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IL-17A as a new circulating bioindicator for non-small cell lung cancer diagnosis and prognosis

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Abstract. – OBJECTIVE: Lung cancer (LC) is the highest contributor to cancer-associated mortality worldwide. Approximately 85% of all LC incidences involve non-small cell LC (NSCLC). Unfortunately, owing to a significant lack of sensitive and robust bioindicators, most patient diagnoses occur at advanced stages of the disease, thereby resulting in extremely poor patient outcomes. Herein, we elucidated the role of interleukin-17A (IL-17A) among NSCLC patients.

MATERIALS AND METHODS: Circulating IL-17A content was measured using enzyme-linked immunosorbent assay (ELISA), and its diagnostic and prognostic abilities were assessed using the receiver operating characteristic (ROC) curve and Kaplan-Meier analysis, respectively.

RESULTS: Our analysis revealed that circulating IL-17A levels were significantly augmented among NSCLC vs. control samples. Moreover, based on our area under the curve (AUC) analysis, circulating IL-17A levels fared considerably better than the standard bioindicator carcinoembryonic antigen (CEA) in both testing and validation cohorts. Notably, we also revealed that the circulating IL-17A levels were accurately and reliably predicted in early-stage NSCLC patients. Besides, we demonstrated a strong correlation between elevated circulating IL-17A expression and worse prognosis among NSCLC patients.

CONCLUSIONS: Herein, we demonstrated that circulating IL-17A levels can serve as reliable and potent diagnostic and prognostic bioindicators for NSCLC.

Key Words:
Non-small cell lung cancer (NSCLC), Interleukin-17A (IL-17A), Biomarker, Carcinoembryonic antigen, Prognosis.

Abbreviations

Non-small cell lung cancer (NSCLC); enzyme-linked immunosorbent assay (ELISA); Receiver operating characteristic (ROC); area under the curve (AUC); carcinoembryonic antigen (CEA); Lung cancer (LC); lung adenocarcinoma (LAD); lung squamous cell carcinoma (LSCC); large cell carcinoma (LCC); Interleukin-17A (IL-17A); alcoholic liver disease (ALD).

Introduction

Lung cancer (LC) is responsible for the most cancer-associated deaths worldwide. Approximately 85% of all LC are non-small cell LC (NSCLC), which, in turn, are sub-categorized into lung adenocarcinoma (LAD), lung squamous cell carcinoma (LSCC), large cell carcinoma (LCC), and other uncommon histological forms[1-3]. Unfortunately, owing to a significant lack of reliable detection systems, most NSCLC patients are diagnosed at later stages of the disease. In addition, NSCLC has a relatively enhanced recurrence rate. Therefore, the overall 5-year survival rate remains < 15%, and patient prognosis is extremely poor[4-7]. Given these challenges, it is both urgent and necessary to establish new and reliable bioindicators for the early diagnosis and prognostic estimation of NSCLC patients.

Interleukin-17A (IL-17A) belongs to the IL-17 family of cytokines, and it has a strong proinflammatory function in both autoimmune and inflammatory diseases[8-15]. Earlier research[16-22] revealed that IL-17A expression is markedly enhanced in a large number of hepatic diseases, namely, hepa-

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titis-induced hepatic inflammatory diseases, B/C, and alcoholic liver disease (ALD), and it modulates intracellular signaling *via* interaction with the IL-17 receptor A (IL-17RA). IL-17RA is typically expressed in numerous cell types, and it is activated by several IL-17 cytokines, such as IL-17A, IL-17F, IL-17A/F, and IL-17E (IL-25). To date, there are no reports on a potential association between circulating IL-17A levels and NSCLC patient outcomes. Herein, we examined the role of circulating IL-17A in the early diagnosis and prognostic evaluation of NSCLC patients.

