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Research article

Clinical application of serum seven tumour-associated autoantibodies in patients with pulmonary nodules

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ABSTRACT

Background: The incidence of pulmonary nodules is increasing because of the promotion and popularisation of low-dose computed tomography (LDCT) screening for populations with suspected lung cancer. However, a high rate of false positives and concerns regarding the radiation-related cancer risk of repeated CT scanning remain major obstacles to its wide application. This study aimed to investigate the clinical value of seven tumour-associated autoantibodies (7-TAAbs) in the differentiation of malignant pulmonary tumours from benign ones and the early detection of lung cancer in routine clinical practice.

Methods: We included 377 patients who underwent both the 7-TAAbs panel test and LDCT screening, and were diagnosed with pulmonary nodules using LDCT. An enzyme-linked immunosorbent assay (ELISA) was used to measure the serum levels antibodies for P53, PGP9.5, SOX2, GAGE7, GBU4-5, CAGE, and MAGE-A1. The relationships between the positive rates of the 7-TAAbs and the patient sex, and age, and the number, size, and composition of pulmonary nodules were analysed. We then statistically evaluated the clinical application value.

Results: The positive rates of the 7-TAAbs did not correlate with sex, age, number, size, or composition of pulmonary nodules. The serum antibody level of GBU4-5 in patients with pulmonary nodules tended to increase with age; the serum antibody level of SOX2 tended to increase with nodule size and was the highest among patients with mixed ground-glass opacity (mGGO) nodules. The antibody positive rate for CAGE in female patients with pulmonary nodules was significantly higher than that in male patients ($P < 0.05$). The positive rate of GBU4-5 antibody in patients aged 60 years and above was higher than that in younger patients ($P < 0.05$). The positive rate of GAGE7 antibody in patients with pulmonary nodules sized 8–20 mm was also significantly higher than that in patients with pulmonary nodules sized less than 8 mm ($P < 0.01$). Significant differences were observed in the GAGE7 antibody levels of patients with pulmonary nodules of different compositions ($P < 0.01$). The positive rate of the 7-TAAbs panel test in patients with lung cancer was significantly higher than in patients with pulmonary nodules ($P < 0.01$). Serum levels of P53, SOX2, GBU4-5, and MAGE-A1 antibodies were significantly higher in patients with lung cancer than in those with pulmonary nodules ($P < 0.05$).

Conclusion: The low positive rates of serum 7-TAAbs in patients with lung cancer and pulmonary nodules may be related to different case selection, population differences, geographical

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differences, different degrees of progression, and detection methods. The combined detection of 7-TAAbs has some clinical value for screening and early detection of lung cancer.

1. Introduction

Lung cancer is one of the most common and fatal cancers worldwide, and its incidence has increased in recent years. Tobacco smoking is estimated to cause up to 90 % of cases, and other recognised risk factors include passive smoking, emphysema, occupational exposure, and asbestos exposure [1,2]. According to global statistics concerning cancer, in 2020, there were approximately 2.2 million new lung cancer cases and 1.8 million deaths from lung cancer worldwide [3]. As the second most common cancer and the main cause of cancer-related deaths, it accounts for approximately one-tenth of the cancers diagnosed (11.4 %) and one-fifth of the deaths (18.0 %). Because the clinical characteristics of lung cancer are not obvious in the early stages, 70–80 % of lung cancer patients are diagnosed in the middle or late stages. In most countries, the 5-year survival rate of patients with lung cancer after diagnosis ranges from 10 % to 20 %. The postoperative 5-year survival rate of patients with stage 0 cancer can reach 90 %, that of patients with stage I cancer is reduced to approximately 60–70 %, and that of patients with stage IV lung cancer is less than 5 % [3,4]. Therefore, the early diagnosis and treatment of lung cancer are keys to reducing mortality rates. Early lung cancer mostly manifests as pulmonary nodules, which can be solitary or multiple focal, quasi-circular, high-density solid, or sub-solid lung shadows with a diameter of no more than 3 cm, without atelectasis, hilar lymph node enlargement, or pleural effusion on imaging [5].

The common diagnostic methods for lung cancer include X-ray imaging, cancer cytology screening, pleural effusion examination, mediastinoscopy, thoracoscopy, low-dose computed tomography (LDCT), magnetic resonance imaging (MRI), tissue puncture biopsy and pathological section examination [6,7]. Imaging is the most used method of lung cancer screening. In recent years, with the wide application of LDCT in screening for lung cancer at an early stage and the gradual popularisation of multidetector CT (MDCT), an increasing number of pulmonary nodules have been detected, and the detection rate of ground-glassnodules (GGN) has increased significantly [8,9]. Benign lesions account for the majority of these pulmonary nodules; however, there are a small proportion of malignant lesions, including early lung cancer. Although LDCT has a sensitivity of >90 % for pulmonary nodules, it lacks sufficient accuracy to distinguish benign nodules from early-stage lung cancer. As a result, there is a high rate of false-positive nodules and unnecessary follow-up or surgery [10]. Even in high-risk patient groups undergoing lung cancer screening, the cancer detection rate is only 3.8 % [11]. The incidence was also low in lung nodules with a high probability of cancer, such as Lung-RADS 3, 4A, and 4B, with incidence rates of 3.9 %, 15.5 %, and 36.3 %, respectively. Therefore, lung nodules can undergo unnecessary workups [12].

