Received: 25 January 2020 | Revised: 10 April 2020 | Accepted: 12 April 2020

DOI: 10.1002/jcla.23349

RESEARCH ARTICLE

WILEY

Clinical value of seven autoantibodies combined detection in the diagnosis of lung cancer

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Funding information
Medical and Health Planned Science and Technology Project of Zhejiang Province, Grant/Award Number: 2018KY158 and 2019KY606; Nature Science Foundation of Ningbo City, Grant/Award Number: 2019A610230

Abstract

Background: To analyze the clinical value of seven autoantibodies (p53, PGP9.5, SOX2, GAGE7, GBU4-5, MAGE A1 and CAGE) in lung cancer patients.

Methods: ELISA was used to determine serum levels of seven autoantibodies in 177 patients with lung cancer, 201 healthy persons, and 210 patients with benign pulmonary diseases. Positive rates of 7 autoantibodies were analyzed; receiver operating characteristic (ROC) curves were drawn to analyze their diagnostic efficiency in lung cancer and to compare the positive rate of seven kinds of autoantibody combined detection of lung cancer patients with different clinicopathological features.

Results: The positive rate of seven autoantibodies in all subjects was 13.44%. The positive rate of seven autoantibodies in lung cancer was 25.42%. The positive rate of the combined detection of seven autoantibodies in the lung cancer group was significantly higher than that in healthy control group (χ^2 = 19.76, P < .001) and benign lung disease group (χ^2 = 21.44, P < .001). Sensitivity, specificity, and AUC^{ROC} of the seven autoantibodies were 25.42%, 91.75%, and 0.683, respectively. Sensitivity and AUC^{ROC} were higher than those of the single autoantibody detection. Positive rates of seven autoantibodies in different pathological types and clinical stages of lung cancer patients were significantly different (P < .05).

Conclusions: The combined detection of 7 autoantibodies in lung cancer has some clinical value for the auxiliary diagnosis of lung cancer.

KEYWORDS

autoantibody, clinical value, combined detection, diagnosis, lung cancer

1 | INTRODUCTION

Lung cancer is the most common cause of death from cancer in the world, the prevalence keeps increasing in recent years, and its morbidity and mortality rank first in China, which is seriously endangering people's health.[1] Most lung cancer patients are already in the advanced stage at the time of diagnosis.[2,3] Average 5-year survival rate is approximately 17.4%. Early detection and treatment of lung cancer are a promising task to decrease lung mortality.[4-6] Histopathology is typically used to diagnose lung cancer, but it is

Abbreviations: ROC, receiver operating characteristic; TAAs, tumor-associated antigens.

invasive. Although serum tumor biomarkers have certain diagnostic value for lung cancer, they are mainly used for efficient monitoring, which is meaningless for early diagnosis.[7,8] Autoantigens that are abnormally expressed in tumor cells are called tumor-associated antigens (TAAs).[9] After these related antigens are recognized by the immune system, the body produces autoantibodies to kill tumor cells.[10] Combined detection of autoantibodies has been reported to have potential efficacy as diagnostic and prognostic tools in tumors.[11,12] Studies[13-16] also have shown that the detection of autoantibodies for early diagnosis of lung cancer has higher sensitivity and better estimation accuracy. The aim of this study was to investigate the clinical value of seven autoantibodies and their combined detection of p53, PGP9.5, SOX2, GAGE7, GBU4-5, MAGE A1 and CAGE in the early diagnosis of lung cancer.

2 | STUDY SUBJECTS AND METHODS

2.1 | Study subjects

A total of 588 participants were collected from May 2018 to December 2018 at Ningbo Medical Center, Li Huili Hospital, Ningbo, China. A total of 313 males and 275 females were included, ranging in age from 22 to 89 years, with a median age of 53 years. All subjects were divided into the lung cancer group, healthy control group, and benign lung disease group. Among them, 177 patients were in the lung cancer group, 88 males and 89 females; aged 29-89 years old, with a median age of 62 years; according to histopathological staging: 147 cases of adenocarcinoma, 21 cases of squamous cell carcinoma, and 9 cases of small cell carcinoma; TNM staging: I period in 131 cases, II-III period 18 cases, and IV period 28 cases. The healthy control group was composed of 201 cases, 124 males and 77 females; age 22-72, median age 47. There were 210 patients with benign lung disease, 101 males and 109 females; age 22-85, median age 55. This study was reviewed and approved by the ethics committee of Ningbo Li Huili Hospital, and informed consent was obtained from all participants.