Materials and Methods

Study Participants

As our testing population, we obtained 105 NSCLC, 20 pulmonary tuberculosis (PT), and 50 healthy control (HC) serum samples from the First Affiliated Hospital of Xingtai Medical College between the period of January 2013 to January 2016. In addition, as our verification population, we acquired 65 early-stage NSCLC, 15 PT, and 35 HC serum samples from the aforementioned institution during January 2017 and January 2019. Patients who received chemotherapy or radiotherapy were excluded from the analysis. This research received ethical approval from our institution and written informed consents from participants, respectively. Our protocols strictly followed the guidelines of the Declaration of Helsinki 1964 and its subsequent revisions. Participant clinical demographics are summarized in detail in Table I.

IL-7A Enzyme-Linked Immunosorbent Assay (ELISA)

Circulating IL-7A concentration was assessed *via* an ELISA kit (Carmania Pars Gene, Kerman, Iran) following kit directions.

Statistical Analysis

The SPSS 20.0 software (IBM Corp., Armonk, NY, USA) was employed for data analyses. The Kaplan-Meier analysis assessed survival curves, and the log-rank test assessed statistical significance. The Chi-square test was employed for the evaluation of the link between circulating IL-17A levels and clinicopathological factors. The receiver operating characteristic curves (ROC) were utilized for diagnostic metrics determination. The student's *t*-test was employed for inter-cohort comparisons. Data are presented as mean ± SD of three distinct experiments; $p < 0.05$ was set as the significance threshold.

Table I. Baseline clinical demographics of patients within the NSCLC testing and verification populations.

Characteristics	Testing cohort (N = 105)		Validation cohort (N = 65)	
	Number of cases	Percentage (%)	Number of cases	Percentage (%)
Gender				
Male	85	80.95	55	84.62
Female	20	19.05	10	15.38
Age (years)				
> 60	60	57.14	45	69.23
≤ 60	45	42.86	20	30.77
Clinical stage				
I	45	42.86	45	69.23
II	35	33.33	20	30.77
III	10	9.52		
IV	15	14.29		
Distant metastasis				
Yes	15	14.29	0	0.00
No	90	85.71	65	100.00
CEA(μg/L)				
> 5	60	57.14	35	53.85
≤ 5	45	42.86	30	46.15
Tumor Size (cm)				
> 4	35	33.33	10	15.38
≤ 4	70	66.67	55	84.62

Carcinoembryonic antigen (CEA).

Results

Verification of Circulating IL-17A Content in the NSCLC Testing Population

As shown in Figure 1A, circulating IL-17A levels were highly expressed in NSCLC samples. Pulmonary tuberculosis (PT) is the most frequently occurring benign pulmonary lesion. However, the currently available detection system does not properly delineate between PT and NSCLC patients. In this report, we demonstrated that circulating IL-17A levels were markedly enhanced among NSCLC and early-stage NSCLC patients, relative to its levels in HC and PT individuals (Figure 1A-B). We also explored the levels of a standard bioindicator carcinoembryonic antigen (CEA) in the testing population. As depicted in Figure 1 C-D, the CEA content also exhibited a marked rise among NSCLC patients relative to HC and PT individuals.

Diagnostic Ability of IL-17A to Circulate in the NSCLC Testing Population

We next employed ROC-AUC to evaluate the diagnostic ability of circulating IL-17A. The area under the curve (AUC) of circulating IL-17A was 0.9046, and of CEA was 0.7392 (Figure 2A). Thus, the AUC value of circulating IL-17A was substantially elevated, compared to CEA. In the case of early-stage NSCLC (stages I and II), circulating IL-17A fared considerably better in delineating NSCLC patients from PT patients and