In clinical practice, tumour markers such as carcinoembryonic antigen (CEA), human cytokeratin 21-1 fragment (CYFRA21-1), squamous cell carcinoma antigen (SCCA), and neuron-specific enolase (NSE) are commonly used in the diagnosis of lung cancer. However, these markers are mainly used for treatment efficacy and prognosis monitoring. The sensitivity and specificity of their use for auxiliary diagnosis of early lung cancer do not meet the requirements of clinical diagnosis [10,13]. Lung biopsy and bronchoscopy are invasive examinations with a low positive diagnosis rate [14]. Therefore, it is necessary to develop a non-invasive examination method with high sensitivity and specificity to effectively identify high-risk individuals with early lung cancer.

In recent years, tumour-associated autoantibodies (TAAbs) have shown good diagnostic value in lung cancer-related research and have been approved for early diagnosis of lung cancer in some European and American countries [15]. Zhang et al. [16] found that the serum levels of p53, SOX2, GAGE7, and MAGE-A1 were significantly increased in patients with non-small cell lung cancer (NSCLC), PGP 9.5 was highly expressed in lung squamous cell carcinoma, and GBU4-5 was highly expressed in lung adenocarcinoma. Ren et al. [17] used 7-TAAbs and LDCT to screen for early lung cancer in high-risk populations in China; the sensitivity and specificity of lung cancer diagnosis were 61 % and 90 %, respectively. The combination of 7-TAAbs and LDCT significantly improved the diagnostic accuracy of GGN and/or solid nodule (SN), which has the potential for further clinical applications. However, few studies have reported the diagnostic value of tumour autoantibodies in patients with stages 0–IV lung cancer.

In this study, the diagnostic value of autoantibodies in malignant pulmonary nodules was investigated by detecting the levels of TAAbs in patients with pulmonary nodules. The diagnostic efficacy and correlation between 7-TAAbs and tumour markers were analysed.

2. Materials and methods

2.1. Patients and samples collection

A total of 377 outpatients or inpatients who visited the Department of Respiratory Medicine and Cardiothoracic Surgery of our hospital from April 2021 to August 2022 were included, consisting of 199 males and 178 females aged between 15 and 85 years (median 55.00 years, interquartile range 49.00, 64.00). The enrolled patients were all diagnosed with pulmonary nodules in accordance with the Chinese Guidelines for Classification, Diagnosis, and Treatment of Pulmonary Nodules [18]. The lesions of some patients were confirmed to be pulmonary malignant tumours by clinical diagnosis, imaging, and pathology. Ethical approval was granted by the Affiliated Chaohu Hospital of the Anhui Medical University Ethics Committee (no. KYXM-202103-008).

On admission, a 5 mL sample of fasting venous blood was collected in heparin anticoagulant tubes for the detection of autoantibodies to seven lung cancer-related antigens, P53, SOX2, GAGE7, GBU4-5, CAGE, PGP9.5, MAGE-A1, and tumour markers CEA and alpha-fetoprotein (AFP).

2.2. CT imaging analysis

In this study, pulmonary nodules were diagnosed using CT scans, and the size of the largest non-calcified nodule was recorded. According to the size (maximum diameter) of the nodules detected by CT, they were classified into four categories: (I) nodule-free or less than 5 mm, (II) from 5 to 7 mm, (III) from 8 to 10 mm, and (IV) 10 mm or greater.

2.3. Seven lung cancer-related antibody detection

The presence of seven lung cancer-related autoantibodies was detected by enzyme-linked immunosorbent assay (ELISA) (Hangzhou Kaibaoluo Biotechnology Co., Ltd), and the operation steps were performed according to the manufacturer's instructions. The reference ranges were as follows, P53: 0.0–13.1 U/mL, SOX2: 0.0–10.3 U/mL, GAGE7: 0.0–14.4 U/mL, GBU4-5: 0.0–7.0 U/mL, CAGE: 0.0–7.2 U/mL, PGP9.5: 0.0–11.1 U/mL, and MAGE-A1: 0.0–11.9 U/mL. If the level of any of the 7-TAAbs exceeded the reference range, it was considered positive.

2.4. Detection of tumour markers CEA and AFP

The widely used tumour markers CEA and AFP were detected using an electrochemiluminescence immunoassay (Roche Diagnostics, Basel, Switzerland). According to the kit instructions, the reference ranges were as follows, CEA: 0.0–5.0 ng/mLand AFP: 0.0–10.0 ng/mL. If the level of the tumour marker exceeded the reference range, it was considered positive.

2.5. Statistical analysis

All data were analysed using SPSS 25.0 statistical software package. The count data were analysed by χ^2 test, the measurement data of normal distribution were expressed by mean ± SD, and the comparison between groups was performed by the last significant difference (LSD) *t*-test. When the measurement data showed a non-normal distribution, they were expressed as median and interquartile interval, and the comparison between groups was performed by the Mann–Whitney U rank test and the Kruskal–Wallis test. Correlation analysis was performed using the Spearman test. All statistical analyses were two-tailed, and $P < 0.05$ indicated that the difference was statistically significant.

