

A comparative study on inflammatory factors and immune functions of lung cancer and pulmonary ground-glass attenuation

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Abstract. – **OBJECTIVE:** To compare the inflammatory factors and immune functions of patients with lung cancer and pulmonary ground-glass attenuation.

PATIENTS AND METHODS: A total of 108 patients with pulmonary sarcoidosis treated in our hospital were selected and randomly divided into lung cancer group (Group A, n=32), diffuse ground-glass nodule group (Group B, n=35) and solitary ground-glass nodule group (Group C, n=41) according to the diagnosis results. Levels of inflammatory factors, tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), interleukin-6 (IL-6) and interleukin-10 (IL-10), in serum were detected via enzyme-linked immunosorbent assay (ELISA); the T-lymphocyte subset levels (CD3+, CD4+, CD8+ and CD4+/CD8+) in the immune system of patients in the three groups were detected using the flow cytometer; the levels of immunoglobulins, immunoglobulin G (IgG), immunoglobulin A (IgA) and immunoglobulin M (IgM), were detected via immunoturbidimetric assay.

RESULTS: There were no significant differences in the levels of TNF- α , IL-1 β , IL-6 and IL-10 in patients between Group A and Group B ($p>0.05$), but the levels in Group A and Group B were significantly higher than those in Group C ($p<0.05$). There were no significant differences in the levels of CD3+, CD4+, CD8+ and CD4+/CD8+ in patients between Group A and Group B ($p>0.05$), but the levels in Group A and Group B were significantly lower than those in Group C ($p<0.05$). There were no significant differences in the levels of IgG, IgA and IgM in patients between Group A and Group B ($p>0.05$), but the levels in Group A and Group B were significantly lower than those in Group C ($p<0.05$).

CONCLUSIONS: There are lower inflammation and immune functions in patients with lung cancer and pulmonary ground-glass attenuation. Compared with those in patients with lung cancer and diffuse ground-glass nodules, the inflammatory degree in patients with solitary ground-glass nodules is lower and the immune functions are better. Detecting the inflammatory

factors and immune functions of patients can also be used as a differential diagnosis means of lung cancer.

Key Words:

Lung cancer, Pulmonary ground-glass attenuation, Inflammatory factor, Immune function.

Introduction

Lung cancer is one of the most clinically common malignant tumors, and its incidence rate increases year by year due to the environmental pollution, diet, and other factors¹. With the continuous improvement of imaging technology and the enhancement of residents' awareness of physical examination, the early detection rate of lung cancer is also increasing day by day². Pulmonary ground-glass attenuations are often found in computed tomography (CT) or X-ray examination, and about 30% are the malignant tumors, which can be divided into solitary ground-glass nodules or diffuse ground-glass nodules. Under CT, it is manifested as the increased density of shadow, but the pulmonary bronchus or pulmonary vascular structure can be seen^{3,4}. Patients with lung cancer and ground-glass attenuation suffer from the chronic inflammation in lung tissues, often accompanied by systemic inflammatory response, and a variety of inflammatory factors, such as tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), interleukin-6 (IL-6) and interleukin-10 (IL-10), will participate in the lung injury and disease progression and lead to the low immune functions of the body, thus affecting the postoperative recovery of patients^{5,6}. In this study, inflammatory factors and immune functions of patients with lung cancer and pulmonary ground-glass attenuation were compared and studied, providing

the basis for the differential diagnosis and prognosis evaluation of lung cancer and pulmonary ground-glass attenuation.

Patients and Methods

Patients

A total of 108 patients with pulmonary sarcoidosis treated in our hospital from February 2016 to January 2017 was selected and randomly divided into lung cancer group (Group A), diffuse ground-glass nodule group (Group B) and solitary ground-glass nodule group (Group C) according to the diagnosis results. This study was approved by the Ethics Committee of the Affiliated Jiangyin Hospital of Southeast University. Signed written informed consents were obtained from all participants before the study. Inclusion criteria: 1) patients with lung cancer meeting the diagnostic criteria of non-small cell lung cancer of WHO; 2) patients diagnosed as solitary ground-glass nodules via imaging examination meeting the relevant diagnostic criteria of “the Sixth Chinese Lung Cancer Summit Conference”, and with the clinical symptoms, such as cough, expectoration and chest pain; 3) patients who signed the informed consent. There were no significant differences in general materials among the three groups ($p>0.05$) (Table I).