2.2 | Serum sample collections and processing

Serum from 5 mL fasting blood was separated by centrifugation at 3500 r/min (2410 g), 5 minutes, completed within 8 hours, if the specimen cannot be detected in time, and stored at −20°C.

2.3 | Reagents and equipment

The ELISA was used in the test according to 7-AABS assay kit (Hangzhou Cancer probe Biotech Company). Measured the OD value of each sample with Microplate Reader (ST360, Shanghai Kehua Biotechnology Co., Ltd.).

2.4 | Enzyme-linked immunosorbent assays (ELISA)

The ELISA kit is tested according to the instructions. The seven autoantibodies' positive reference values were as follows: p53 ≥ 13.1 U/mL, PGP9.5 ≥ 11.1 U/mL, SOX2 ≥ 10.3 U/mL, GAGE7 ≥ 14.4 U/mL, GBU4-5 ≥ 7.0 U/mL, MAGE A1 ≥ 11.9 U/mL, and CAGE ≥ 7.2 U/mL. If one of the seven autoantibodies is positive, it will be judged as positive. If all seven autoantibodies are negative, it will be judged as negative.

2.5 | Statistical analysis

Statistical analysis was carried out in SPSS software, version 22.0. Due to seven antibodies against seven TAAs were not normally distributed (Shapiro-Wilk's test), the data were expressed as median (Quartile) [M(*P*25, *P*75)]. One-way ANOVA and nonparametric Kruskal-Wallis test were used to compare differences of antibody levels among multiple groups, and nonparametric Mann-Whitney *U* test was used to compare the differences of antibody levels between two groups[14]; ROC curve was drawn to analyze the diagnostic efficiency; and chi-square test was used for comparison between groups. A two-tailed $P < .05$ was considered statistically significant.

3 | RESULTS

3.1 | Comparison of seven autoantibody detection positive rates

Among the 588 patients, the positive rate of seven autoantibodies was 13.44%, which was significantly higher than the single detection of autoantibody. The positive rate of the combined detection of seven autoantibodies in the lung cancer group (25.42%) was significantly higher than that in healthy control group (8.46%) and benign lung disease group (8.10%). The difference between the lung cancer group and the healthy control group was statistically significant ($\chi^2 = 19.76$, $P < .001$) and between the lung cancer group and the benign disease group was also statistically significant ($\chi^2 = 21.44$, $P < .001$; Table 1).

3.2 | Comparison of serum autoantibody detection levels in each group

The serum levels of autoantibodies in each group were significantly different ($P < .05$). Differences of serum PGP9.5, GAGE7, GBU4-5, and CAGE between the lung cancer group and the healthy controls group were statistically significant ($P < .05$). While in p53, SOX2, and MAGE A1 there was no significant difference ($P > .05$). Compared with lung benign disease group, serum PGP9.5, SOX2, GAGE7, MAGE A1, and CAGE in lung cancer group had a significant difference ($P < .05$), but

TABLE 1 The positive rate of seven kinds of autoantibodies in 588 subjects

Autoantibodies	Total (n = 588)	Lung cancer (n = 177)	Healthy controls (n = 210)	Benign lung disease (n = 201)	χ^2	*P*
p53	19 (3.23)	12 (6.78)	6 (2.99)	1 (0.48)		
PGP9.5	21 (3.57)	12 (6.78)	5 (2.49)	4 (1.90)		
SOX2	14 (2.38)	9 (5.08)	3 (1.49)	2 (0.95)		
GAGE7	20 (3.40)	15 (8.47)	2 (1.00)	3 (1.43)		
GBU4-5	19 (3.23)	11 (6.21)	3 (1.49)	5 (2.38)		
MAGE A1	12 (2.04)	7 (3.95)	2 (1.00)	3 (1.43)		
CAGE	17 (2.89)	12 (6.78)	0 (0.00)	5 (2.38)		
Combined detection	79 (13.44)	45 (25.42)	17 (8.48)	17 (8.10)	31.304	<.001

Note: Values are expressed as No (%). Combined detection, between three groups, $\chi^2 = 31.304$, $P < .001$; lung cancer versus benign disease, $\chi^2 = 21.436$, $P < .001$; benign disease versus healthy controls, $\chi^2 = 19.758$, $P < .001$.

there was no significant difference between p53 and GBU4-5 ($P > .05$; Table 2).