3. Results

3.1. Basic characters in patients with pulmonary nodules

The clinical data of 377 patients with pulmonary nodules were analysed, and no difference was found in the positive rates of serum 7-TAAbs among the different clinical characteristics (sex, age, nodule number, nodule size, and nodule nature) ($P > 0.05$). The positivity rates for serum 7-TAAbs in patients with pulmonary nodules tended to increase with increasing age and nodule size (Table 1).

Table 1
The distributions of demographical characteristics in 377 patients with pulmonary nodules.

Characteristics	n	Seven lung cancer related antibody [n (%)]		χ^2	*P*-value	OR (95%CI)
		+	–			
Sex						
Male	199	31 (15.58)	168 (84.42)	0.175	0.676	1.129 (0.638–1.998)
Female	178	25 (14.04)	153 (85.96)			
Age, years						
<60	263	35 (13.31)	228 (86.69)	1.644	0.200	0.680 (0.376–1.229)
≥60	114	21 (18.42)	93 (81.58)			
Number of nodules						
1	174	27 (15.52)	147 (84.48)	0.112	0.737	1.102 (0.624–1.945)
>1	203	29 (14.29)	174 (85.71)			
Nodule size (mm)						
≤8 mm	279	38 (13.62)	241 (86.38)	1.302	0.522	0.563 (0.553–0.573)
8–20 mm	77	14 (18.18)	63 (81.82)			
>20 mm	21	4 (19.05)	17 (80.95)			
Composition						
Solid nodule	263	38 (14.45)	225 (85.55)	0.263	0.877	0.895 (0.889–0.901)
Pure GGO	78	13 (16.67)	65 (83.33)			
Mix GGO	36	5 (13.89)	31 (86.11)			

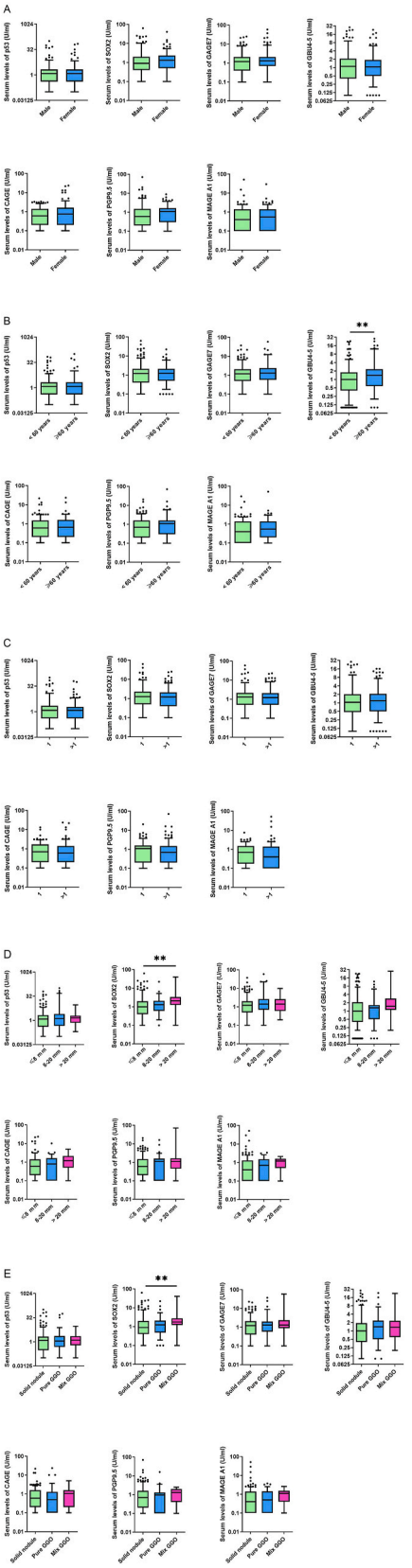

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Fig. 1. Comparison of 7-TAAbs levels in patients with pulmonary nodules. The serum concentration levels of p53, SOX2, GAGE7, GBU4-5, CAGE, PGP9.5 and MAGE A1among different clinical characteristics, including sex (A), age (B), nodule number (C), nodule size (D), nodule nature (E) respectively. **represents $p < 0.01$.

