Improvement in the performance of an autoantibody panel in combination with heat shock protein 90a for the detection of early-stage lung cancer

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Abstract. The early diagnosis of lung cancer is closely associated with the decline of mortality. A panel consisting of seven lung cancer-related autoantibodies (7-AABs) has been shown to be a reliable and specific indicator for the early detection of lung cancer, with a specificity of ~90% and a positive predictive value of ~85%. However, its low sensitivity and negative predictive value limit its wide application. To improve its diagnostic value, the diagnostic efficiencies of 7-AABs in combination with non-specific tumor markers were retrospectively investigated for the detection of early-stage lung cancer. A total of 217 patients with small lung nodules who presented with ground-glass opacity or solid nodules as well as 30 healthy controls were studied. The concentrations of 7-AABs and heat shock protein 90a (HSP90a) were assessed using ELISA. Automated flow fluorescence immune analysis was used for the assessment of CEA, CYFRA21-1, CA199 and CA125 levels. The results showed that 7-AABs + HSP90a possessed a remarkably improved diagnostic efficiency for patients with small pulmonary nodules or for patients with lung nodules of different types, which suggested that 7-AABs in combination with HSP90a could have a high clinical value for the improvement of the diagnostic efficiency of early-stage lung cancer.

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Introduction

Lung cancer is currently the leading cause of cancer-related mortality worldwide (1). Non-small cell lung cancer (NSCLC) accounts for the majority of all lung cancer cases. The 5-year survival rate of patients with NSCLC is ~15% (2). Moreover, most patients with NSCLC are at the advanced/metastatic stage when diagnosed, and the 5-year survival rate is only 5.5% (3). However, the 5-year survival rate of patients with early-stage NSCLC is ~60% (4,5). Therefore, the early detection and diagnosis of lung cancer are essential for the improvement of the overall survival rate. However, the techniques for early diagnosis still face challenges.

Recently, lung cancer-related autoantibodies (AABs) have been reported as biomarkers with high specificity, good stability and non-invasion have been identified and have become a focus of research in the early diagnosis of high-risk lung cancer cohorts (6,7). Notably, the positive results of lung cancer-related AABs are observed before lung cancer is clinically diagnosed. Patient cohorts with lung cancer have been screened using computed tomography (CT) at the Mayo Clinic, and studies have reported that lung cancer-related AABs can provide a valuable warning up to 5 years before the clinical diagnosis of lung cancer (8). It has also been reported that lung cancer-related AABs have serve an important role in giving early warnings up to 4 years before the clinical diagnosis of pulmonary carcinoma in 200,000 high-risk postmenopausal women (9). The sensitivities and specificities of a panel consisting of four lung cancer-related autoantibodies (4-AABs), namely tumor protein 53 (p53), melanoma antigen 1 (MAGEA1), protein gene product 9.5 (PGP9.5) and sex-determining region Y-box 2 (SOX2), as a diagnostic tool for monitoring 458 patients at high risk of lung cancers were reported to be 71.8 and 89%, respectively (10). A panel of seven lung cancer-related autoantibodies (7-AABs) in peripheral circulating blood, including p53, PGP9.5, SOX2, cancer/testis G antigen 7 (GAGE7), ATP-dependent RNA helicase 4-5 (GBU4-5), cancer-associated antigen (CAGE) and MAGEA1 was reported to be able to distinguish malignant nodules from

benign nodules and healthy controls (HCs), with a sensitivity of 56.53% and a specificity of 91.60% (11). The specificity of 7-AABs can be further increased to 95.80% when combined with CT (11). Another large-scale prospective study consisting of 1,915 Chinese individuals recruited from five research centers reported that the panel of 7-AABs has substantially higher sensitivity and specificity for the early diagnosis of lung cancer compared with non-specific tumor markers, such as carcinoembryonic antigen (CEA), neuron-specific enolase and cytokeratin fragment antigen 21-1 (CYFRA21-1) (12). Therefore, as a specific tumor marker, the lung cancer-related AAB panel possesses marked clinical application value in the diagnosis of pulmonary carcinoma. However, the means to improve its sensitivity remains largely unsolved.

A previous study reported that heat shock protein 90a (HSP90a), which maintains the stability of various protein molecules, is closely related to the occurrence and development of malignant tumors (13). The cutoff value of HSP90a is 50 ng/ml, which can accurately distinguish malignant and benign tumors in multiple tissues, such as the liver, lung, pancreas and breast (14). However, HSP90a alone has limitations in early lung cancer diagnosis due to its non-specificity. Therefore, the aim of the present study was to combine the 7-AABs with HSP90a to improve their diagnostic value in early-stage lung cancer.

In the present study, the level of each lung cancer-related AAB in patients with malignant lung nodules (MLNs) or benign lung nodules (BLNs), as well as HCs, was investigated. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and relative risk (RR) of the 7-AABs with combinations of the 7-AABs and other non-specific tumor markers, such as HSP90a, CEA, CYFRA21-1, carbohydrate antigen 199 (CA199) or carbohydrate antigen 125 (CA125) were assessed. Furthermore, the diagnostic efficiency of the aforementioned markers in patients with different sizes, types and stages of lung nodules was evaluated.

Patients and methods

Patients. A total of 217 individuals who were in good health, with lung nodules (ground-glass opacity or solid nodule) firstly diagnosed using high-resolution CT (HRCT) scans during routine examinations were enrolled as patients. Patients were recruited from October 2020 to August 2021 in The First Affiliated Hospital of Wannan Medical College (Wuhu, China). There were 78 males and 139 females, and the average age was 54.5±1.3 (mean ± standard deviation) years. The exclusion criteria for patients were as follows: Chronic obstructive pulmonary disease (including chronic bronchitis, pneumonectomies and pulmonary heart disease), pulmonary fibrosis, or a history of treated or untreated pulmonary malignancy. A total of 30 individuals who were in good health without underlying lung diseases, nor other major diseases screened for in the routine examinations were selected as healthy controls. The present study was approved by the Ethics Review Committee of the First Affiliated Hospital of Wannan Medical College (approval no. 202076; Wuhu, China) and the experimental procedures were in agreement with The Declaration of Helsinki. Written informed consent was obtained from all participants.

Laboratory measurements. The serum concentrations of 7-AABs were quantified using the Seven Autoantibodies Detection Kit (ELISA) (cat. no. 20210106. CancerProbe Biotechnology Co., Ltd.). The concentration of HSP90a in the blood was assessed using the Human Heat Shock Protein HSP90-α ELISA kit (cat. no. HUEB0886; Assay Genie Co., Ltd.). CEA, CYFRA21-1, CA199 and CA125 were assessed using a Multiple Tumor Markers Detection Kit (cat. no. LP120280; Shanghai Tellgen Life Science Co., Ltd.) and were quantified using a Tesmi F4000 automated flow fluorescence immune analyzer (Shanghai Tellgen Life Science Co., Ltd.).

Hematoxylin and eosin (H&E) staining. Tissue samples from the patients with lung nodules were immersed and fixed using 4% formaldehyde at room temperature (~20˚C) for 24 h, embedded in paraffin, and then sectioned at 3-5 μm thickness. Sections were baked at 70˚C for 1 h and dewaxed using xylene, before rehydration in a 100, 95, 85 and 75% ethanol series for 5 min at a time. Then, the sections were transferred into distilled water for 5 min. Next, the sections were stained using H&E at room temperature for 5 min, followed by dehydration with absolute ethyl alcohol at room temperature for 5 min and sealed using neutral gum (cat. no. N116470, Shanghai Aladdin Biochemical Technology Co., Ltd.) for assessment. Following the World Health Organization Classification of Tumours (5th Edition)-Thoracic Tumours (15) guidelines, microscopic assessment of the sections was then performed by experienced pathologists.

