validation method. With an unadjusted C index of
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6 238 0.691 and a bootstrap-corrected C index of 0.691, the nomogram displayed excellent
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8 239 accuracy in estimating the risk of OS. In the validation cohort, the nomogram showed
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10 240 a C index of 0.691 for the estimation of OS. A suitable calibration curve for risk
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12 241 estimation was also displayed ($R^2=0.455$, LR $\chi^2=80.55$) (figure 4B). We collected 86
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14 242 NSCLC patients from Sichuan Cancer Hospital in the external validation step. The
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16 243 ROC curve showed an AUC of 0.783 (0.743-0.822) for predicting 5-year overall
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18 244 survival, compared with an AUC of 0.749 (0.709-0.788) for the external validation data
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20 245 (figure 5). We calculated the total score using Nomogram for patients in the training
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22 246 and validation sets, respectively, and divided them into four groups according to 40-
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24 247 60,61-80,81-100,101-120, and performed Kaplan-Meier analysis and plotted survival
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26 248 curves, which were found to have good separation and were statistically significant
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28 249 (Supplementary Figures S1a, S1b).

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31 251 **Discussion**

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33 252 In this study, we detected the expression of miR-219-5p in a large cohort of SCLC
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35 253 patients at a single institution, between Mar 2010 and June 2015. The results suggested
36
37 254 that reduced expression of miR-219-5p was significantly correlated with unfavorable
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39 255 clinical features. Moreover, patients in high miR-219-5p expression group displayed
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41 256 better OS compared with those in low miR-219-5p expression group. The multivariate
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43 257 analysis demonstrated miR-219-5p an independent prognostic factor for OS. In addition,
44
45 258 to propose, and retrospectively verify in an independent cohort of patients, these
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47 259 independent risk factors were applied to establish a nomogram for OS estimation. The
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49 260 nomogram revealed good accuracy in estimating the risk of OS.

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51 261 Carcinogenesis involves multiple biological processes which are related to many
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53 262 key genes[15, 16]. The characteristics of cancer occurrence represent properties that a
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55 263 cell acquire a certain ability to become and maintain itself as a cancer cell[17]. The key
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57 264 genes guide the cellular signaling pathways related to occurrence and progression of
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59 265 cancers[18, 19]. Using miRNA expression to predict the clinical diagnosis and
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4 266 prognosis of cancer has more advantages than mRNAs, as miRNAs are proved to be
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6 267 the vital posttranscriptional regulators of gene expression[20, 21]. In comparison with
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8 268 mRNAs, these vital gene regulators are highly conserved among species[22].
9

10 269 It has been reported that miRNAs were related to the initiation and progression of
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12 270 various cancers, and many miRNAs have been identified as a promising biomarker for
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14 271 prognostic prediction of cancer[10, 23]. Recently, some miRNAs have been proved to
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16 272 be a novel prognostic biomarker for SCLC[24, 25]. A study of Yu et al. indicated that
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18 273 miR-92a-2 was significantly higher in SCLC patients group compared to healthy
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20 274 control, and detection of miR-92a-2 levels could be a potential biomarker for patients
21
22 275 with SCLC[26]. As a promising biomarker, miR-219-5p has been identified as a
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24 276 prognostic factor for different cancers. Long et al. found that miR-219-5p expression
25
26 277 level was distinctly decreased in melanoma tissues and cell lines, and the modulation
27
28 278 of miR-219-5p expression could be a prognostic biomarker and treatment strategy in
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30 279 melanoma[27]. A study from Huang et al. suggested a role of miR-219-5p for
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32 280 prognostic prediction and therapeutic strategy in colorectal cancer[28]. However, there
33
34 281 is no studies exploring the role of miR-219-5p for biomarker in patients with SCLC. To
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36 282 the best of our knowledge, this study was the first attempt ever made to
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38 283 comprehensively evaluate the role for prognostic prediction based on miR-219-5p
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40 284 expression in patients with SCLC. In the current study, we initially examined the
41
42 285 expression levels of miR-219-5p in SCLC patients. We, for the first time, demonstrated
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44 286 a correlation of the altered miR-219-5p expression with available clinical parameters.
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46 287 We found that miR-219-5p was significantly associated with lymphocytes count, PNI
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48 288 score and stage of SCLC. The univariate analysis indicated that increased miR-219-5p
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4 289 expression was a protective predictor for mortality. Kaplan-Meier curve displayed that
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6 290 patients with elevated miR-219-5p expression levels or accepted immunotherapy had
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9 291 low cumulative incidence of death compared to those with reduced miR-219-5p
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11 292 expression or unaccepted immunotherapy, respectively. In addition, gender, age, serum
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13 293 CRP level, albumin level, lymphocytes count, PNI score, immunotherapy, heart failure,
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15 294 KPS score and miR-219-5p level were associated with overall mortality. The
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17 295 multivariate analysis showed that miR-219-5p, gender, PNI score, immunotherapy and
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19 296 heart failure could predict OS as the independent risk factors.
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24 297 Nomograms are applied for visualization of statistical models, graphical
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26 298 evaluation of variable significance and examination of predicted values[29, 30]. They
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28 299 have been widely performed to predict cancer risks and therapeutic outcomes[31, 32].
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30 300 Most recently, several studies have successfully established a prognostic nomogram
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32 301 that combined a miRNA with clinical-related variables for OS estimation in different
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34 302 cancers[33-35]. Although nomogram is becoming increasingly popular, no studies have
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36 303 built prognostic models using combination of miR-219-5p and clinical risk factors in
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38 304 SCLC patients. In this study, based on the combination of miR-219-5p and independent
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40 305 clinicopathological variables, we created a nomogram model that could provide an
41
42 306 individual prognostic prediction for OS estimation in SCLC patients. The results
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44 307 indicated excellent accuracy in estimating the risk of OS. There was a suitable
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46 308 calibration curve for risk estimation, indicating a well-performed nomogram, and good
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48 309 agreements between observation and prediction. To further verify the accuracy and
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50 310 efficiency of the model, an external date containing 86 patients from Sichuan Cancer
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52 311 Hospital was conducted. The results indicated that the prognostic model could
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54 312 accurately predict the prognosis of SCLC patients. Hence, this was the first prognostic
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56 313 nomogram for patients with SCLC that considered clinical parameters in addition to
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58 314 miR-219-5p. This nomogram could provide comprehensive information for patients, as
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60 315 well as a better guidance for clinical therapy. Based on the model, the potential high-