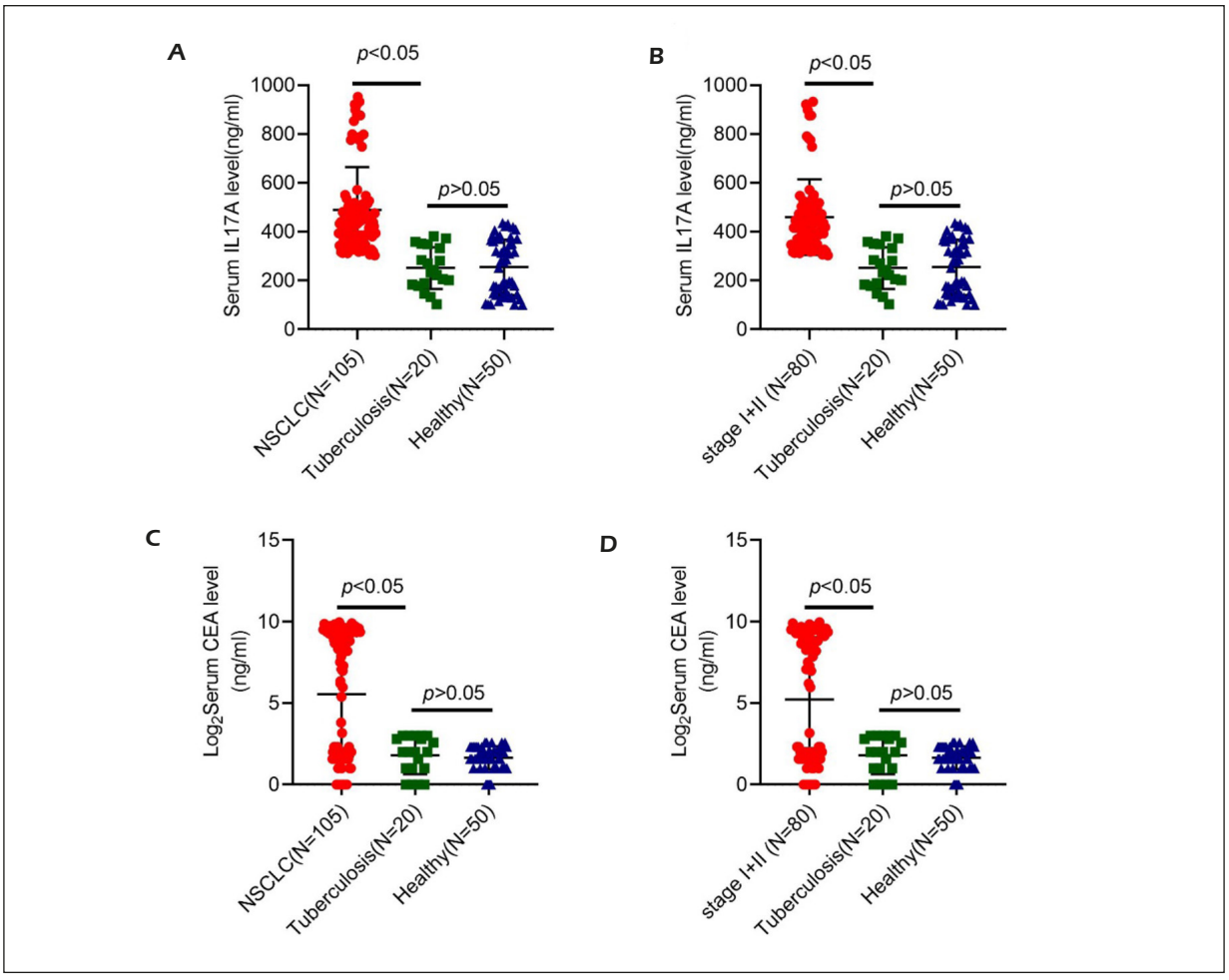

Figure 1. Assessment of circulating IL-17A content in the NSCLC testing population. **A**, Evaluation of circulating IL-17A content among 105 lung cancer (LC) patients, 22 pulmonary tuberculosis (PT) patients, and 51 healthy controls (HC), as evidenced by ELISA. **B**, Verification of circulating IL-17A content among 35 early-stage NSCLC patients, 22 PT patients, and 51 HC, as detected by ELISA. **C**, Evaluation of circulating CEA content among the NSCLC testing population, as detected by ELISA assay. **D**, Evaluation of circulating CEA content among the early-stage NSCLS testing population, as evidenced by ELISA assay.