Statistical analysis. Data were statistically analyzed using SPSS version 22.0 (IBM Corp.). After the Shapiro-Wilk test was performed, comparison of the levels of each AAB (namely, p53, PGP9.5, SOX2, GAGE7, GBU4-5, MAGEA1 and CAGE) and HSP90a among the three groups was performed using the Kruskal-Wallis test followed by Dunn's test for non-parametric data. Comparison of the diagnostic efficiency of 7-AABs for discrimination between MLNs and BLNs in different groups was performed using the $\chi 2$ test. Clinical performance was presented in terms of sensitivity (the percentage of true positives) and specificity (the percentage of true negatives). PPV (the probability of MLNs given a positive test result), NPV (the probability of BLNs given a negative test result) and RR (the proportion of cases with a positive outcome in the two groups) were also calculated. McNemar's test was used for comparison of the sensitivity between 7-AABs + HSP90a and other groups (such as 7-AABs, 7-AABs + CEA, 7-AABs + CYFRA21-1, 7-AABs + CA199 and 7-AABs + CA125). For comparison of the NPV between 7-AABs + HSP90a and other groups, the marginal regression model based on the generalized estimation equation was used as follows: Model, $g[P(D=1|Z, X=0)]=\alpha N + \beta NZ$, where g represented the contiguous function, Z represented the indicator variable and X represented the diagnostic result. Null hypothesis (H0) $\beta=0$ was used to check the difference between tests. When g took the natural logarithmic function, e^{β} represented an estimate of the relative NPV. The diagnostic efficiencies of 7-AABs, 7-AABs + HSP90a, 7-AABs + CEA, 7-AABs + CYFRA21-1, 7-AABs + CA199 and 7-AABs + CA125 were assessed using receiver operating characteristic (ROC) curves. All tests

Table I. Clinical characteristics of patients with lung nodules and HCs.

Clinical characteristic	Lung nodule	MLN	BLN	HC
Number of patients	217	159	58	30
Age, years	54.5±11.3	54.5±11.7	54.5±10.1	53.2±9.1
Sex (M/F), n	78/139	50/109	28/30	11/19
NSCLC, n	158	158	-	-
Adenocarcinoma *in situ*, n (%)	66 (41.8)	66 (41.8)	-	-
Invasive adenocarcinoma, n (%)	91 (57.6)	91 (57.6)	-	-
Squamous cell carcinoma, n (%)	1 (0.6)	1 (0.6)	-	-
SCLC, n	1	1	-	-
Stage, n (%)				
0	66 (41.5)	66 (41.5)	-	-
I	91 (57.2)	91 (57.2)	-	-
II	1 (0.6)	1 (0.6)	-	-
III	1 (0.6)	1 (0.6)	-	-
Size, n (%)	217	159	58	
<8 mm	68 (31.3)	42 (26.4)	26 (44.8)	-
8-20 mm	149 (68.7)	117 (73.6)	32 (55.2)	-
Type, n (%)	217	159	58	
Pure GGO	58 (26.7)	45 (28.3)	13 (22.4)	-
Mix GGO	97 (44.7)	85 (53.5)	12 (20.7)	-
Solid nodule	62 (28.6)	29 (18.2)	33 (56.9)	-

HC, healthy control; MLN, malignant lung nodule; BLN, benign lung nodule; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; M, male; F, female; GGO, ground-glass opacity.

were two-tailed, and P<0.05 was considered to indicate a statistically significant difference. The data were presented as mean ± standard deviation, number of subjects (n) and percentage (%). Three replicate experiments were performed for each measurement.

Results

Clinical characteristics of the patients. A total of 217 patients with lung nodules and 30 HCs were enrolled in the present study. Table I summarizes the clinical features of these subjects. A total of 159 patients with MLNs including 158 NSCLC cases (41.8% were cases with adenocarcinoma *in situ*; 57.6% were cases with invasive adenocarcinoma and 0.6% were cases with squamous cell carcinoma) and one case with small cell lung cancer (SCLC) and, 58 patients with BLNs were confirmed by pathological examination (Fig. 1). According to the histopathological results, the 159 patients were divided into one of four stages: Stage 0, 66 patients (41.5%); stage I, 91 patients (57.2%); stage II, 1 patient (0.6%); and stage III, 1 patient (0.6%).

Of the 217 enrolled patients, 68 (31.3%) and 149 (68.7%) patients, with lung nodules ≤8 mm and 8-20 mm, respectively, were evaluated using HRCT. Moreover, 58 (26.7%), 97 (44.7%) and 62 (28.6%) patients with pure ground-glass opacity (pGGO), mix ground-glass opacity (mGGO) and solid nodule (SN), respectively, were also evaluated using HRCT (Fig. 2). A total of 159 patients with MLNs and 58 patients with BLNs were classified and were presented in Table I.

The reactivity of each AAB and HSP90a in patients and HCs. The levels of 7-AABs, namely p53, PGP9.5, SOX2, GAGE7, GBU4-5, MAGEA1 and CAGE in serum samples and plasma HSP90a levels in 217 patients and 30 HCs were assessed using ELISA. The levels of p53, PGP9.5, SOX2, GAGE7, MAGEA1 and HSP90a in the MLN group were significantly elevated compared with those in the HC group (Fig. 3). However, there were no significant differences in the levels of GBU4-5 and CAGE among the three groups. These data suggested that the levels of 7-AABs and HSP90a from patients with MLNs showed marked differences compared with patients with BLNs and HCs.

Diagnostic values of 7-AABs alone or in combination with non-specific tumor markers for patients with lung nodules. The diagnostic values of 7-AABs alone or in combination with non-specific tumor markers for patients with lung nodules were evaluated. It was found that although the 7-AABs demonstrated a high specificity (77.6%; 95% CI, 64.4-87.1%) and PPV (84.0%; 95% CI, 73.8-90.8%) in patients with lung nodules, a low sensitivity (42.8%; 95% CI, 35.0-50.9%) and NPV (33.1%; 95% CI, 25.4-41.7%) were also demonstrated. Moreover, 7-AABs reflected a 1.3-fold increase in RR of MLNs, which demonstrated a good diagnostic efficiency (AUC=0.612, P=0.012). However, statistical analysis indicated that 7-AABs + HSP90a exhibited an elevated sensitivity (87.4%; 95% CI, 81.0-92.0%; P<0.0001) and NPV (66.1%; 95% CI, 52.5-77.6%; P<0.0001) compared with 7-AABs alone or