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4 316 risk patients with low survival rate could be more accurately selected for a specific
5 317 therapeutic strategy.

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8 9 319 **Strengths and limitations**

10 320 We screened valid variables by Cox regression to construct a survival prediction model
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12 321 for SCLC and collected data for external validation in a logical manner. However, there
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14 322 are some limitations in this article. Firstly, experimental research explaining the
15
16 323 biological processes of miR-219-5p is needed. Thus, the molecular mechanism of miR-
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18 324 219-5p should be investigated in further research. Secondly, the prognostic nomogram
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20 325 needs to be further assessed in a prospective and large-scale multicenter study before it
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22 326 can be applied to clinical practice. Finally, our data lacked some of the risk factors
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24 327 associated with SCLC for inclusion, such as the determination of some of the high-risk
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26 328 genes and the patient's previous chemotherapy and specific targeted therapies, which
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28 329 will require further analysis in our future studies.

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31 32 331 **CONCLUSIONS**

33
34 332 In conclusion, we found that the miR-219-5p expression levels were significantly
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36 333 correlated with clinical parameters of SCLC patients. Furthermore, miR-219-5p was
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38 334 proved to be an independent factor for prognostic prediction in patients with SCLC.
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40 335 Moreover, a nomogram based on multivariate analysis and including miR-219-5p
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42 336 expression levels showed excellent accuracy in estimating the risk of OS. However, the
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44 337 prospective validation of the prognostic nomogram is needed in the future.

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51 52 341 **Contributors**

53
54 342 ZHX and XMW designed the study. ZJC, XHZ, JGZ and MQX collected and analysed
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56 343 the data. ZJC, JGZ and XHZ drafted the initial manuscript. ZHX and XMW reviewed
57
58 344 and edited the article. All authors read and approved the final manuscript.

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4 345

5 346 **Funding**

7 347 None.

9 348

11 349 **Competing interests**

13 350 The authors declare that they have no competing interests.

15 351

17 352 **Patient consent for publication**

19 353 Not applicable.

21 354

23 355 **Ethics approval**

25 356 The study was approved by ethics committee of Suzhou Xiangcheng People's Hospital
27 357 (reference number 20140193). All procedures performed in the present study were in
29 358 accordance with the principles outlined in the 1964 Helsinki Declaration and its later
31 359 amendments.

33 360

35 361 **Provenance and peer review**

37 362 Not commissioned; externally peer reviewed.

39 363

41 364 **Data availability statement**

43 365 The datasets used and analyzed during the current study are available from the
45 366 corresponding author on reasonable request.