3.2. Comparison of 7-TAAbs levels in patients with pulmonary nodules

The serum autoantibody levels for GBU4-5 in patients with pulmonary nodules tended to increase with age ($Z = -2.698$, $P = 0.007$) (Fig. 1B), and the serum autoantibody levels for SOX2 tended to increase with nodule size ($F = 12.307$, $P = 0.002$) (Fig. 1D). In addition to nodule size, the serum autoantibody levels for SOX2 were the highest among patients with mGGO nodules, followed by patients with pure ground-glass opacity (pGGO) nodules, and patients with solid nodules had the lowest serum levels ($F = 11.792$, $P = 0.003$) (Fig. 1E). However, no difference was found in the serum levels of other autoantibodies in patients with pulmonary nodules with different clinical characteristics, including sex, age, and number, size, and composition of nodules (Fig. 1A–E). The median age of male patients [56.0 years (50.0, 64.0)] was higher than that of female patients [53.5 (47.0, 62.3) years] ($Z = -2.201$, $P = 0.028$). Moreover, the number of nodules was associated with age. The median age of patients with more than one nodule [56.0 (51.0, 66.0) years] was higher than that of patients with only one nodule [53.0 (46.0, 60.3) years] ($Z = -2.972$, $P = 0.003$). The nodule size was also associated with age. Patients were divided into three groups according to the maximum diameter of the nodules: no more than 8 mm, 8–20 mm, and greater than 20 mm. The median age of the patients for these three groups was 54.0 (47.0, 60.0), 57.0 (50.0, 67.5), and 70.0 (58.5, 75.0) years, respectively. The patients with larger nodules were generally older than those with smaller nodules ($F = 30.370$, $P = 0.000$). However, age was not associated with nodule composition ($F = 4.271$, $P = 0.118$).

3.3. Comparison of positive rates of 7-TAAbs in patients with pulmonary nodules with different clinical characteristics

There were sex differences in CAGE autoantibody presence among patients with pulmonary nodules, with a higher positive rate in females than in males ($P < 0.05$). The autoantibody positive rate for GBU4-5 in patients aged 60 years and above was higher than that in younger patients ($P < 0.05$). The positive rate of GAGE7 autoantibody in patients with lung nodules 8–20 mm was also significantly higher than that in patients with nodules less than 8 mm in diameter ($P < 0.01$).

There were significant differences in GAGE7 autoantibody in patients with pulmonary nodules with different components ($P < 0.01$). The positivity rate for GAGE7 in pulmonary nodules with mGGO (mixed ground-glass turbidity) was significantly higher than that in pulmonary nodules with pGGO (pure ground glass turbidity) and solid nodules. The positivity rate for GAGE7 in patients with pulmonary nodules (pGGO) was significantly higher than that in patients with solid nodules. However, there were no differences in the positivity rates of other autoantibodies among patients with lung nodules with different clinical characteristics, including sex, age, number, size, and composition of the nodules (Table 2).

3.4. Comparison of the positive rates of 7-TAAbs in patients with pulmonary nodules

The positive rates of P53 and SOX2 autoantibodies were higher than those of PGP9.5, and MAGE-A1 in the serum specimens of patients with pulmonary nodules ($P < 0.05$, $P < 0.05$, respectively), and the positive rate of GBU4-5 autoantibody was higher than those of GAGE7, CAGE, PGP9.5, and MAGE-A1 ($P < 0.05$, $P < 0.01$, $P < 0.01$, and $P < 0.001$, respectively). The differences in the positivity rates for the other antibodies were not statistically significant (Table 3).

3.5. Comparison of 7-TAAbs in lung cancer and pulmonary nodules patients

Among the 377 patients with pulmonary nodules, forty-seven cases were diagnosed with lung cancer. The positive rate of 7-TAAbs was 13.03 % (43/330) in 330 patients with only pulmonary nodules and 27.66 % (13/47) in 47 patients with lung cancer ($\chi^2 = 6.962$, $P = 0.005$). Serum levels of autoantibodies for P53, SOX2, GBU4-5, and MAGE-A1 in patients with lung cancer were significantly higher than those in patients with only pulmonary nodules ($P < 0.05$, $P < 0.01$, $P < 0.05$, and $P < 0.01$, respectively; Fig. 2A, B, D and G). Additionally, the median age of patients with lung cancer [64.0 (53.0, 72.0) years] was higher than that of patients with only pulmonary nodules [55.0 (48.0, 61.0) years] ($Z = -3.935$, $P = 0.000$). Of the 47 patients with lung cancer, 22 were male and 25 were female; of the 330 patients with only pulmonary nodules, 177 were male and 153 were female. Therefore, there was no difference in sex composition between the two groups of patients ($\chi^2 = 0.770$, $P = 0.380$).

3.6. Comparison of the positive rates of 7-TAAbs and tumour markers in patients with pulmonary nodules

Among the 377 patients with pulmonary nodules, only 109 cases were tested for CEA. In these 109 cases, the positive rates for CEA and 7-TAAbs were 9.17 % (10/109), and 15.60 % (17/109), respectively. The positive rates of 7-TAAbs were higher than those of CEA, but the difference showed no statistical significance ($\chi^2 = 2.070$, $P = 0.150$). Eighty-eight of the 377 patients were tested for AFP; all were negative. In these patients, the positive rate for 7-TAAbs was 13.64 % (12/88), which was higher than that of AFP ($\chi^2 = 12.878$, $P = 0.000$).

Table 2
Comparison of positive rates of 7-TAAbs in patients with pulmonary nodules with different clinical characteristics.