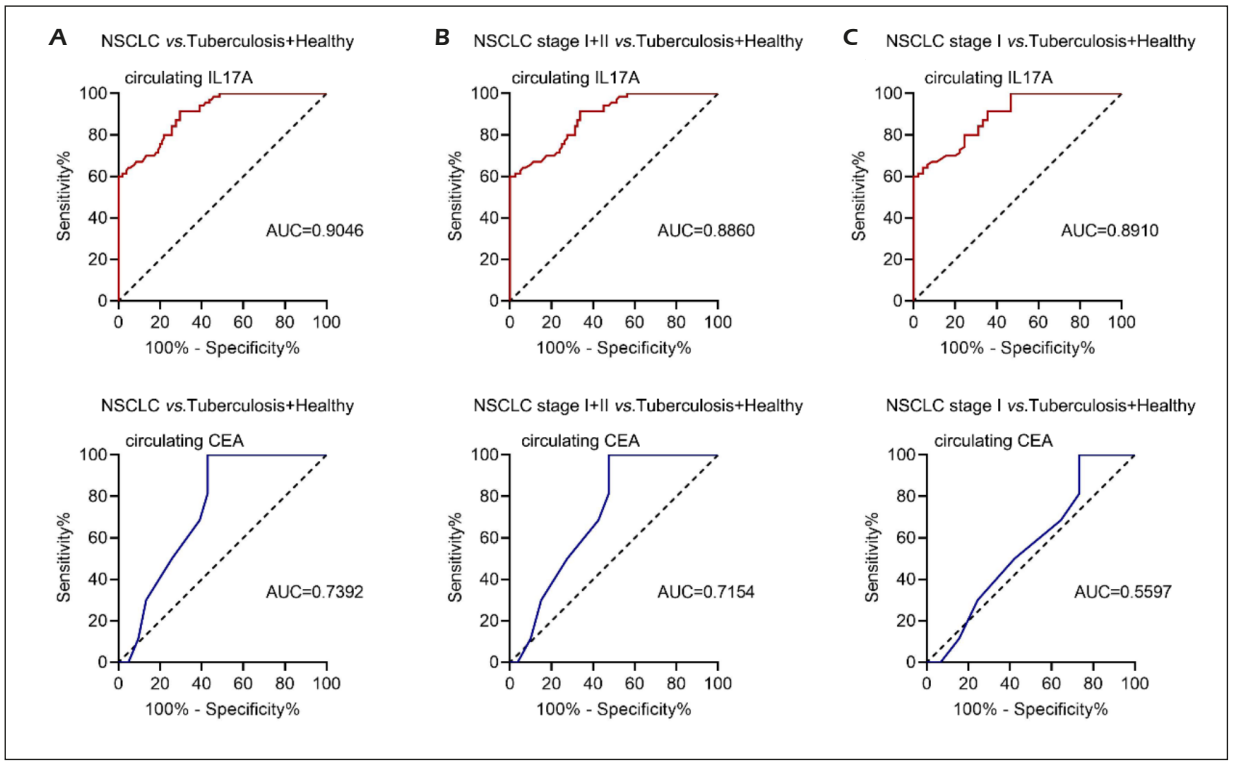

Figure 2. Diagnostic ability of circulating IL-17A content in NSCLC testing population. **A**, ROC curve of circulating IL-17A and CEA contents among NSCLC patients and healthy controls (HC). **B**, ROC curve of circulating IL-17A and CEA contents among the early-stage NSCLC patients, pulmonary tuberculosis (PT) patients, and HC. **C**, ROC curve of circulating IL-17A and CEA contents among stage I NSCLC patients, PT patients, and HC.

HC (AUC = 0.8860). In contrast, circulating CEA displayed an AUC of 0.7154 (Figure 2B). We also observed a strong diagnostic advantage for stage I patients (circulating IL-17A, AUC = 0.8910; circulating CEA, AUC = 0.5597), which indicated that circulating IL-17A was advantageous to the early detection of NSCLC (Figure 2C).