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8 468 **Figure titles**

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10 469 **Figure 1.** Study screening flowchart
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14 471 **Figure 2.** Overall survival (OS) of SCLC patients with different levels of miR-219-5p
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16 472 and different treatments

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18 473 (A) OS of SCLC patients with high or low level of miR-219-5p. (B) OS of SCLC
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20 474 patients with different treatments (immunotherapy vs non-immunotherapy).
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24 476 **Figure 3.** Multivariate Cox regression analysis of 5-year overall survival
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28 478 **Figure 4.** Nomogram for overall survival (OS) risk estimation and its predictive
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30 479 performance

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32 480 (A) Nomogram to estimate the OS risk of SCLC patients. (B) Validity of the predictive
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34 481 performance of the nomogram in estimating the OS risk.
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37 483 **Figure 5.** External validation of the prognostic model
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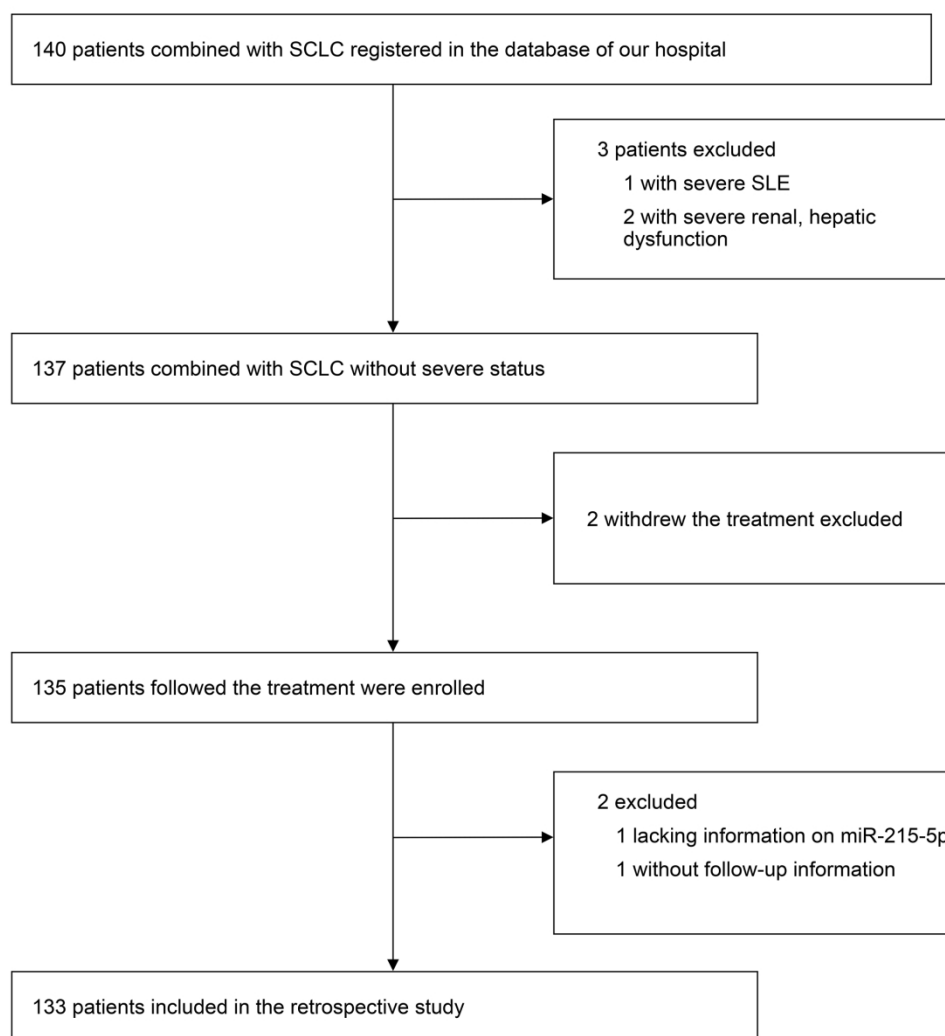


Figure 1 A flow chart of the screening process.

180x195mm (300 x 300 DPI)