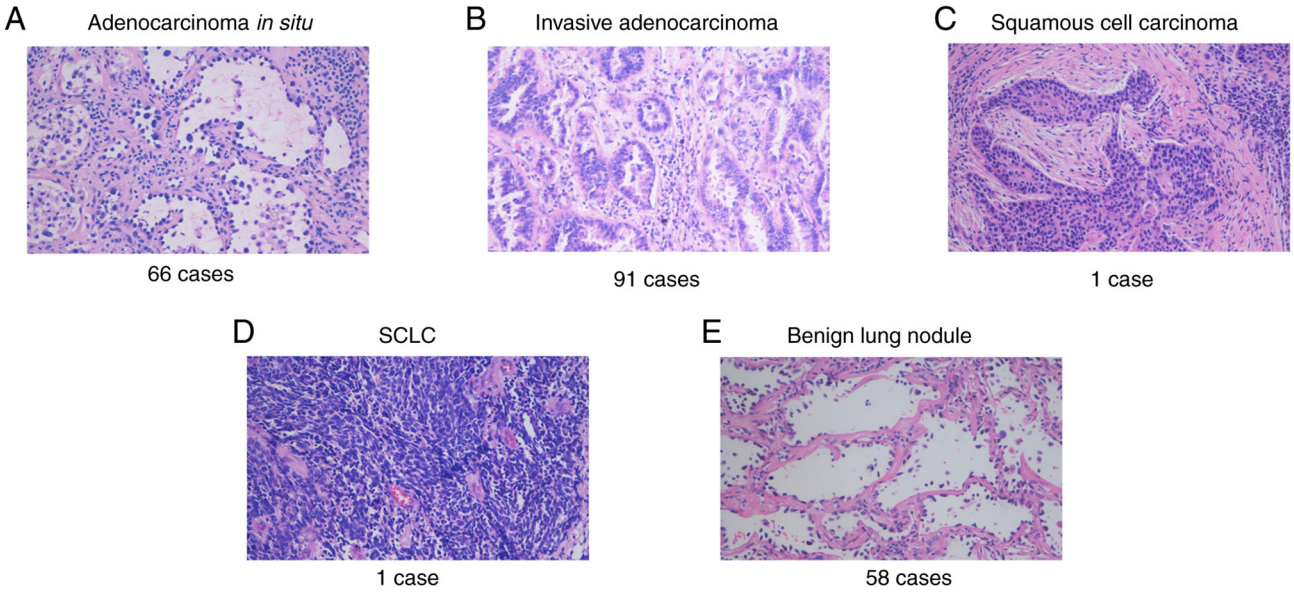

Figure 1. Pathological examination of H&E staining was used to confirm patients with (A) adenocarcinoma *in situ*, (B) invasive adenocarcinoma, (C) squamous cell carcinoma, (D) SCLC or (E) benign lung nodule. Magnification x200. SCLC, small cell lung cancer.

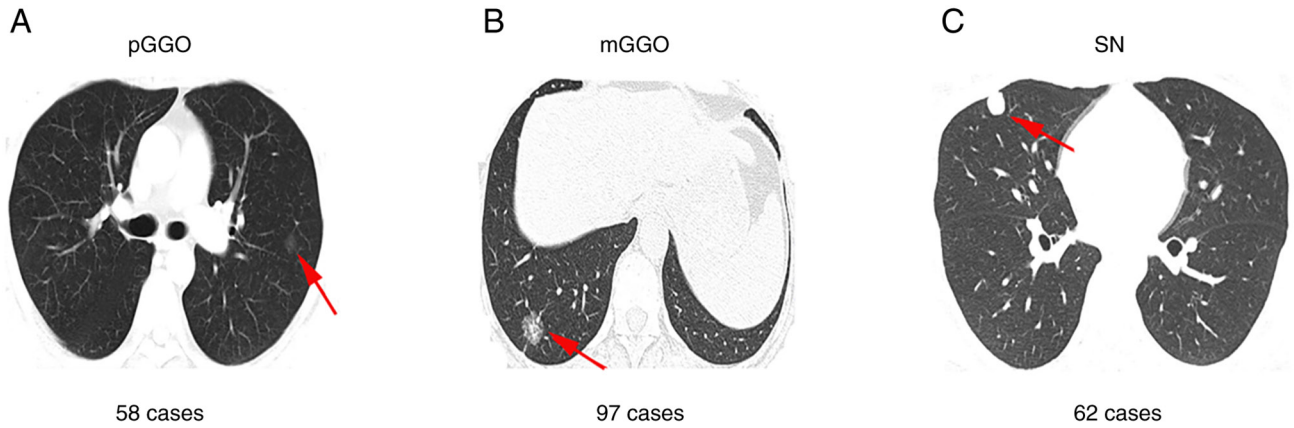

Figure 2. High-resolution computed tomography was used to evaluate patients with (A) pGGO (indicated by the red arrow), (B) mGGO (indicated by the red arrow) or (C) SN (indicated by the red arrow). pGGO, pure ground-glass opacity; mGGO, mix ground-glass opacity; SN, solid nodule.

in combination with other non-specific tumor markers, such as CEA, CYFRA21-1, CA199 or CA125. Furthermore, a positive result for 7-AABs + HSP90a reflected a 2.6-fold increase in RR for MLNs, which was significantly higher compared with 7-AABs alone or other combinations (Table II). ROC curves of 7-AABs + HSP90a (AUC=0.842; P<0.0001) also demonstrated that such a combination possessed significantly improved diagnostic efficiency for the discrimination between MLNs and BLNs compared with 7-AABs (AUC=0.612; P=0.012), 7-AABs + CEA (AUC=0.612; P=0.012), 7-AABs + CYFRA21-1 (AUC=0.612; P=0.012), 7-AABs + CA199 (AUC=0.612; P=0.012) and 7-AABs + CA125 (AUC=0.674; P<0.0001) (Fig. 4A), which indicated that 7-AABs + HSP90a exhibited better diagnostic efficiency for patients with pulmonary nodules.

Diagnostic values of 7-AABs alone or in combination with non-specific tumor markers for patients with lung nodules of different sizes. The diagnostic values of 7-AABs alone or in combination with non-specific tumor markers for patients with pulmonary nodules of 8-20 mm or <8 mm was assessed. The results demonstrated a better diagnostic value of 7-AABs for discrimination between MLNs and BLNs in the 8-20 mm group (χ^2=5.4, P=0.02) compared with the <8 mm group (χ^2=2.9, P=0.09). Furthermore, 7-AABs + HSP90a demonstrated an elevated sensitivity (all P<0.0001) and NPV (all P<0.0001) compared with 7-AABs alone or in combination with other non-specific tumor markers, such as CEA, CYFRA21-1, CA199 and CA125, in both the 8-20 mm and <8 mm groups. A positive result for 7-AABs + HSP90a indicated a 2.2-fold and 4.0-fold increase in the RR of MLNs in the 8-20 mm and <8 mm groups, respectively (Table III). ROC curves of 7-AABs + HSP90a (AUC=0.868, P<0.0001; AUC=0.784, P<0.0001) also demonstrated that such a combination possessed markedly improved diagnostic efficiency for the discrimination between MLNs and BLNs compared with 7-AABs (AUC=0.595, P=0.099; AUC=0.630, P=0.074), 7-AABs + CEA (AUC=0.595, P=0.099; AUC=0.630, P=0.074),