Characteristics		p53		SOX2		GAGE7		GBU4-5		CAGE		PGP9.5		MAGE A1	
		Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Sex															
Male	199	6	193	9	190	3	196	14	185	0	199	4	195	2	197
Female	178	6	172	3	175	4	174	6	172	6	172	0	178	1	177
χ^2		0.039		2.454		0.022		2.511		4.834		1.955		0.000	
P-value		0.844		0.117		0.882		0.113		0.028		0.162		1.000	
Age, years															
<60	263	7	256	10	253	4	259	10	253	4	259	2	261	2	261
≥60	114	5	109	2	112	3	111	10	104	2	112	2	112	1	113
χ^2		0.310		0.520		0.101		3.910		0.000		0.101		–	
P-value		0.578		0.471		0.750		0.048		1.000		0.751		1.000	
Number of nodules															
1	174	8	166	7	167	5	169	10	164	2	172	1	173	0	174
>1	203	4	199	5	198	2	201	10	193	4	199	3	200	3	200
χ^2		2.099		0.740		0.944		0.126		0.049		0.122		1.058	
P-value		0.147		0.390		0.331		0.723		0.824		0.727		0.304	
Nodule size Largest diameter (mm)															
≤8 mm	279	7	272	9	270	2	277	15	264	5	274	2	277	3	276
8–20 mm	77	5	72	1	76	5	72	3	74	1	76	1	76	0	77
>20 mm	21	0	21	2	19	0	21	2	19	0	21	1	20	0	21
χ^2		3.840		3.629		11.471		1.051		0.453		3.096		1.062	
P-value		0.147		0.163		0.003		0.591		0.797		0.213		0.588	
Composition															
Solid nodule	263	9	254	9	254	2	261	15	248	3	260	3	260	3	260
Pure GGO	78	3	75	2	76	2	76	4	74	3	75	1	77	0	78
Mix GGO	36	0	36	1	35	3	33	1	35	0	36	0	36	0	36
χ^2		1.344		0.165		10.235		0.546		3.455		0.438		1.311	
P-value		0.511		0.921		0.006		0.761		0.178		0.803		0.519	

Table 3
Comparison of positive rates of 7-TAAbs in patients with pulmonary nodules.

pulmonary nodulespatients (n)	p53		SOX2			GAGE7		GBU4-5		CAGE		PGP9.5		MAGE A1	
	Positive	Negative	Positive	Negative		Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
377	12	365	12	365		7	370	20	357	6	371	4	373	3	374
χ^2	–		0.000			1.350		2.089		2.049		4.087		5.510	
P-value	–		1.000			0.245		0.148		0.152		0.043		0.019	
χ^2	–		–			–		6.492		0.078		0.830		1.622	
P-value	–		–			–		0.011		0.780		0.362		0.203	
χ^2	–		–		–	–		–		7.808		11.017		12.961	
P-value	–		–		–	–		–		0.005		0.001		0.000	
χ^2	–		–		–	–		–		–		0.405		0.450	
P-value	–		–		–	–		–		–		0.524		0.502	
χ^2	–		–		–	–		–		–		–		0.000	
P-value	–		–		–	–		–		–		–		1.000	

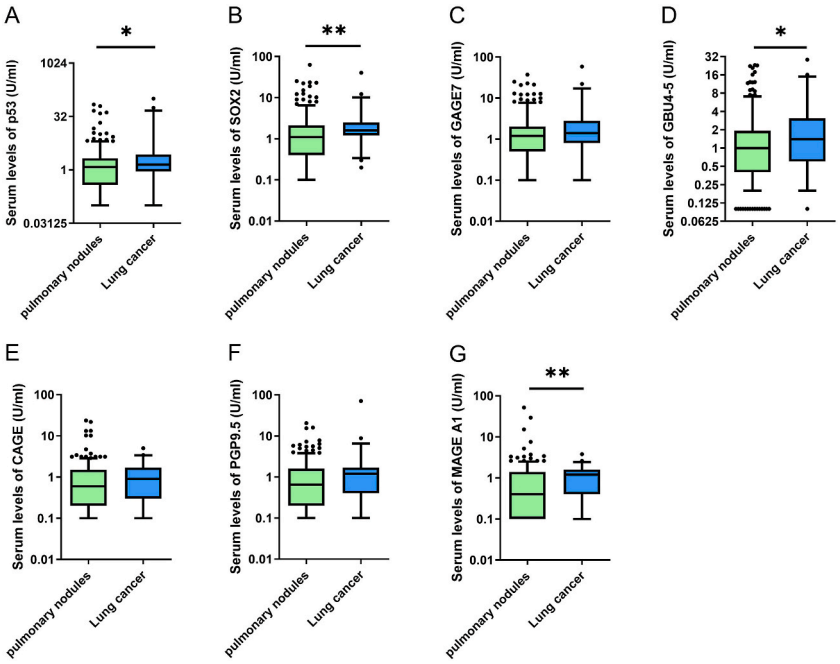

Fig. 2. Comparison of serum levels of 7-TAAbs in patients with lung nodules and lung cancer. The serum concentration levels of p53 (A), SOX2 (B), GAGE7 (C), GBU4-5 (D), CAGE (E), PGP9.5 (F) and MAGE A1 (G) in patients with lung nodules and lung cancer, respectively. * represents $p< 0.05$; ** represents $p < 0.01$.