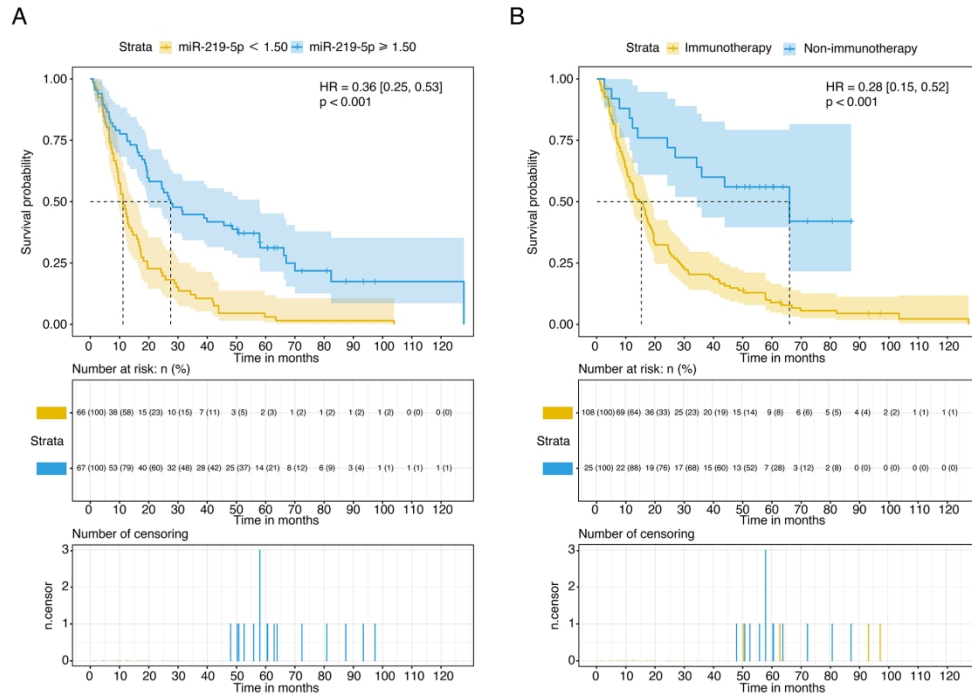


Figure 2 Overall survival (OS) of SCLC patients in different levels of miR-219-5p and different treatments. (A) OS of SCLC patients with high or low level of miR-219-5p. (B) OS of SCLC patients with different treatments (immunotherapy vs non-immunotherapy).

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Figure 3

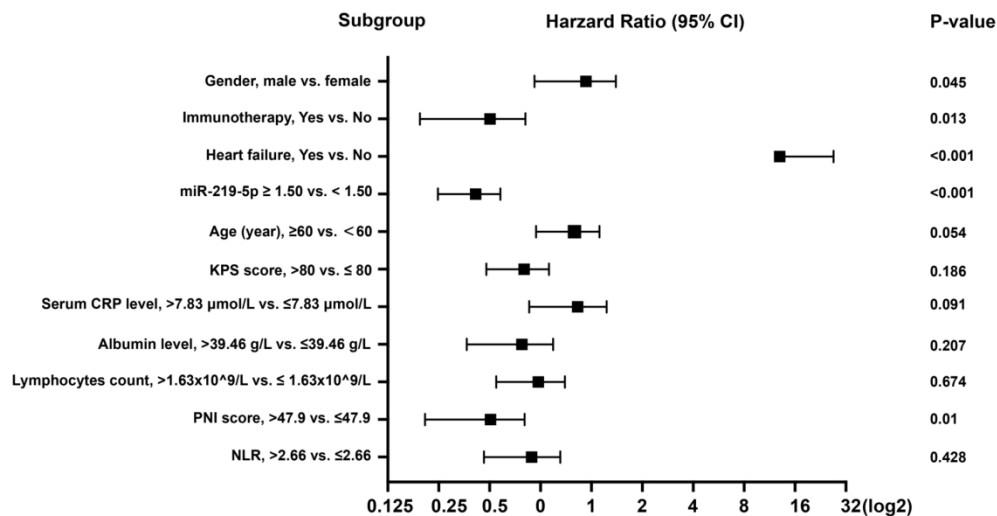
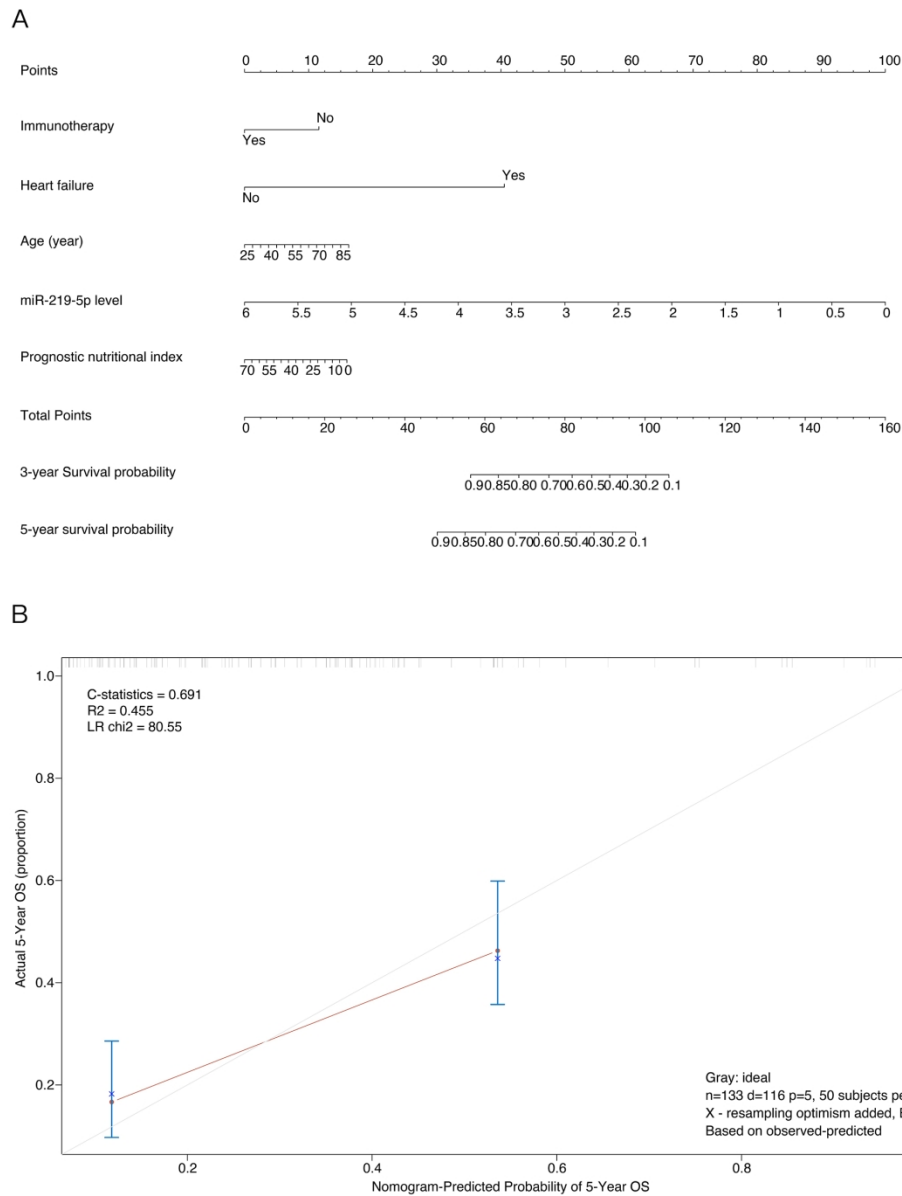