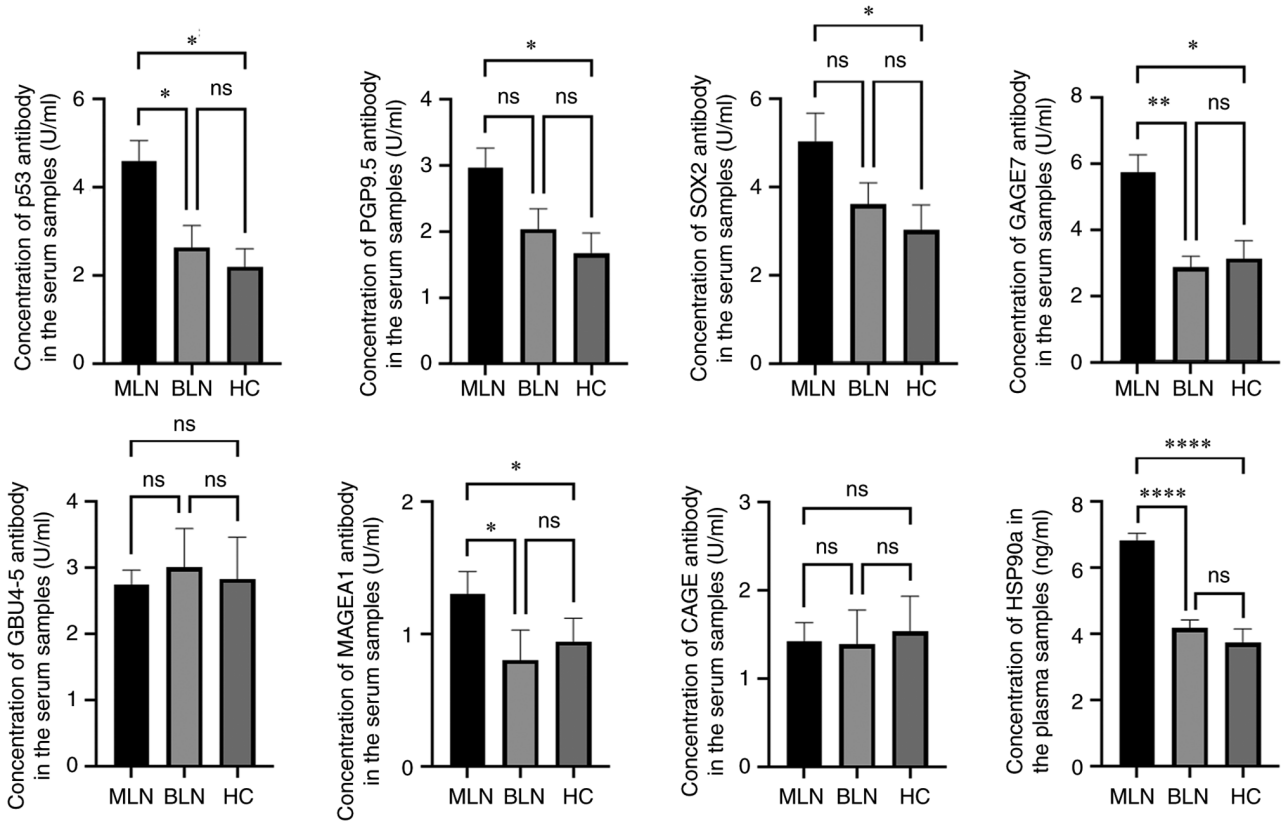

Figure 3. Levels of each autoantibody and HSP90a in the MLN, BLN and HC groups. *P<0.05, **P<0.01 and ****P<0.0001. After the normality test was performed, Kruskal-Wallis test followed by Dunn's test was used to analyze the data. MLN, malignant lung nodules; BLN, benign lung nodules; HC, healthy control; p53, Tumor protein 53; MAGEA1, melanoma antigen 1; PGP9.5, protein gene product 9.5; SOX2, sex-determining region Y-box 2; GAGE7, cancer/testis G antigen 7; GBU4-5, ATP-dependent RNA helicase 4-5; CAGE, cancer-associated antigen; HSP90a, heat shock protein 90a; ns, not significant.

7-AABs + CYFRA21-1 (AUC=0.595, P=0.099; AUC=0.630, P=0.074), 7-AABs + CA199 (AUC=0.595, P=0.099; AUC=0.630, P=0.074) and 7-AABs + CA125 (AUC=0.696, P=0.001; AUC=0.630, P=0.074) in the 8-20 mm and <8 mm groups, respectively (Fig. 4B). These data demonstrated that 7-AABs + HSP90a exhibited improved diagnostic values for patients with small pulmonary nodules (<20 mm).

Diagnostic values of 7-AABs alone or in combination with non-specific tumor markers for patients with lung nodules of different types. The diagnostic values of 7-AABs alone or in combination with non-specific tumor markers for patients with pGGO, mGGO or SN were evaluated. Statistical analysis indicated that a better diagnostic value of 7-AABs for discrimination between MLNs and BLNs was demonstrated in the pGGO group (χ^2=3.9, P=0.048) compared with the mGGO (χ^2=1.2, P=0.27) and SN (χ^2=2.1, P=0.15) groups. It was noteworthy that 7-AABs + HSP90a exhibited an increased sensitivity and NPV compared with 7-AABs alone or in combination with other non-specific tumor markers, such as CEA, CYFRA21-1, CA199 and CA125, in the three groups. A positive result for 7-AABs + HSP90a indicated a 3.3-fold, 1.5-fold and 5.5-fold increase in the RR for MLNs in the pGGO, mGGO and SN groups, respectively (Table IV). ROC curves of 7-AABs + HSP90a (AUC=0.925, P<0.0001; AUC=0.836, P<0.0001) also indicated that such a combination possessed improved diagnostic efficiency for discrimination between MLNs and BLNs compared with 7-AABs (AUC=0.803, P=0.001; AUC=0.724, P=0.012), 7-AABs + CEA (AUC=0.803, P=0.001; AUC=0.724, P=0.012), 7-AABs + CYFRA21-1 (AUC=0.803, P=0.001; AUC=0.724, P=0.012), 7-AABs + CA199 (AUC=0.803, P=0.001; AUC=0.724, P=0.012) and 7-AABs + CA125 (AUC=0.803, P=0.001; AUC=0.724, P=0.012) in the pGGO and mGGO groups; however, no significance was detected in the SN group (AUC=0.5, P=1.0) (Fig. 4C). The above results demonstrated the improved diagnostic efficiency of 7-AABs + HSP90a for patients with lung nodules of different types, especially pGGO and mGGO.

Discussion

Early-stage NSCLC has a notably better prognosis compared with advanced-stage NSCLC, and can usually be treated radically with relatively benign outcomes (16). It has been reported that lung cancer-related AABs have high specificity and PPV in diagnosing early-stage lung cancer. However, the relatively low sensitivity and NPV greatly limit their wider application in clinical practice (11,12,17). 7-AABs consisted of seven antibodies against p53, MAGEA1, GAGE7, CAGE, GBU4-5, SOX2 and PGP9.5. p53 was first reported as a tumor suppressor, and has been reported to be associated with tumorigenesis, regulation and apoptosis (18,19). It is an important biological indicator for the evaluation of tumor biological behavior and the screening of high-risk patients with lung cancer (20-22). MAGEA1, GAGE7 and CAGE are members of the cancer-testis antigen family, which can accelerate tumor formation, resist

Table II. Diagnostic values of 7-AABs alone or in combination with non-specific tumor markers for patients with lung nodules.

Diagnostic value	7-AABs	7-AABs+ HSP90a	7-AABs + CEA	7-AABs+ CYFRA21-1	7-AABs+ CA199	7-AABs+ CA125
Specificity, %	77.6	67.2	70.7	72.4	75.9	77.6
Sensitivity, %	42.8	87.4[a]	45.9	49.1	44.0	47.8
PPV, %	84.0	88.0	81.1	83.0	83.3	85.4
NPV, %	33.1	66.1[a]	32.3	34.1	33.1	35.2
RR (95% CI)	1.3 (1.1-1.5)	2.6 (1.8-3.7)	1.2 (1.0-1.4)	1.3 (1.1-1.5)	1.2 (1.1-1.5)	1.3 (1.1-1.5)

[a]P<0.0001 compared with other groups, namely 7-AABs, 7-AABs + CEA, 7-AABs + CYFRA21-1, 7-AABs + CA199 or 7-AABs + CA125, respectively. McNemar's test was used for the comparison of the sensitivity between 7-AABs + HSP90a and other groups. The marginal regression model based on the generalized estimation equation was used for the comparison of the NPV between 7-AABs + HSP90a and other groups. 7-AABs, seven lung cancer-related autoantibodies; CEA, carcinoembryonic antigen; CYFRA21-1, cytokeratin fragment antigen 21-1; CA199, carbohydrate antigen 199; CA125, carbohydrate antigen 125; HSP90a, heat shock protein 90a; PPV, positive predictive value; NPV, negative predictive value; RR, relative risk; CI, confidence interval.