Laboratory Equipment and Reagents

Main laboratory equipment: microplate reader (Jiangsu Potebio Co., Ltd. Jiangsu, China), flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA), the specific protein instrument (Beckman Coulter, Brea, CA, USA); relevant kits: TNF- α , IL-1 β , IL-6 and IL-10 kits (Zhejiang Ikon Biology Co., Ltd. Zhejiang, China), Multiset three-color reagent (BD Biosciences, Franklin Lakes, NJ, USA), λ -type light chain assay kit and κ -type light chain assay kit (Beckman Coulter, Brea, CA, USA).

Detection of Inflammatory Factors

3-5 mL fasting venous blood was collected from patients, and the serum was extracted and stored in the refrigerator at -80°C. The levels of TNF- α , IL-1 β , IL-6 and IL-10 in serum of patients were measured via enzyme-linked immunosorbent assay (ELISA). The specific steps were as follows: 1) sample dilution: 10 standard wells were set on the ELISA plate, and 100 μ L sample was added into the 1st and 2nd wells, and 50 μ L diluted solution was added, respectively. 100 μ L sample was taken from the 1st

and 2nd wells and added into the 3rd and 4th wells, and 50 μ L diluted solution was added, respectively. Next, 50 μ L sample was taken from the 3rd and 4th wells and added into the 5th and 6th wells, and 50 μ L diluted solution was added, respectively. The sample was diluted for 5 times to obtain the diluted sample finally; 2) loading and incubation: the diluted sample was added into the sample well, incubated for 30 min at 37°C after membrane sealing; then the plate was washed for 5 times (20 s/time); 3) enzymatic incubation: 50 μ L enzyme-labeled reagent was added for incubation at 37°C for 30 min, then the plate was washed for 5 times (20 s/time); 4) color development and termination: the color developing agents A and B (50 μ L for each) were added into the standard wells for incubation at 20°C for 15 min, and then 50 μ L stop buffer was added. The optical density (OD) values were read within 15 min using the microplate reader (wavelength of 450 nm) and the levels of TNF- α , IL-1 β , IL-6 and IL-10 were calculated.

Detection of T-lymphocyte Subset Levels

3-5 mL fasting peripheral venous blood (fasting for 8 hours) was collected from patients in the early morning, EDTA-K2 was used for anticoagulation, and the number of white blood cell (WBC) in samples was controlled at $4-10 \times 10^9/L$. When $WBC < 4 \times 10^9/L$, 4 mL lymphocyte separating solution was added, followed by centrifugation at 2000 rpm for 20 min to separate the mononuclear cell; when $WBC > 10 \times 10^9/L$, phosphate buffered saline (PBS) solution was used for dilution. According to the instructions of multiset three-color kit, 5 μ L CD3-PC5/CD4-RD1/CD45-PITC and CD3-PC5/CD8-ECD/CD45-PITC were added into 2 TruCount tubes, respectively; 40 μ L sample was added into 2 TruCount tubes and mixed well, followed by incubation at 20°C for 15 min in a dark place; then, 500 μ L FACS hemolysin was added and mixed well, followed by incubation at 20°C for 15 min in a dark place; the supernatant was taken after centrifugation at 2500 rpm for 3 min, then 300 μ L 9% sodium chloride solution was added. The percentages of CD3⁺, CD4⁺ and CD8⁺ were detected using the flow cytometer, and the ratio of CD4⁺/CD8⁺ was calculated.

Immunoglobulin Detection

2-3 mL fasting peripheral venous blood (fasting for 8 hours) was collected from patients in the early morning, followed by complete agglutination and centrifugation at 2000 rpm for 10 min. The supernatant was taken and 9% sodium chlo-

Table I. Comparisons of baseline data of patients among the three groups.