Figure 3 Multivariate cox regression analysis of 5-year overall survival on data in the SCLC patients.

159x91mm (300 x 300 DPI)



45 Figure 4 Nomogram for overall survival (OS) risk estimation of SCLC patients and its predictive performance.
46 (A) Nomogram to estimate the OS risk of SCLC patients in different variations. To build the nomogram, find
47 the position of each variable on the corresponding axis, draw a line to the points axis for the number of
48 points, add the points from all of the variables, and draw a line from the total points axis to determine the
49 OS probabilities at the lower line of the nomogram. (B) Validity of the predictive performance of the
50 nomogram in estimating the OS risk of SCLC patients.

51 180x216mm (300 x 300 DPI)

Figure 5

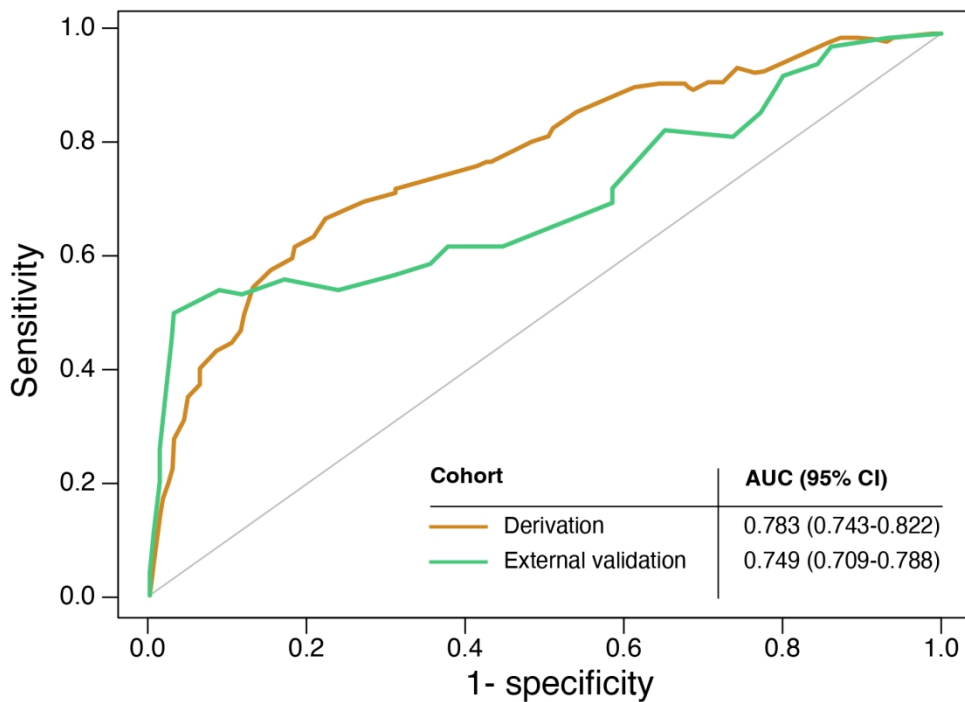


Figure 5 External validation of the prognostic model.

Supplement Materials

Part I. Details of variants

Patients' age: The age of patients was based on the time of diagnosis of NSCLC.

Body mass index: The equation of body mass index (BMI) is calculated as follow:
$$\text{BMI (kg/m}^2\text{)} = \text{weight (kg)} / \text{height (m)}^2$$
. The data of BMI was recorded before clinical treatment.