3.3 | Evaluation of diagnostic efficiency of single antibody and seven autoantibodies in patients with lung cancer

Lung cancer patients as the disease group, healthy controls group and lung benign disease group as the control group, ROC curve analysis of seven autoantibody individual detection and combined detection diagnostic efficiency of patients with lung cancer. The results showed that the sensitivity of individual antibody detection was <10%. The specificity was higher than 97%, and the AUC^{ROC} was higher than 0.40; the sensitivity of the seven autoantibodies combined detection (25.42%) and the AUC^{ROC} (0.683) were both higher than the individual antibody detection (Table 3 and Figure 1).

3.4 | Positive rates of seven autoantibodies in different pathological types of lung cancer patient

Positive rates of seven autoantibodies combined detection in different pathological types and clinical stages of lung cancer patients were statistically significant ($P < .05$). There was no significant difference in the positive rate of lung cancer patients with different age, gender, and smoking ($P > .05$; Table 4).

4 | DISCUSSION

This study showed that the positive rate of seven autoantibodies combined detection (13.44%) was significantly higher than that of individual autoantibody detection (3.57%), suggesting that combined detection could improve the positive rate of the patients and avoid missed diagnosis to some extent. Some studies[17-19] have

TABLE 2 Detection serum level of autoantibodies in each group [M(*P25*, *P75*)]

Autoantibodies	Lung cancer (n = 177)	Healthy controls (n = 210)	Begin lung disease (n = 201)	*H*	*P*
p53	0.400 (0.000, 2.000)	0.700 (0.200, 1.700)*	0.700 (0.200, 1.700)*	9.948	.007
PGP9.5	0.400 (0.100, 2.300)	0.200 (0.000, 0.800)**	0.200 (0.000, 0.800)**	39.255	.000
SOX2	0.800 (0.100, 1.960)	0.900 (0.300, 2.175)*	0.800 (0.300, 2.100)**	16.944	.000
GAGE7	1.400 (0.350, 3.650)	1.250 (0.400, 2.300)**	1.200 (0.300, 2.250)**	41.612	.000
GBU4-5	0.500 (0.000, 1.600)	0.300 (0.000, 1.100)**	0.200 (0.000, 0.800)*	6.057	.048
MAGE A1	0.300 (0.100, 1.500)	0.300 (0.100, 0.600)*	0.300 (0.100, 0.600)**	10.635	.005
CAGE	0.100 (0.000, 1.100)	0.100 (0.000, 0.400)**	0.100 (0.000, 0.300)**	9.260	.010

Note: Compared with lung cancer:

*$P > .05$;

**$P < .05$

TABLE 3 Diagnostic efficacy of single autoantibody detection and combined detection of seven autoantibodies

	Seven Autoantibodies							
	P53	PGP9.5	SOX2	GAGE7	MAGE A1	CAGE	GBU4-5	Combined detection
Sensitivity (%)	6.78	6.78	5.08	8.47	3.95	6.78	6.21	25.42
Specificity (%)	98.30	97.81	98.78	98.78	98.78	98.78	98.05	91.73
PPV (%)	63.16	57.14	64.29	75.00	70.59	44.44	57.89	56.96
NPV (%)	71.00	70.90	70.73	71.48	71.10	70.12	70.83	74.07
Accuracy (%)	70.75	70.41	70.58	71.60	71.09	69.73	70.41	71.77
AUC	0.497	0.642	0.539	0.619	0.572	0.569	0.558	0.683

Abbreviations: AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value.