Item	Group A (n=32)	Group B (n=35)	Group C (n=41)	F/ X ²	p
Gender (male/female)	20/12	22/13	26/15	0.346	0.423
Average age (years old)	57.53±6.56	56.85±6.63	57.43±6.14	0.076	0.947
MAP (mmHg)	98.56±1.43	97.93±1.56	98.43±0.63	1.550	0.258
BMI (kg/m ²)	22.34±1.46	21.93±1.35	21.52±1.21	1.061	0.172
FEV ₁ /FVC (%)	49.26±4.36	48.86±4.27	49.13±4.09	0.861	0.674

Table II. Comparisons of inflammatory factor indexes of patients among the three groups (ng/L).

Group	Case	TNF- α	IL-1 β	IL-6	IL-10
Group A	32	26.57±4.13	51.45±3.54	38.72±5.46	26.57±4.34
Group B	35	25.36±4.38 [@]	49.64±3.38 [@]	36.48±5.57 [@]	24.25±4.73 [@]
Group C	41	12.42±3.15 ^{*#}	30.37±3.06 ^{*#}	10.73±3.64 ^{*#}	8.73±3.48 ^{*#}

Note: ^{*}Compared with Group A, $p < 0.05$; [#]Compared with Group A, $p < 0.05$; [@]Compared with Group A, $p < 0.05$.

ride solution was added for dilution. According to the instructions of the specific protein instrument, immunoturbidimetric assay was performed, and the detection results of IgG, IgA and IgM levels would be automatically generated.

Evaluation Indexes

The T-lymphocyte subset levels (CD3⁺, CD4⁺, CD8⁺ and CD4⁺/CD8⁺) in the immune system of patients in the three groups were detected using the flow cytometer; the levels of immunoglobulin, immunoglobulin G (IgG), immunoglobulin A (IgA) and immunoglobulin M (IgM), were detected via immunoturbidimetric assay; the levels of TNF- α , IL-1 β , IL-6 and IL-10 in serum were detected via ELISA.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 19.0 (SPSS Inc. Chicago, IL, USA) software was used for data processing. Measurement data were presented as mean \pm standard deviation ($\bar{x} \pm s$), and t -test was used. $p < 0.05$ suggested that the difference was statistically significant.

Results

Comparisons of inflammatory factor levels in patients among the three groups: there were no significant differences in the levels of TNF- α , IL-1 β , IL-6 and IL-10 in patients between Group A and Group B ($p > 0.05$), but the levels in Group A and Group B were significantly higher than those in Group C ($p < 0.05$) (Table II). Comparisons of T-lymphocyte subset levels in patients among the three groups: there were no significant differences in the levels of CD3⁺, CD4⁺, CD8⁺ and CD4⁺/CD8⁺ in patients between Group A and Group B ($p > 0.05$), but the levels in Group A and Group B were significantly lower than those in Group C ($p < 0.05$) (Table III). Comparisons of immunological indexes of patients among the three groups: there were no significant differences in the levels of IgG, IgA and IgM in patients between Group A and Group B ($p > 0.05$), but the levels in Group A and Group B were significantly lower than those in Group C ($p < 0.05$) (Table IV).

Table III. Comparisons of T-lymphocyte subset levels of patients among the three groups.

Group	Case	CD3 ⁺	CD4 ⁺	CD8 ⁺	CD4 ⁺ /CD8 ⁺
Group A	32	62.53±5.56	36.83±5.46	19.38±3.67	1.42±0.47
Group B	35	63.48±5.32 [@]	37.47±5.72 [@]	20.15±3.65 [@]	1.43±0.38 [@]
Group C	41	70.37±5.63 ^{*#}	41.57±5.84 ^{*#}	26.78±3.72 ^{*#}	1.68±0.38 ^{*#}

Note: ^{*}Compared with Group A, $p < 0.05$; [#]Compared with Group A, $p < 0.05$; [@]Compared with Group A, $p < 0.05$.