Serum CEA level: The serum level of carcinoembryonic antigen (CEA) is one of tumor marks, the higher serum *CEA* level than that of healthy individuals led to its clinical application as a diagnostic biomarker for cancer.

Serum CRP level: C-reactive protein (CRP) is detected by colorimetric determination through fasting blood collection and CRP is one of the inflammation markers.

Albumin level: Serum albumin is detected by colorimetric determination through fasting blood collection. And serum albumin is one of the indicators of nutritional status.

Neutrophils count, lymphocytes count, hemoglobin level, platelet count: All these indexes were extracted from blood routine examination to evaluate preoperative status of patients.

PNI score: Prognostic nutritional index (PNI) is an index to evaluate the nutritional status of surgical patients, to predict the risk of surgery and to help make prognostic judgment. The equation is listed as below: $\text{PNI} = \text{serum albumin (g/L)} + 5 * \text{lymphocytes (*}10^9\text{/L)}$.

NLR: Neutrophil to Lymphocyte Ratio (NLR) is an index of inflammation markers to predict the prognosis of various cancer, including lung cancer.

Pathologic type: Non-small cell lung cancer can be classified as adenocarcinoma, squamous cell carcinoma, large cell lung cancer, or others, depending on surgical or tumor biopsy specimens

Stage of NSCLC: Non-small cell lung cancer was staged according to the seventh edition of international union against cancer (UICC) TNM was published in 2009

Surgery: For early and locally advanced NSCLC, which is generally acceptable, there is a history of surgical resection.

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Therapy of radiation: Radiation therapy is a medical procedure that uses the delivery of high-energy radiation to kill cancer cells and shrink tumors, including external beam radiation therapy, internal radiation therapy and stereotactic body radiotherapy (SBRT). It aims to cure small tumors, treat lung cancer both locally and nearby lymph nodes, treat metastases, relieve symptoms, and also at prevention.

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Application of platinum: Platinum-based chemotherapy may be used as a first or second line therapy for advanced lung cancer, after surgery (adjuvant chemotherapy), or before surgery to reduce the size of a tumor (neoadjuvant therapy). The most commonly used platinum drugs are cisplatin, carboplatin and nedaplatin. Treatment of non-small cell lung cancer begins with either cisplatin or carboplatin combined with another medication, such as docetaxel, pemetrexed, paclitaxel, gemcitabine and gemcitabine.

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Target therapy: Targeted therapies for lung cancer are drugs that are tailored to attack certain mutations of cancer cells. These mutations lead to the production of abnormal proteins which guide the growth and development of a cancer cell. Common driver mutations include EGFR mutations, ALK rearrangements, ROS1 rearrangements, MET amplifications, KRAS mutations, HER2 mutations, BRAF mutations and so on.

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Application of TKI: Tyrosine kinase inhibitors (TKI) act as competitive inhibitors of ATP binding to tyrosine kinases, or as tyrosine analogs that block tyrosine kinase activity and inhibit cell proliferation. Epithelial growth factor receptor (EGFR) is a protein that is present on the surface of both normal cells and lung cancer cells. Mutations in EGFR can occur at different locations on exon 18 to 21. ALK-positive lung cancer refers to people who have lung cancer that tests positive for the EML4-ALK fusion mutation. Several FDA-approved medications available to treat EGFR-positive and ALK-positive lung adenocarcinoma, including:

- 42 • EGFR inhibitors - Tarceva (erlotinib), Gilotrif (afatinib), Iressa (gefitinib), Tagrisso (osimertinib), and Portrazza (necitumumab)
- 43 • ALK inhibitors - Xalkori (crizotinib), Zykadia (ceritinib), and Alecensa (alectinib)
- 44 • ROS1 inhibitor - Xalkori (crizotinib)

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Application of VEGF inhibitor: VEGF (Vascular endothelial growth factor) can activate angiogenesis through different signaling pathways. VEGF inhibitors are drugs that block the ability of tumors to form new blood vessels, and hence, grow and spread. Some currently available medications include bevacizumab, ramucirumab, axitinib, endostar and anlotinib.