3.7. Correlation between 7-TAAbs and tumour markers in patients with pulmonary nodules

Serum autoantibody levels for GAGE7, GBU4-5, and CAGE in patients with pulmonary nodules were all correlated with the expression levels of the tumour marker CEA ($P < 0.05$, $P < 0.01$, and $P < 0.05$, respectively), whereas the serum autoantibody levels of P53, SOX2, PGP9.5 and MAGE-A1 were not correlated with CEA levels. There was no correlation between the levels of 7-TAAbs and the tumour marker AFP in patients with pulmonary nodules (Table 4).

4. Discussion

With the rapid development of medical imaging technology and the continuous improvement in health awareness, LDCT is gradually being accepted by patients as an early screening method for lung cancer. Pulmonary nodules refer to single or multiple spherical lesions located in the lung parenchyma, with clear boundaries and a diameter less than or equal to 30 mm, and may be benign or malignant. Benign pulmonary nodules include sarcoidosis, inflammatory pseudotumours, inflammatory lesions, tuberculomas, and benign tumours, whereas malignant pulmonary nodules usually refer to lung cancers. Low-dose CT has a 96.4 % false-positive rate for pulmonary nodules (<8 mm), and its low specificity makes it difficult to distinguish between benign and malignant lesions, especially in patients with simple GGO and solid pulmonary nodules. In addition, the aetiology of pulmonary nodules is complex, the lesions are small, and the clinical manifestations lack specificity. Moreover, conventional methods such as tracheoscopy and percutaneous lung puncture cannot be used to obtain pathological results [5,19,20]. Therefore, there is an urgent need to develop a non-invasive, convenient, and economical method to distinguish between benign and malignant pulmonary nodules.

In patients with lung cancer, tumour cells can express small amounts of abnormal proteins, such as tumour-specific antigens, which

Table 4
Correlation between 7-TAAbs and tumor markers in patients with pulmonary nodules.

Indicators	CEA (n = 109)		AFP (n = 88)	
	r	P	r	P
p53	0.153	0.111	0.030	0.784
SOX2	0.156	0.106	0.045	0.677
GAGE7	0.220	0.021	0.133	0.217
GBU4-5	0.278	0.003	0.120	0.264
CAGE	0.220	0.022	0.125	0.245
PGP9.5	0.145	0.133	−0.043	0.693
MAGE A1	0.147	0.127	0.083	0.443

are specific protein products released, shed, or secreted after cell necrosis during tumour occurrence and development. They are tumour-derived and closely related to biological processes, such as the cell cycle, signal transduction, proliferation, and apoptosis [21–23]. These tumour antigens can be immunogenic through a variety of mechanisms, including: (1) protein over-expression, mutation, folding error, or inappropriate degradation; (2) abnormal sites of expression; for example, antigens usually only expressed in human germ cells (such as testis and ovarian blastocyst) may be abnormally expressed in various tumours (such as MAGE and CAGE); (3) post-translation modifications of proteins, such as glycosylation, phosphorylation, oxidation, and cleavage, may lead to new epitopes or enhance the display of their own epitopes; (4) heterotopic expression of proteins, such as the intracellular proteins of tumour cells being secreted to the extracellular space or relocated to the cell surface [17,24–26]. In the early stages of a tumour, the amount of tumour-associated antigens in the blood is very small, and it is difficult to carry out accurate quantitative analysis. Consequently, the early diagnosis of tumours by testing for tumour-associated antigens is difficult. Tumour-associated autoantibodies are products of the humoral immune response to tumour-associated antigens. They usually appear in the serum earlier than tumour-associated antigens, and their structures are more stable, especially at low tumour loads [24]. When the concentration of tumour-associated antigens in serum is very low, owing to the immune amplification effect, autoantibodies can appear at high levels, and are easy to detect [26]. Moreover, collecting blood samples to test for autoantibodies is minimally invasive, and serum levels are relatively stable, with little hourly or daily fluctuations, greatly simplifying the sample collection process. Therefore, autoantibodies have an advantage over tumour-associated antigens in the early diagnosis of cancer.

With the development of phage display technology, protein microarrays, and other new and effective high-throughput screening technologies, an increasing number of TAAbs have been identified in patients with tumours. These autoantibodies are not only tumour-specific, but are also related to the early diagnosis and prognosis of the disease. P53, SOX2, PGP9.5, NY-ESO-1, CAGE, GAGE-7, MAGE-A1, and GBU4-5 are tumour-related antigens found in patients with early lung cancer. Antibodies against these autoantigens can be used as tumour biomarkers. Compared with traditional tumour markers such as CEA, NSE, SCCA, and CYFRA21-1 [17,27,28], these autoantibodies have higher specificity and sensitivity and can be detected at the early stage of lung cancer, such as when the tumour presents as a small pulmonary nodule. Some studies have found that the detection of lung cancer in the early stages is approximately five years earlier than that of imaging examinations. In addition, the half-life of autoantibodies in the blood is longer than that of the antigen itself, making them easier to detect [25]. Therefore, tumour autoantibodies are expected to be effective biomarkers for the differentiation of benign and malignant pulmonary nodules.