Detection of Circulating IL-17A Levels in the NSCLC Verification Population

We next assessed the circulating IL-17A expression in a multicentric early-stage cohort using 115 serum specimens (65 early-stage NSCLC, 15 PT, and 35 HC). As depicted in Figure 3A, circulating IL-17A expression was increased among stage I and II NSCLC patients, compared to PT patients and HC. Circulating CEA was also markedly enhanced in early-stage NSCLC; however, it failed to delineate between PT and NSCLC patients (Figure 3B). Herein, we demonstrated circulating IL-17A and CEA AUC values of 0.8926 and 0.8157, respectively, in delineating early-stage NSCLC from HC in the verification population (Figure 3C). Additionally, we observed circulating IL-17A and CEA AUC values of 0.8483 and 0.8133, respectively, in delineating between stage I NSCLC patients and HC (Figure 3D). Given this evidence, the circulating IL-17A AUC value was markedly enhanced compared to CEA. Thus, we verified the diagnostic precision and reliability of circulating IL-17A for early-stage NSCLC in the verification population.

Circulating IL-17A Levels Indicate Worse Prognosis in the NSCLC Testing Population

Given the marked rise in circulating IL-17A in early-stage NSCLC, we next examined whether the circulating IL-17A content predicted NSCLC patient prognosis. To do this, we classified participants into an elevated- and reduced IL-17A expression cohort, according to their circulating IL1-7A levels, using a median threshold value. Based on our analysis, the circulating IL-17A concentration was directly associated with tumor stage and distant metastasis (Table II). In particular, we observed that enhanced circulating IL-17A levels corresponded to shorter overall survival