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KPS score: A performance measure for rating the ability of a person to perform usual activities, evaluating a patient's progress after a therapeutic procedure, and determining a patient's suitability for therapy. It is used most commonly in the

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3 prognosis of cancer therapy, usually after chemotherapy and customarily administered
4 before and after therapy. It was named for Dr. David A. Karnofsky, an American
5 specialist in cancer chemotherapy. Patients with more than 80 scores had better
6 postoperative status and longer survival time. And patients with more than 70 scores
7 can suffer from chemoradiotherapy.
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11 **Smoking:** Smoker refers to continuous or cumulative smoking > 1 cigarette/day over
12 a lifetime of more than 6 months. (1997, WHO)
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15 **Hypertension:** Hypertension is defined as a repeatedly elevated blood pressure
16 exceeding 140 over 90 mmHg.
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19 **Diabetes:** Diabetes is a group of metabolic diseases characterized by hyperglycemia.
20 And fasting blood glucose more than 7.0 mmol/l or blood glucose more than 11.1
21 mmol/l within 2 hours after meal can be diagnosed diabetes.
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24 **Hyperlipemia:** Hyperlipemia means the presence of excess fat or lipids in the blood.
25 And total cholesterol ≥ 6.2 mmol/L, low density lipoprotein cholesterol ≥ 4.1 mmol/L,
26 triglyceride ≥ 2.3 mmol/L, high density lipoprotein cholesterol < 1.0 mmol/L can be
27 diagnosed hyperlipemia.
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30 **Heart failure:** The information was recorded through history taking and verified after
31 hospitalization. Heart failure means inability of the heart to keep up with the demands
32 on it and, specifically, failure of the heart to pump blood with normal efficiency. Heart
33 failure may be due to failure of the right or left or both ventricles. The signs and
34 symptoms depend upon which side of the heart is failing. They can include shortness
35 of breath (dyspnea), asthma due to the heart (cardiac asthma), pooling of blood (stasis)
36 in the general body (systemic) circulation or in the liver's (portal) circulation, swelling
37 (edema), blueness or duskiness (cyanosis), and enlargement (hypertrophy) of the
38 heart.
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44 **ACS:** Acute coronary syndrome is a term for a group of conditions that suddenly stop
45 or severely reduce blood from flowing to the heart. When blood cannot flow to the
46 heart, the heart muscle can become damaged. Heart attack and unstable angina are
47 both acute coronary syndromes (ACS).
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50 **Withdraw treatment:** Reasons for patients withdrew from treatment were listed
51 below: 1) High cost of the treatment: In China, the cost of hormone therapy can be up
52 to 3000 RMB a month if without health insurance. 2) Poor patient compliance: Some
53 patients discontinue treatment because they do not comply with the treatment plan
54 prescribed by their doctor.
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Part II. Report of sample size assessment

PASS 15.0.5

Tests for Two Survival Curves Using Cox's Proportional Hazards Model

Numeric Results with $H_a: HR \neq 1$

	Total Sample Size N	Control Sample Size N1	Trtmnt Sample Size N2	Prop'n Control N1/N P1	Hazard Ratio h2/h1 HR	Control Prob Event Pev1	Trtmnt Prob Event Pev2	Control Events E1	Trtmnt Events E2	Alpha	Beta
Power	103	51	52	0.500	0.500	0.750	0.950	38.3	49.4	0.050	0.100

References

- Chow, S.C., Shao, J., Wang, H. 2008. Sample Size Calculations in Clinical Research, 2nd Edition. Chapman & Hall/CRC.
- Schoenfeld, David A. 1983. 'Sample Size Formula for the Proportional-Hazards Regression Model', Biometrics, Volume 39, Pages 499-503.

Report Definitions

Power is the probability of rejecting a false null hypothesis. Power should be close to one.

N is the total sample size.

N1 and N2 are the sample sizes of the control and treatment groups.

P1 is the proportion of the total sample that is in the control group, group 1.

HR is the hazard ratio: h_2/h_1 .

Pev1 and Pev2 are the probabilities of an event in the control and the treatment groups.

E1 and E2 are the number of events required in the control and the treatment groups.

Alpha is the probability of a type one error: rejecting a true null hypothesis.

Beta is the probability of a type two error: failing to reject a false null hypothesis.

Summary Statements

A two-sided test of whether the hazard ratio is one with an overall sample size of 103 subjects (of which 51 are in the control group and 52 are in the treatment group) achieves 90% power at a 0.050 significance level when the hazard ratio is actually 0.500. The number of events required to achieve this power is 87.7. It is anticipated that proportions of subjects having the event during the study is 0.750 for the control group and 0.950 for the treatment group. These results assume that the hazard ratio is constant throughout the study and that Cox proportional hazards regression is used to analyze the data.