In this study, 7-TAAbs were analysed in 377 patients diagnosed with pulmonary nodules by LDCT examination. There was no difference in the serum 7-TAAbs positive rates among patients with pulmonary nodules based on sex, age, nodule number, nodule size, and nodule nature ($P > 0.05$). However, the positivity rate increased with age and nodule size (Table 1). There was no difference in the positive rates of serum autoantibodies among the groups of solid, pGGO, and mGGO nodules. The positivity rate increased with increasing nodule size, which is consistent with the results of Xu et al. [29]. Zhang et al. [16] and Ouyang et al. [30] reported that the positivity rates of 7-TAAbs were not affected by age or sex, which is consistent with the results of this study. In general, the malignancy risk of pulmonary nodules is related to their diameter, with larger nodules exhibiting a higher risk of malignancy. It has been reported that the risk of malignant tumours is only 0.4 % for pulmonary nodules with a diameter of less than 5 mm, whereas the risk increases to 1.3 % for those with a diameter of 5–10 mm [31]. When the diameter of the nodule was 10–20 mm, the risk of malignant tumours increased significantly from 33 % to 60 %, and from 64 % to 82 % when the nodule was greater than 20 mm. Therefore, the diameter of pulmonary nodules is a major factor to consider in deciding on treatment [5,32]. In one study, the serum samples of 296 patients with pulmonary nodules were analysed with 7-TAAbs, showing that patients with positive results had more than double the risk of cancer compared to those with negative nodules of 4–20 mm [33].

We analysed the relationship between serum autoantibody levels for P53, PGP9.5, SOX2, GAGE7, GBU4-5, MAGE-A1, and CAGE in 377 patients with pulmonary nodules and the clinical characteristics of sex, age, number, size, and composition of pulmonary nodules. In this study, the serum GBU4-5 antibody levels increased with age in patients with pulmonary nodules. The serum antibody level of SOX2 increased with an increase in pulmonary nodules and was highest in patients with mGGO nodules, followed by patients with pGGO nodules, and lowest in patients with solid nodules (Fig. 1). In addition, the 199 male patients with pulmonary nodules were older than the 178 female patients. Both the number and size of the pulmonary nodules correlated with patient age. The patients with more than one pulmonary nodule were older than those with only one pulmonary nodule. The age of patients with pulmonary nodules greater than 20 mm was higher than that of patients with pulmonary nodules of 8–20 mm and $\leq$8 mm. The age of patients with pulmonary nodules of 8–20 mm was higher than that of patients with pulmonary nodules of $\leq$8 mm. The nature of the pulmonary nodules was independent of age.

The relationship between the positivity rate of a single antibody (for P53, PGP9.5, SOX2, GAGE7, GBU4-5, MAGE-A1, and CAGE) and clinical characteristics, such as sex, age, number, size, and composition of pulmonary nodules, was also analysed. The positive rate of CAGE antibody in women with pulmonary nodules was significantly higher than that in men ($P < 0.05$). The positive rate of GBU4-5 antibody in patients with pulmonary nodules aged >60 years was higher than that in patients with pulmonary nodules aged <60 years ($P < 0.05$). The positive rate of GAGE7 antibody in patients with pulmonary nodules of 8–20 mm was significantly higher than that in patients with pulmonary nodules of less than 8 mm ($P < 0.01$). The positive rate of GAGE7 in patients with mGGO pulmonary nodules was significantly higher than that in those with pGGO and solid pulmonary nodules, whereas the positive rate of GAGE7 in patients with pGGO pulmonary nodules was significantly higher than that in those with solid pulmonary nodules ($P < 0.01$) (Table 2). In addition, the positive rates of P53 and SOX2 single antibodies in patients with pulmonary nodules were higher than those of PGP9.5 and MAGE-A1 antibodies. The positive rate for GBU4-5 autoantibody was higher than that for GAGE7, CAGE, PGP9.5, and MAGE-A1 (Table 3). Pulmonary nodules can be divided into solid and sub-solid nodules according to their density. Subsolid nodules, also known as GGN, can be divided into pGGO and mGGO nodules based on the presence of solid components. GGN have a higher risk of

malignancy than solid nodules, whereas mGGO nodules have the highest malignant frequency (63 %), followed by pGGO nodules (18 %), and the malignancy rate of solid nodules is only 7 % [5]. In addition, it has been reported that when the proportion of solid components in mGGO nodulesis larger or when the solid component gradually increases, there is a higher probability of malignancy [32].