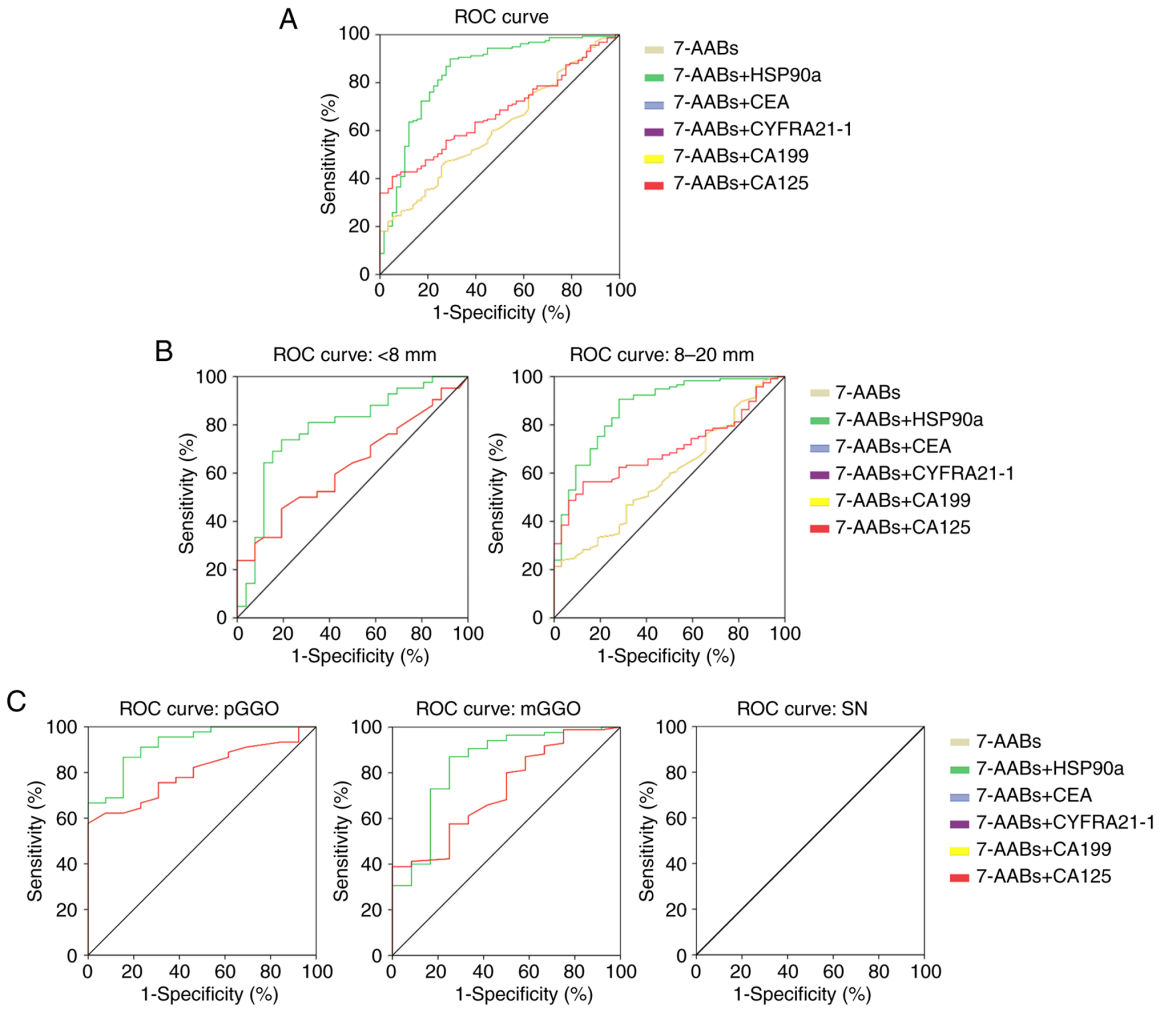

Figure 4. Diagnostic efficiency of 7-AABs alone or in combination with non-specific tumor markers. (A) Diagnostic efficiency was assessed using the ROC curve for patients with lung nodules. The results of 7-AABs, 7-AABs + CEA, 7-AABs + CYFRA21-1 and 7-AABs + CA199 were very similar to the ROC analysis using the overlapped data. (B) Diagnostic efficiency was assessed using the ROC curve for patients with lung nodules of different sizes. The results of 7-AABs, 7-AABs + CEA, 7-AABs + CYFRA21-1, 7-AABs + CA199 and 7-AABs + CA125 were similar to the ROC analysis using the overlapped data in the <8 mm group. The results of 7-AABs, 7-AABs + CEA, 7-AABs + CYFRA21-1 and 7-AABs + CA199 were very similar to the ROC analysis using the overlapped data in the 8-20 mm group. (C) Diagnostic efficiency was assessed using the ROC curve for patients with lung nodules of different types. The results of 7-AABs, 7-AABs + CEA, 7-AABs + CYFRA21-1, 7-AABs + CA199 and 7-AABs + CA125 were similar to the ROC analysis using the overlapped data in the pGGO and mGGO groups. The results of 7-AABs alone or in combination with non-specific tumor markers were identical to the ROC analysis using the overlapped data in the SN group. 7-AABs, seven lung cancer-related autoantibodies; ROC, receiver operating characteristic; CEA, carcinoembryonic antigen; CYFRA21-1, cytokeratin fragment antigen 21-1; CA199, carbohydrate antigen 199; CA125, carbohydrate antigen 125; pGGO, pure ground-glass opacity; mGGO, mix ground-glass opacity; SN, solid nodule; HSP90a, heat shock protein 90a.

Table III. Diagnostic values of 7-AABs alone or in combination with non-specific tumor markers for patients with lung nodules of different sizes.

A, tumor size <8 mm

Diagnostic value	7-AABs	7-AABs + HSP90a	7-AABs + CEA	7-AABs + CYFRA21-1	7-AABs + CA199	7-AABs + CA125
Specificity, %	73.1	61.5	73.1	69.2	73.1	73.1
Sensitivity, %	47.6	90.5[a]	50.0	52.4	47.6	47.6
PPV, %	74.1	79.2	75.0	73.3	74.1	74.1
NPV, %	46.3	80.0[a]	47.5	47.4	46.3	46.3
RR (95% CI)	1.4 (1.0-1.4)	4.0 (1.6-9.6)	1.4 (1.0-2.1)	1.4 (1.0-2.0)	1.4 (1.0-2.0)	1.4 (1.0-2.0)

B, tumor size 8-20 mm

Diagnostic value	7-AABs	7-AABs + HSP90a	7-AABs + CEA	7-AABs + CYFRA21-1	7-AABs + CA199	7-AABs + CA125
Specificity, %	81.3	71.9	68.8	75.0	78.1	81.3
Sensitivity, %	41.0	86.3[a]	44.4	47.9	42.7	47.9
PPV, %	88.9	91.8	83.9	87.5	87.7	90.3
NPV, %	27.4	60.0[a]	25.3	28.2	27.2	29.9
RR (95% CI)	1.2 (1.0-1.4)	2.2 (1.5-3.3)	1.1 (1.0-1.3)	1.2(1.0-1.4)	1.2 (1.0-1.4)	1.3 (1.1-1.5)