Procedure Input Settings

Autosaved Template File

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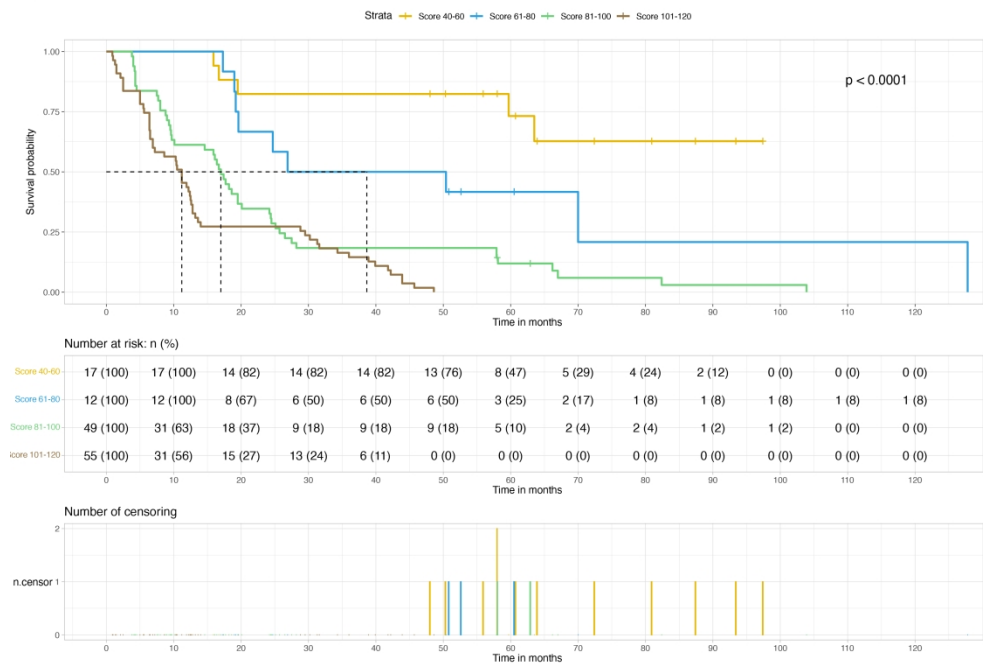
Proportional Hazards Model - Autosaved 2020_1_24-9_57_39.t92

Design Tab

Solve For:	Sample Size
Alternative Hypothesis:	Ha: HR \neq 1
Power:	0.90
Alpha:	0.05
Group Allocation:	Equal (N1 = N2)
Pev1 (Probability of a Control Event):	0.750
Pev2 (Probability of a Treatment Event):	0.950
HR (Actual Hazard Ratio = h2/h1):	0.5

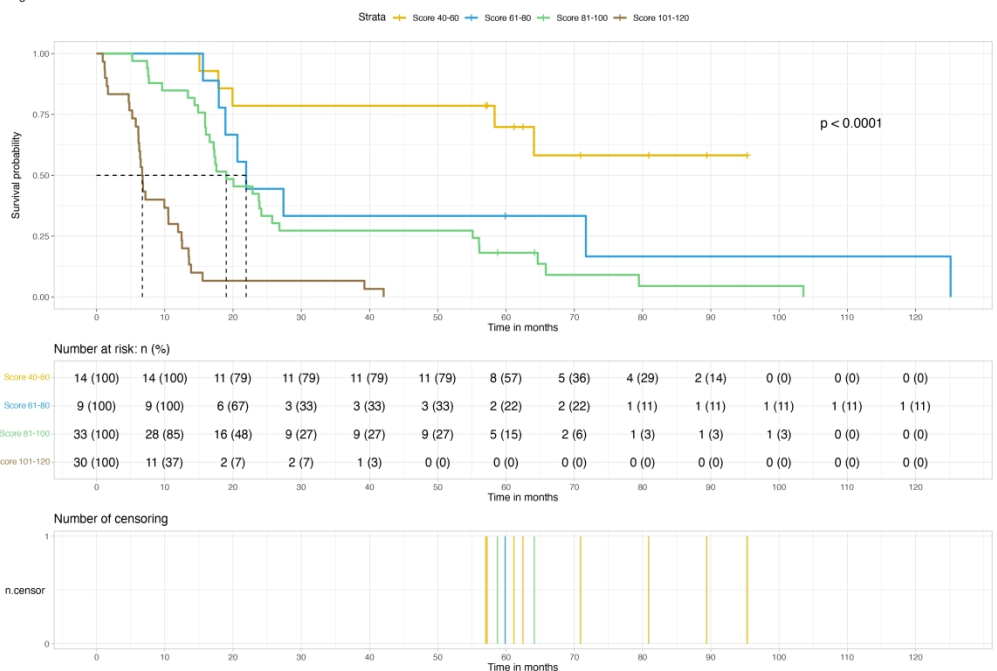
For peer review only

Figure S1a



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Figure S1b



STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of cohort studies

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6
		(b) For matched studies, give matching criteria and number of exposed and unexposed	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	7
		(b) Describe any methods used to examine subgroups and interactions	7
		(c) Explain how missing data were addressed	7
		(d) If applicable, explain how loss to follow-up was addressed	7
		(e) Describe any sensitivity analyses	7
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	8
		(c) Consider use of a flow diagram	8
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9
		(b) Indicate number of participants with missing data for each variable of interest	9
		(c) Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	Report numbers of outcome events or summary measures over time	10
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10
		(b) Report category boundaries when continuous variables were categorized	11
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	11
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12
Discussion			
Key results	18	Summarise key results with reference to study objectives	13-14
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.