Of 377 patients with pulmonary nodules, 47 were diagnosed with lung cancer. The positive rate of 7-TAAbs was 27.66 %, which was significantly higher than that of the 330 patients with pulmonary nodules (13.03 %). The serum antibody levels for P53, SOX2, GBU4-5, and MAGE-A1 in patients with lung cancer were significantly higher than those in patients with pulmonary nodules (Fig. 2). These results are consistent with previous reports [16,34–36]. In addition, the age of the patients with lung cancer was significantly higher than that of the patients with pulmonary nodules. In this study, the positive rates of 7-TAAbs in patients with lung cancer and lung nodules were lower than those reported by Li et al. [26] and Hu et al. [37]. We believe that the differences in the sensitivity and specificity of combined detection for the diagnosis of lung cancer may be related to different case selections, population differences, geographical differences, different progression of lung cancer, and detection methods [36]. Second, owing to the heterogeneity between tumours, different individuals may have different tumour-related autoantibody profiles. Although tumour-associated antigens can activate immune responses during the cancer immune editing process, these reactions may not be very strong in patients with inert lung cancer and may be affected by treatment. Due to the complexity and heterogeneity of lung cancer and the expression of tumour-associated antigens, the diagnostic effect of individual tumour antibodies is usually limited, even for the same cancer subtype [6,38]. Although some studies have reported that autoantibodies are effective for the early diagnosis of lung cancer, the test method used in this study was an ELISA, which has a low detection flux, narrow linear range, low precision, and it is difficult to increase the number of indicators, limiting further improvement of its sensitivity and specificity [6]. In addition, most of the 47 patients with lung cancer in this study had adenocarcinomas, with few cases of squamous cell carcinoma and small cell carcinoma, which may explain the low positive rates for the 7-TAAbs test.

In this study, 109 of 377 patients with pulmonary nodules were also tested for CEA, with a positive rate for the 7-TAAbs of 15.60 %, which was slightly higher than the CEA positive rate of 9.17 %. Similarly, 88 of the 377 patients with pulmonary nodules were also tested for AFP, and all were negative, while the positive rate of the 7-TAAbs test was 13.64 %. We further analysed the relationship between serum antibody levels of P53, PGP9.5, SOX2, GAGE7, GBU4-5, MAGE-A1, and CAGE and the tumour markers CEA and AFP in patients with pulmonary nodules. Serum levels of GAGE7, GBU4-5, and CAGE antibodies in patients with pulmonary nodules were correlated with CEA levels, whereas the serum levels of P53, SOX2, PGP9.5, and MAGE-A1 antibodies were not. There was no correlation between the serum levels of 7-TAAbs and AFP in patients with pulmonary nodules.

Liquid biopsy is a technique that uses human body fluids as a specimen source to detect and obtain tumour-related information. Compared with traditional invasive tissue biopsy, liquid biopsy has advantages such as good compliance, easy specimen acquisition, and good specificity. More importantly, it can effectively overcome tumour heterogeneity and achieve accurate tumour-assisted diagnosis, real-time monitoring, therapeutic efficacy evaluation, and prognosis judgment [39,40]. Liquid biopsy mainly detects circulating tumour DNA (ctDNA), circulating tumour cells (CTC), and exosomes, with ctDNA and CTC being the two liquid biopsy targets that have received the most attention [39,41]. ctDNA is a tumour marker with broad application prospects, high sensitivity, and high specificity, and is suitable for various tumours, providing an opportunity for non-invasive sampling of tumours [42]. Because of the small amount of ctDNA in the blood, which only accounts for 1 % or even 0.01 % of the circulating cell-free DNA (cfDNA), ctDNA entering the bloodstream is susceptible to the influence of tumour site, size, metastasis, vascular infiltration, and tumour stage, resulting in significant measurement differences [40,43,44]. However, with the rapid development of gene sequencing, it has become possible to detect and quantify it in the blood. Recent studies based on cfDNA methylation or ctDNA gene sequencing have achieved good results in distinguishing benign and malignant pulmonary nodules [42–45]. Therefore, ctDNA gene sequencing is of great significance for the discovery of tumour traces in the early or pre-cancerous stages and provides an opportunity for tumour treatment. Early tumour gene detection based on ctDNA will be a future research and development direction for tumour disease prevention and treatment [39,44].

5. Conclusion

The results indicated that the combined detection of 7-TAAbs had certain clinical significance in the differential diagnosis of benign and malignant lung nodules and high specificity and sensitivity, which could compensate for the deficiency of the high false positive rate of LDCT imaging. Therefore, this study suggests that the combination of lung cancer autoantibody detection and LDCT examination can be considered an early lung cancer screening program that can be widely used in high-risk patient groups to improve the detection rate of early lung cancer. However, the relationship between autoantibodies and the pathological types, stages, and clinical manifestations of lung cancer still needs to be further studied and explored with a larger sample size and clinical information, or in combination with ctDNA analysis, to provide more accurate guidelines for auxiliary diagnosis, efficacy monitoring, and prognostic judgment of lung cancer in the future.

Data availability statement

The data generated during and/or analysed during the current study are available from the corresponding author.

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Availability of data and materials

All data generated or analysed during this study are included in this published article.

Ethics approval and consent to participate

The authors declare that they have obtained appropriate institutional review board approval and have followed the principles outlined in the Declaration of Helsinki for all human and animal experimental investigations. The data utilized in this study were obtained from the hospital's electronic medical record system.

Consent for publication

All authors read and approved the final manuscript.

CRediT authorship contribution statement

Kaiming Hu: Writing – original draft, Software, Methodology. **Lili Gao:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Ruyi Zhang:** Writing – original draft, Resources, Investigation. **Meiyi Lu:** Investigation, Funding acquisition, Formal analysis. **Dangui Zhou:** Software, Resources, Data curation. **Siqi Xie:** Validation, Methodology, Data curation. **Xinyue Fan:** Writing – original draft, Validation, Data curation. **Mei Zhu:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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