[a]P<0.0001 compared with other groups, namely 7-AABs, 7-AABs + CEA, 7-AABs + CYFRA21-1, 7-AABs + CA199 or 7-AABs + CA125, respectively. McNemar's test was used for the comparison of the sensitivity between 7-AABs + HSP90a and other groups. The marginal regression model based on the generalized estimation equation was used for the comparison of the NPV between 7-AABs + HSP90a and other groups. 7-AABs, seven lung cancer-related autoantibodies; CEA, carcinoembryonic antigen; CYFRA21-1, cytokeratin fragment antigen 21-1; CA199, carbohydrate antigen 199; CA125, carbohydrate antigen 125; HSP90a, heat shock protein 90a; PPV, positive predictive value; NPV, negative predictive value; RR, relative risk.

apoptosis, promote tumor proliferation and metastasis, and cause cellular and humoral immune responses (23,24). Their increased expression in NSCLC has been reported, including squamous cell carcinoma and adenocarcinoma (6,25). GBU4-5 is an ATP-dependent DNA helicase, which regulates transposon methylation and inhibits gene expression in the process of cell differentiation. The expression of GBU4-5 is increased in lung cancer (26). SOX2 activates the expression of oncogenes in lung cancer cells through regulation of the RAS-MAPK-survivin signaling pathway, which promotes the occurrence of lung cancer (27,28). SOX2 also serves a vital role in modulation of the growth and regulation of lung cancer stem cells, and it has a relatively high expression level in lung cancer cells compared with those in normal lung cells (29,30). PGP9.5 can increase the deubiquitination of functional proteins and regulate the expression of cell cycle genes (31). The upregulation of PGP9.5 is associated with lung cancer progression, and it is highly expressed in both squamous cell carcinoma and non-squamous cell carcinoma (32). The combined detection of 7-AABs has a much higher specificity and accuracy than a single marker, improving the detection rate of lung cancer (11).

In the present study, 217 patients with different types of small pulmonary nodules (≤20 mm), such as pGGO, mGGO and SN, were assessed. The results indicated that p53, PGP9.5, SOX2, GAGE7 and MAGEA1 protein expression levels were significantly increased in MLNs compared with BLNs and HCs. The specificity, sensitivity, PPV and NPV of 7-AABs for discrimination between MLNs and BLNs were 77.6, 42.8, 84.0 and 33.1%, respectively, which was similar those values previously reported (11,12). HSP90a is closely associated with numerous human cancers, and it has previously been employed for the early screening of certain tumors, including liver, lung and colorectal cancer, due to its high sensitivity (33-37). Chen *et al* (38) have reported that HSP90a is frequently over-expressed in human liver carcinoma cells, and its expression level is negatively correlated to the prognosis of patients with liver cancer. HSP90a, as a target of G-Rh2, serves a vital role in liver cancer therapy (38). The inhibition of HSP90a also enhances anti-tumor immunity (39). In a parallel study comparing alpha-fetoprotein (AFP), plasma HSP90a demonstrated a significantly higher diagnostic performance in distinguishing hepatocellular carcinoma (HCC) from HCs. Moreover, plasma HSP90a exhibited excellent diagnostic accuracy in discriminating AFP-negative patients with HCC (sensitivity, 93.9%; specificity, 91.3%) and AFP-limited liver cancer (sensitivity, 96.6%; specificity, 90.3%) (40). Furthermore, Zhong *et al* (41) reported that HSP90a, a valuable predictor of early chemotherapy effectiveness in advanced NSCLC, was closely correlated with tumor remission after chemotherapy, while HSP90a was not correlated with tumor diameter and pathological type. Targeting HSP90a suppresses the growth of lung cancer (42), promotes

Table IV. Diagnostic values 7-AABs alone or in combination with non-specific tumor markers for patients with lung nodules of different types.

A, pure ground-glass opacity type lung nodules

Diagnostic value	7-AABs	7-AABs + HSP90a	7-AABs + CEA	7-AABs + CYFRA21-1	7-AABs + CA199	7-AABs + CA125
Specificity, %	84.6	76.9	84.6	84.6	84.6	84.6
Sensitivity, %	51.1	91.1[a]	51.1	57.8	51.1	53.3
PPV, %	92.0	93.2	92.0	92.9	92.0	92.3
NPV, %	33.3	71.4[a]	33.3	36.7	36.7	36.7
RR (95% CI)	1.4 (1.1-1.8)	3.3 (1.4-7.5)	1.4 (1.1-1.8)	1.5 (1.1-2.0)	1.4 (1.1-1.8)	1.4 (1.1-1.9)

B, mix ground-glass opacity type lung nodules

Diagnostic value	7-AABs	7-AABs + HSP90a	7-AABs + CEA	7-AABs + CYFRA21-1	7-AABs + CA199	7-AABs + CA125
Specificity, %	83.3	66.7	75.0	75.0	83.3	83.3
Sensitivity, %	37.6	84.7[a]	41.2	42.4	38.8	43.5
PPV, %	94.1	94.7	92.1	92.3	94.3	94.9
NPV, %	15.9	38.1[a]	15.5	15.5	16.1	16.1
RR (95% CI)	1.1 (1.0-1.3)	1.5 (1.1-2.1)	1.1 (1.0-1.3)	1.1 (1.0-1.3)	1.1 (1.0-1.3)	1.1 (1.0-1.3)

C, solid type lung nodules

Diagnostic value	7-AABs	7-AABs + HSP90a	7-AABs + CEA	7-AABs + CYFRA21-1	7-AABs + CA199	7-AABs + CA125
Specificity, %	72.7	63.6	63.6	66.7	69.7	72.7
Sensitivity, %	44.8	89.7[a]	51.7	55.2	48.3	51.7
PPV, %	59.1	68.4	55.6	59.3	58.3	62.5
NPV, %	60.0	87.5[b]	60.0	62.9	60.5	63.2
RR (95% CI)	1.5 (0.9-2.5)	5.5 (1.9-16.1)	1.4 (0.8-2.4)	1.6 (0.9-2.7)	1.5 (0.9-2.5)	1.7 (1.0-2.9)

[a]P<0.0001 and [b]P<0.001 compared with other groups, namely 7-AABs, 7-AABs + CEA, 7-AABs + CYFRA21-1, 7-AABs + CA199 or 7-AABs + CA125, respectively. McNemar's test was used for the comparison of the sensitivity between 7-AABs + HSP90a and other groups. The marginal regression model based on the generalized estimation equation was used for the comparison of the NPV between 7-AABs + HSP90a and other groups. 7-AABs, seven lung cancer-related autoantibodies; CEA, carcinoembryonic antigen; CYFRA21-1, cytokeratin fragment antigen 21-1; CA199, carbohydrate antigen 199; CA125, carbohydrate antigen 125; HSP90a, heat shock protein 90a; PPV, positive predictive value; NPV, negative predictive value; RR, relative risk.