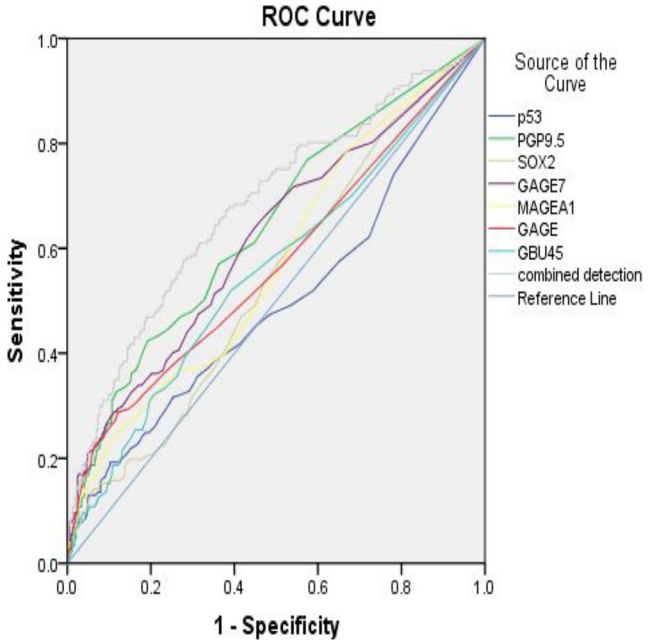

FIGURE 1 The receiver operating characteristic (ROC) curve analysis of seven autoantibodies in lung cancer

TABLE 4 Baseline characteristics of lung cancer patients

Parameters	Cases (n)	Positive rate (%)[a]	χ^2	*P*
Age range (y)				
<60	72	14 (19.44)	2.289	.130
≥60	105	31 (31.13)		
Gender				
Male	88	25 (29.21)	0.823	.364
Female	89	20 (23.59)		
Smoking				
None	53	14 (27.78)	0.039	.843
Yes	124	31 (25.81)		
Histology				
Adenocarcinoma	147	30 (21.62)	11.716	.003
Squamous cell	21	11 (52.38)		
SCLC	9	4 (44.44)		
TNM stages				
I	131	25 (19.08)	11.014	.004
II, III	18	7 (47.37)		
IV	28	13 (46.43)		

Abbreviations: SCLC, small cell carcinoma of the lung; TNM, tumor-node-metastasis.

[a]Values are expressed as No (%).

confirmed that the combined detection of seven autoantibodies and serum tumor markers can improve the detection rate of lung cancer, and some studies[20,21] have reported that the combination of seven autoantibodies and low-dose CT can improve the diagnostic accuracy of patients presenting as ground-glass nodules or solid nodules. The above results indicate that the combined detection of seven autoantibodies may serve as a preliminary screening test for high-risk patients to distinguish lung cancer patients from normal patients.

The study also showed that the sensitivity of all the seven autoantibodies was low and the diagnostic efficiency was not good. Five of seven autoantibodies (PGP9.5, SOX2, GAGE7, MAGE A1, and CAGE) appeared more frequently in serum of the lung cancer group than that of the non-lung cancer group. The combination of seven autoantibodies can significantly identify lung cancer patient.[4] The results are slightly different from those of R. Zhang et al[22] and Du Q et al,[23] which may be related to different selection of cases, population differences, and diverse detection methods. Although the specificity and positive predictive value of the seven autoantibodies were decreased, the sensitivity (25.42%), accuracy (71.77%), and AUC^{ROC} (0.683) were significantly increased, suggesting that the combined detection of the seven autoantibodies has higher diagnostic efficiency, which is consistent with the report of Broodman et al[24] However, the diagnostic efficiency of the seven autoantibodies reported by Dai et al[25] is significantly better than that of this study, which may be related to the differences in the subjects selected.

Positive rates of seven autoantibodies in different age, gender, pathological types, and clinical stages of lung cancer patients were calculated. The difference of positive rates in different pathological types and clinical stages of lung cancer patients was statistically significant ($P < .05$), which was not consistent with the conclusion of

Zhao Juan, et al It may be related to the tissue type and lung cancer stage of the selected lung cancer. Most of the cases in this study are adenocarcinoma, while the cases of squamous cell carcinoma and small cell carcinoma are less. There are more cases of stage I lung cancer, but the number of stage II, III and IV lung cancer is too small. The number of samples can be extended for further study.

All in all, seven autoantibodies combined test can increase the accuracy of lung cancer diagnosis.[26] The early detection rate of lung cancer can be improved through the screening of lung cancer autoantibodies in high-risk groups, which plays a certain auxiliary role in the early diagnosis of lung cancer and has important practical significance.

ACKNOWLEDGMENT

This work was supported by Medical and Health Planned Science and Technology Project of Zhejiang Province (Grant No. 2019KY606), Medical and Health Planned Science and Technology Project of Zhejiang Province (Grant No. 2018KY158), and Nature Science Foundation of Ningbo city (Grant No.2019A610230).

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How to cite this article: Mu Y, Xie F, Sun T. Clinical value of seven autoantibodies combined detection in the diagnosis of lung cancer. *J Clin Lab Anal*. 2020;34:e23349. https://doi.org/10.1002/jcla.23349