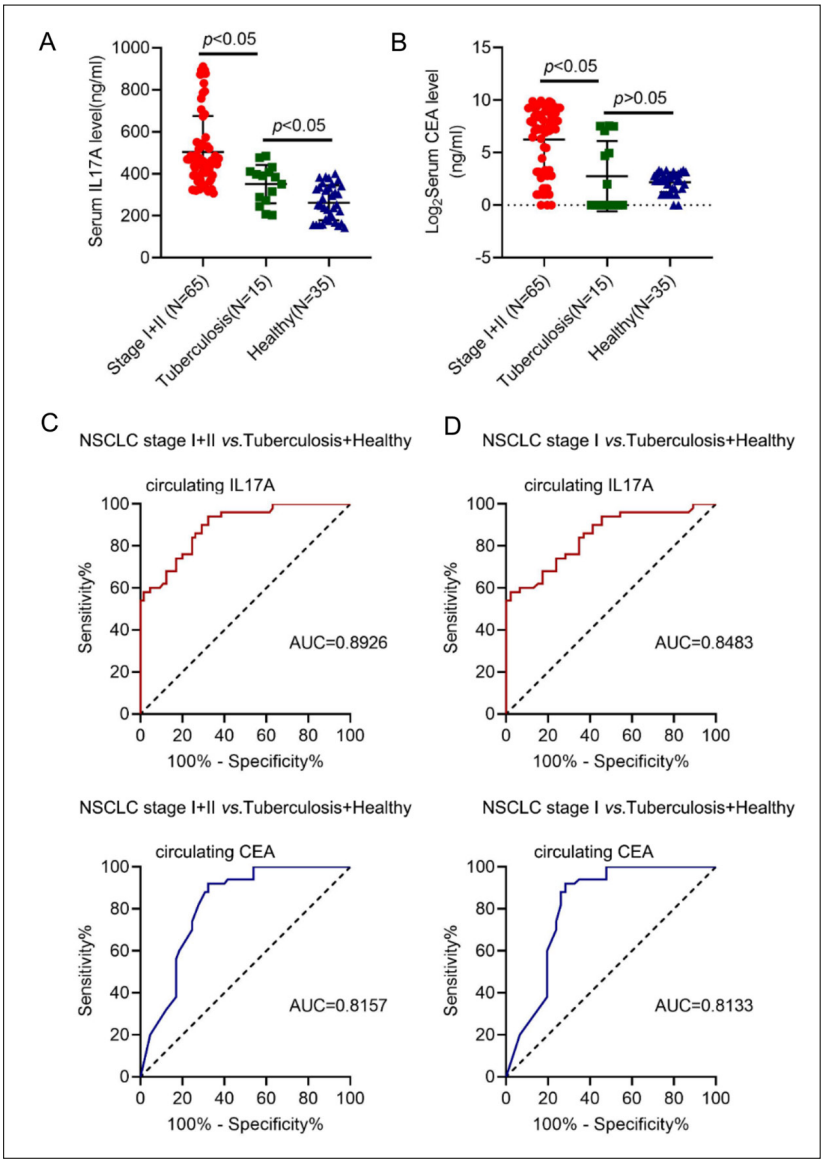

Figure 3. Evaluation of circulating IL-17A content in the NSCLC verification population. Circulating IL-17A (**A**) and CEA (**B**) contents among healthy controls (HC), pulmonary tuberculosis (PT) patients, and stage I and II NSCLC patients in the verification population. **C**, ROC curve of circulating IL-17A and CEA contents in the early-stage NSCLC patients, PT patients, and HC. **D**, ROC curve of circulating IL-17A and CEA contents among stage I NSCLC patients, PT patients, and HC.

(OS) relative to reduced circulating IL-17A levels ($p < 0.05$) (Figure 4A). As depicted in Figure 4B, enhanced CEA expression was also associated with shorter OS duration. Taken together, this evidence suggested that circulating IL-17A levels may serve as potent and reliable bioindicators of NSCLC patient prognosis.

Discussion

Herein, we demonstrated that circulating IL-17A has enhanced stability, and is easy to measure, which, along with its potency and reliability of NSCLC diagnosis and prognostic estimation, makes it an excellent candidate for clinical application. Using ROC curves, we revealed that IL-17A accurately delineated early-stage NSCLC patients from HC, with high sensitivity and specificity. Additionally, based on our survival analysis, elevated IL-17A concentration was closely correlated with shorter OS duration among NSCLC patients.

Early-stage NSCLC, with clear surgical indication, is most effectively managed with surgical intervention[23]. Since the initial NSCLC diagnosis often occurs at an advanced stage, < 30% of patients select the surgical route[24,25]. Based on literature examining the outcome of treatments at different stages of the disease, it is clear that early disease diagnosis is very beneficial for patient prognosis. Conventional NSCLC screening utilizes magnetic resonance images (MRI) or

Table II. Comparison of clinicopathological features of NSCLC patients, based on their circulating IL-17A content in the NSCLC testing population.

Characteristics	Serum IL-17A level		Total	*p*
	High N = 53	Low N = 52		
Gender				1.000
Male	43	42	85	
Female	10	10	20	
Age (years)				0.238
> 60	27	33	60	
≤ 60	26	19	45	
Clinical stage				0.005
I	24	21	45	
II	10	25	35	
III	7	3	10	
IV	12	3	15	
Distant metastasis				0.002
Yes	13	2	15	
No	40	50	90	
CEA (μg/L)				1.000
> 5	30	30	60	
≤ 5	23	22	45	
Tumor Size (cm)				1.000
> 4	18	17	35	
≤ 4	35	35	70	

Carcinoembryonic antigen (CEA).

computed tomography (CT), which are extremely expensive and are susceptible to high rates of false positives. In contrast, tumor bioindicators provide complementary risk assessment for appropriate clinical decision-making. NSCLC patients typically exhibit elevated CEA levels[26-30]; however, its expression is not specific to the early stage of the disease. Therefore, it is critical to identify novel, precise, and reliable diagnostic or prognostic bioindicators to enhance NSCLC patient outcomes. Herein, we revealed that circulating IL-17A was ubiquitously expressed in the serum of early-stage NSCLC patients, and it accurately delineated NSCLC patients from PT patients and HC, with high sensitivity and specificity. Thus, it has great potential as an NSCLC diagnostic bioindicator.