apoptosis and inhibits migration (43). A large-sample and multi-center survey reported a significantly increased level of HSP90a in early-stage NSCLC. Moreover, when the cutoff of HSP90a was 56.33 ng/ml, the sensitivity was 72%, the specificity was 78% and the coincidence rate was 75% (44). In the present study, further evaluation demonstrated that 7-AABs + HSP90a displayed remarkable advantages compared with 7-AABs alone or in other combinations. For pulmonary nodules of <8 mm, the specificity, sensitivity, PPV and NPV of 7-AABs + HSP90a were 61.5, 90.5, 79.2 and 80.0%, respectively, in discrimination between MLNs and BLNs, which was markedly higher compared with 7-AABs alone or in other combinations. Furthermore, 7-AABs + HSP90a reflected a 4.0-fold increase in the RR of MLNs, which indicated a better diagnostic efficiency. Notably, the diagnostic value of 7-AABs + HSP90a for pulmonary nodules of 8-20 mm was superior to that of pulmonary nodules of <8 mm. However, the diagnostic value of 7-AABs + HSP90a for pulmonary nodules of >20 mm required further assessment. For different nodule types, 7-AABs exhibited a good diagnostic value in patients with pGGO (χ^2=3.9, P=0.048) compared with mGGO (χ^2=1.2, P=0.27) and SN (χ^2=2.1, P=0.15). 7-AABs + HSP90a demonstrated a significantly increased diagnostic efficiency for MLNs in patients with pGGO, mGGO and SN. In the present study, besides the significantly increased sensitivity, NPV and RR, it was demonstrated that the specificity of 7-AABs + HSP90a showed a slight decrease compared with 7-AABs. However, based on the results of diagnostic

efficiencies assessed using the ROC curve, 7-AABs + HSP90a exhibited much better diagnostic performance than 7-AABs in the discrimination of MLNs from BLNs. However, a greater number of relevant biomarkers require assessment to improve the specificity in future research. Moreover, the diagnostic values of 7-AABs alone or in combination with non-specific tumor markers for patients with stage 0 and I lung cancer was evaluated. However, based on the χ^2 test, the results demonstrated no significant effects on the discrimination of stage 0 and I lung cancer (Table SI). ROC analysis also demonstrated no diagnostic value for the use of 7-AABs alone or in combination with non-specific tumor markers (AUC=0.5, P=1.0) (Fig. S1). Once produced, an antibody can exist in the peripheral circulating blood for a long time and will not disappear quickly. Therefore 7-AABs are only used to diagnose lung cancer instead of evaluating its prognosis. To assess whether 7-AABs (+/- HSP90a) were correlated with age of diagnosis, 159 patients with lung cancer in the present study were divided into two groups as follows: i) A low-age group (≤50 years old, n=48); and ii) an advanced-age group (>50 years old, n=111). The results demonstrated no significant differences in the detection rate of 7-AABs (+/- HSP90a) in lung cancer between the two groups (χ^2=0.6, P=0.42; χ^2=0.8, P=0.38). Moreover, there were also no significant differences in the detection rate of 7-AABs (+/- HSP90a) in lung cancer between male and female patients (χ^2=0.07, P=0.93; χ^2=2.96, P=0.09). As is well known, histopathological character affects the treatment and prognosis of patients with lung cancer. Furthermore, the tumor markers against adenocarcinoma, squamous cell carcinoma and SCLC are different. In the present study, when two patients with squamous cell carcinoma (1 case with a solid nodule of 8-20 mm) and SCLC (1 case with a solid nodule of 8-20 mm) were excluded, 7-AABs + HSP90a demonstrated elevated sensitivity (patients with lung nodule, 88.5 vs. 87.4%; patients with lung nodule of 8-20 mm, 87.8 vs. 86.3%; patients with solid nodule, 96.3 vs. 89.7%).

However, due to the limitation in the number of patients with SCLC, larger pulmonary nodules (>20 mm) or advanced progression (such as, stage III-IV lung cancer), the diagnostic values of 7-AABs, alone or in combination with non-specific tumor markers, were not evaluated in these patients in the present study. Therefore, these aspects require further study.

In conclusion, the findings of the present study demonstrated that 7-AABs + HSP90a had good clinical value for the diagnosis of early-stage lung cancer, including patients with different types of small nodules, which suggested its value for further application in clinical practice.

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Availability of data and materials

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

Authors' contributions

QC, SZ, WH and GF conceived and designed the study. QC, SZ, GG, WZ, ZZ, GF and NJ performed the data analysis and drafted the manuscript. QC, SZ and WH performed data collection. GG, WZ, GF, NJ and WH analyzed the results. WH and GF edited the manuscript and confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Review Committee of The First Affiliated Hospital of Wannan Medical College (approval no. 202076; Wuhu, China), and the experimental procedures were in agreement with The Declaration of Helsinki. Written informed consent was obtained from all participants.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Siegel RL, Miller KD, Fuchs HE and Jemal A: Cancer statistics, 2021. CA Cancer J Clin 71: 7-33, 2021.
2. Xie E, Lin M, Sun Z, Jin Y, Zhang S, Huang L, Sun R, Wang F and Pan S: Serum miR-27a is a biomarker for the prognosis of non-small cell lung cancer patients receiving chemotherapy. Transl Cancer Res 10: 3458-3469, 2021.
3. Garon EB, Hellmann MD, Rizvi NA, Carcereny E, Leighl NB, Ahn MJ, Eder JP, Balmanoukian AS, Aggarwal C, Horn L, *et al*: Five-year overall survival for patients with advanced non-small-cell lung cancer treated with pembrolizumab: Results from the phase I KEYNOTE-001 Study. J Clin Oncol 37: 2518-2527, 2019.
4. Albano D, Bilfinger T and Nemesure B: 1-, 3-, and 5-year survival among early-stage lung cancer patients treated with lobectomy vs SBRT. Lung Cancer (Auckl) 9: 65-71, 2018.
5. Lutfi W, Schuchert MJ, Dhupar R, Sarkaria I, Christie NA, Yang CJ, Deng JZ, Luketich JD and Okusanya OT: Sublobar resection is associated with decreased survival for patients with early stage large-cell neuroendocrine carcinoma of the lung. Interact Cardiovasc Thorac Surg 29: 517-524, 2019.
6. Luo B, Mao G, Ma H and Chen S: The role of seven autoantibodies in lung cancer diagnosis. J Thorac Dis 13: 3660-3668, 2021.
7. Zhang X, Liu M, Zhang X, Wang Y and Dai L: Autoantibodies to tumor-associated antigens in lung cancer diagnosis. Adv Clin Chem 103: 1-45, 2021.
8. Trudgen K, Khattar NH, Bensadoun E, Arnold S, Stromberg AJ and Hirschowitz EA: Autoantibody profiling for lung cancer screening longitudinal retrospective analysis of CT screening cohorts. PLoS One 9: e87947, 2014.
9. Sullivan FM, Farmer E, Mair FS, Treweek S, Kendrick D, Jackson C, Robertson C, Briggs A, McCowan C, Bedford L, *et al*: Detection in blood of autoantibodies to tumour antigens as a case-finding method in lung cancer using the EarlyCDT®-Lung Test (ECLS): Study protocol for a randomized controlled trial. BMC Cancer 17: 187, 2017.

10. Chen SS, Li K, Wu J, Peng ZY, Wang ZD, Wang JC, Xu CW, Zhu CL, Li BC, Ren H, *et al*: Stem signatures associated antibodies yield early diagnosis and precise prognosis predication of patients with non-small cell lung cancer. J Cancer Res Clin Oncol 147: 223-233, 2021.
11. Du Q, Yu R, Wang H, Yan D, Yuan Q, Ma Y, Slamon D, Hou D, Wang H and Wang Q: Significance of tumor-associated autoantibodies in the early diagnosis of lung cancer. Clin Respir J 12: 2020-2028, 2018.
12. Ren S, Zhang S, Jiang T, He Y, Ma Z, Cai H, Xu X, Li Y, Cai W, Zhou J, *et al*: Early detection of lung cancer by using an autoantibody panel in Chinese population. Oncoimmunology 7: e1384108, 2017.
13. Saini J and Sharma PK: Clinical, prognostic and therapeutic significance of heat shock proteins in cancer. Curr Drug Targets 19: 1478-1490, 2018.
14. Wang X, Song X, Zhuo W, Fu Y, Shi H, Liang Y, Tong M, Chang G and Luo Y: The regulatory mechanism of Hsp90alpha secretion and its function in tumor malignancy. Proc Natl Acad Sci U S A 106: 21288-21293, 2009.
15. WHO Classification of Tumours Editorial Board. WHO Classification of Tumours. Thoracic Tumours. 5th edition. IARC Press, Lyon, 2021.
16. Balata H, Fong KM, Hendriks LE, Lam MS, Ostroff JS, Peled N, Wu N and Aggarwal C: Prevention and early detection for NSCLC: Advances in thoracic oncology 2018. J Thorac Oncol 14: 1513-1527, 2019.
17. Zhang R, Ma L, Li W, Zhou S and Xu S: Diagnostic value of multiple tumor-associated autoantibodies in lung cancer. Onco Targets Ther 12: 457-469, 2019.
18. Stein Y, Rotter V and Aloni-Grinstein R: Gain-of-function mutant p53: All the roads lead to tumorigenesis. Int J Mol Sci 20: 6197, 2019.
19. Lacroix M, Riscal R, Arena G, Linares LK and Le Cam L: Metabolic functions of the tumor suppressor p53: Implications in normal physiology, metabolic disorders, and cancer. Mol Metab 33: 2-22, 2020.
20. Nanami T, Hoshino I, Shiratori F, Yajima S, Oshima Y, Suzuki T, Ito M, Hiwasa T, Kuwajima A and Shimada H: Presence of serum RalA and serum p53 autoantibodies in 1833 patients with various types of cancers. Int J Clin Oncol 27: 72-76, 2022.
21. Zhang T, Li Y, Zhu R, Song P, Wei Y, Liang T and Xu G: Transcription factor p53 suppresses tumor growth by prompting pyroptosis in non-small-cell lung cancer. Oxid Med Cell Longev 2019: 8746895, 2019.
22. Mu Y, Xie F and Sun T: Clinical value of seven autoantibodies combined detection in the diagnosis of lung cancer. J Clin Lab Anal 34: e23349, 2020.
23. Meng X, Sun X, Liu Z and He Y: A novel era of cancer/testis antigen in cancer immunotherapy. Int Immunopharmacol 98: 107889, 2021.
24. Li S, Ma Y, Xiong Y, Zhang P, Wang X, Wang Y and Yang Y: Five tumor-associated autoantibodies expression levels in serum predict lung cancer and associate with poor outcome. Transl Cancer Res 8: 1364-1373, 2019.
25. Huang H, Luo W, Ni Y, Sun S, Wang C and Zhang L: The diagnostic efficiency of seven autoantibodies in lung cancer. Eur J Cancer Prev 29: 315-320, 2020.
26. Wang Y, Jiao Y, Ding CM and Sun WZ: The role of autoantibody detection in the diagnosis and staging of lung cancer. Ann Transl Med 9: 1673, 2021.
27. Novak D, Hüser L, Elton JJ, Umansky V, Altevogt P and Utikal J: SOX2 in development and cancer biology. Semin Cancer Biol 67: 74-82, 2020.
28. Chaudhary S, Islam Z, Mishra V, Rawat S, Ashraf GM and Kolatkar PR: Sox2: A regulatory factor in tumorigenesis and metastasis. Curr Protein Pept Sci 20: 495-504, 2019.
29. Mollaoglu G, Jones A, Wait SJ, Mukhopadhyay A, Jeong S, Arya R, Camolotto SA, Mosbruger TL, Stubben CJ, Conley CJ, *et al*: The lineage-defining transcription factors SOX2 and NKX2-1 determine lung cancer cell fate and shape the tumor immune microenvironment. Immunity 49: 764-779, 2018.
30. Schaal CM, Bora-Singhal N, Kumar DM and Chellappan S: Regulation of Sox2 and stemness by nicotine and electronic-cigarettes in non-small cell lung cancer. Mol Cancer 17: 149, 2018.
31. Fang Y and Shen X: Ubiquitin carboxyl-terminal hydrolases: Involvement in cancer progression and clinical implications. Cancer Metastasis Rev 36: 669-682, 2017.
32. Chen P, Lu W and Chen T: Seven tumor-associated autoantibodies as a serum biomarker for primary screening of early-stage non-small cell lung cancer. J Clin Lab Anal 35: e24020, 2021.
33. Han Y, Zhang Y, Cui L, Li Z, Feng H, Zhang Y, Sun D and Ren L: Plasma heat shock protein 90alpha as a biomarker for the diagnosis of liver cancer: In patients with different clinicopathologic characteristics. World J Surg Oncol 19: 228, 2021.
34. Wang Y, Barghi SM, Yang Y and Akhavan-Sigari R: Value of HSP90α in lung cancer diagnosis and recurrence prediction: A cohort study. Oncol Res Treat 44: 583-589, 2021.
35. Kasanga M, Liu L, Xue L and Song X: Plasma heat shock protein 90-alpha have an advantage in diagnosis of colorectal cancer at early stage. Biomark Med 12: 881-890, 2018.
36. Ichikawa T, Shanab O, Nakahata S, Shimosaki S, Manachai N, Ono M, Iha H, Shimoda K and Morishita K: Novel PRMT5-mediated arginine methylations of HSP90A are essential for maintenance of HSP90A function in NDRG2low ATL and various cancer cells. Biochim Biophys Acta Mol Cell Res 1867: 118615, 2020.
37. Zhao Q, Miao C, Lu Q, Wu W, He Y, Wu S, Liu H and Lian C: Clinical significance of monitoring circulating free DNA and plasma heat shock protein 90alpha in patients with esophageal squamous cell carcinoma. Cancer Manag Res 13: 2223-2234, 2021.
38. Chen C, Wang YS, Zhang ET, Li GA, Liu WY, Li Y and Jin YH: (20S) Ginsenoside Rh2 exerts its anti-tumor effect by disrupting the HSP90A-Cdc37 system in human liver cancer cells. Int J Mol Sci 22: 13170, 2021.
39. Song KH, Oh SJ, Kim S, Cho H, Lee HJ, Song JS, Chung JY, Cho E, Lee J, Jeon S, *et al*: HSP90A inhibition promotes anti-tumor immunity by reversing multi-modal resistance and stem-like property of immune-refractory tumors. Nat Commun 11: 562, 2020.
40. Fu Y, Xu X, Huang D, Cui D, Liu L, Liu J, He Z, Liu J, Zheng S and Luo Y: Plasma heat shock protein 90alpha as a biomarker for the diagnosis of liver cancer: An official, large-scale, and multicenter clinical trial. EBioMedicine 24: 56-63, 2017.
41. Zhong B, Shen J, Zhang C, Zhou G, Yu Y, Qin E, Tang J, Wu D and Liang X: Plasma heat shock protein 90 alpha: A valuable predictor of early chemotherapy effectiveness in advanced non-small-cell lung cancer. Med Sci Monit 27: e924778, 2021.
42. Ojha R, Nepali K, Chen CH, Chuang KH, Wu TY, Lin TE, Hsu KC, Chao MW, Lai MJ, Lin MH, *et al:* Isoindoline scaffold-based dual inhibitors of HDAC6 and HSP90 suppressing the growth of lung cancer in vitro and in vivo. Eur J Med Chem 190: 112086, 2020.
43. Pan J, Jiang F, Zhou J, Wu D, Sheng Z and Li M: HSP90: A novel target gene of miRNA-628-3p in A549 cells. Biomed Res Int 2018: 4149707, 2018.
44. Shi Y, Liu X, Lou J, Han X, Zhang L, Wang Q, Li B, Dong M and Zhang Y: Plasma levels of heat shock protein 90 alpha associated with lung cancer development and treatment responses. Clin Cancer Res 20: 6016-6022, 2014.