NSCLC patients receiving the same treatment in the same disease stage exhibit considerable heterogeneity in their response[31-33]. Emerging evidence[34,35] suggested that aberrant IL-17A expression is strongly associated with clinicopathological features in numerous cancers, which further supports the usage of IL-17A in estimating patient prognosis. Moreover, repeated IL-17A detection

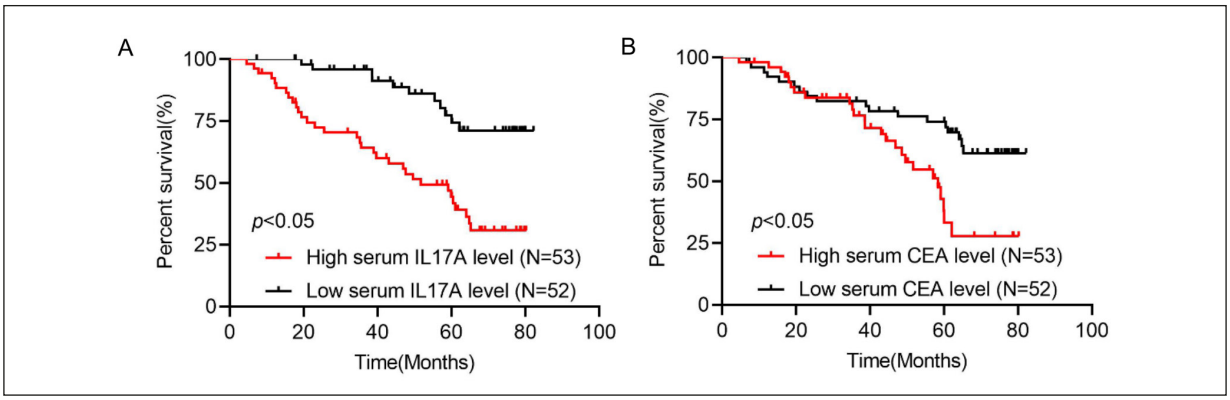

Figure 4. Circulating IL-17A content predicted a worse prognosis among the NSCLC testing population. Kaplan-Meier survival analysis of overall survival (OS) of the NSCLC testing population, based on the circulating IL-17A (**A**) and CEA (**B**) contents.

via peripheral blood collection is a minimally invasive procedure whereby one can monitor the progression of the disease over time. Herein, we revealed that elevated circulating IL-17A levels predicted a worse patient prognosis. Moreover, it was strongly associated with tumor stage and distant metastasis. Prior investigations[36] revealed that CEA is a prognostic indicator of worse NSCLC patient outcomes. Herein, we showed that circulating CEA levels failed to delineate between NSCLC patients and HC, likely due to our relatively limited sample size. In summary, circulating IL-17A is a potentially robust circulating dynamic indicator of NSCLC patient prognosis. Given its strong clinical significance, we recommend further investigations into the usage of IL-17A as a novel diagnostic and prognostic bioindicator for NSCLC patients.

Conclusions

In summary, we revealed that circulating IL-17A exhibited favorable sensitivity and specificity for the early diagnosis of NSCLC. Hence, it is a promising candidate for early NSCLC diagnosis.

Conflict of Interest
The authors declare that they have no conflict of interests.

Availability of Data and Materials
All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

Authors' Contribution
S.-W. Liu designed the experiments. All authors performed statistical analysis, writing-original draft. All authors contributed to editorial changes in the manuscript.

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Ethics Approval
This study was approved by the Medical Ethical Committee of The First Affiliated Hospital of Xingtai Medical College (No. XTFH-2021-04), Shijiazhuang, Hebei Province, China.

Informed Consent
Informed consent form was obtained from the patients.

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