Table IV. Comparisons Comparisons of immunological indexes of patients among the three groups

Group	Case	IgA	IgG	IgM
Group A	32	0.67±0.42	6.35±2.14	0.58±0.26
Group B	35	0.68±0.36 [@]	6.62±2.27 [@]	0.59±0.42 [@]
Group C	41	1.23±0.47 ^{*#}	8.15±2.43 ^{*#}	0.83±0.47 ^{*#}

Note: ^{*}Compared with Group A, $p<0.05$; [#]Compared with Group A, $p<0.05$; [@]Compared with Group A, $p<0.05$.

Discussion

Lung cancer is a kind of malignant tumor with a high incidence rate, and its incidence and mortality rates are increasing year by year, which has become the first malignant tumor threatening the human health in many countries⁷. Lung cancer has a poor prognosis, so the early diagnosis and treatment are particularly important, but difficulties are often encountered in its pathological diagnosis, so the differential diagnosis is required⁸. Pulmonary ground-glass attenuation is the early lesion of lung tissues, which can be either benign or malignant, so studies argue that the pulmonary ground-glass attenuation should be listed into the pre-lung cancer lesion⁹. Imaging examination is one of the main ways to find the early pulmonary ground-glass attenuation. There are many pathogenic factors of pulmonary ground-glass attenuation, including smoking (including passive smoking), air pollution and genetic factors, etc.¹⁰. Pulmonary ground-glass attenuation shows high density and clear boundary in the CT examination, and the nodules show the long cord-like and flat-shaped lesions, while the lung cancer has the burr characteristics with small burr in the border, and the nodules are different in length and thickness with radial characteristics. Imaging combined with serological diagnosis can effectively distinguish from lung cancer to pulmonary ground-glass attenuation, so as to improve the prognosis of patients via the targeted treatment¹¹. Patients with lung cancer and pulmonary ground-glass attenuation suffer from varying degrees of inflammatory response. TNF- α is a kind of soluble polypeptide cytokine secreted by monocytes/macrophages. When the body is damaged, infected or suffers from certain tumors, the body will produce a large amount of TNF- α , and its serum concentration will also be increased, thus starting and triggering inflammatory response and causing cascade reactions¹². IL-1 is a member of interleukin family, including IL-1 α and IL-1 β , in which IL-1 β is an important inflammatory cytoki-

ne that can stimulate T cells to produce various inflammatory factors¹³. IL-6 is also an important member of interleukin family. It is a kind of lymphocyte factor with multiple functions in the acute phase consisting of polypeptide glycoprotein, which plays a dual role of promoting and resisting the inflammatory response in the immune response. Its much release is a dangerous signal for lung disease patients¹⁴. Elevated level of IL-10 may cause the body in a high-reaction state of humoral immunity and its level is abnormal in various tumors¹⁵. This study showed that the levels of TNF- α , IL-1 β , IL-6 and IL-10 in patients with lung cancer and diffuse ground-glass nodules were not significantly different, but the levels of inflammatory cytokines were significantly higher than those in patients with solitary ground-glass nodules ($p<0.05$). This is because the tumor cells will activate the mononuclear cells in the infiltration and metastasis, thereby promoting the secretion of a large number of TNF- α . TNF- α , as a peptide cytokine, can effect with IL-1 β , IL-6 and IL-10 and other inflammatory mediators, causing cascade reactions and increasing the inflammatory response; diffuse ground-like nodular lesions are larger with rapid progression, often accompanied by pleural effusion and hilar lymph nodes, and severe lung tissue damage. The serological examination shows that the TNF- α , IL-1 β , IL-6 and IL-10 levels are higher, and the pathological basis of interstitial nodules is the tumor, so it is close to the level of inflammatory factors in patients with lung cancer; solitary ground-like nodular lesions are smaller with better differentiation and slow growth, accompanied with the inflammatory response, but there is no fibrosis changes and interstitial inflammatory response, so the levels of inflammatory factors are significantly lower than those of the other two, but it still cannot be ignored, and the early treatment is needed. Under the normal state, immunoglobulin remains at a low level in the body fluids. IgG is a kind of antibacterial and antiviral antibody that plays a major role in anti-infection; IgA can form a local immune sy-

stem with the surrounding cells to resist infection; IgM is a kind of high-effect antibody that can dissolve pathogenic bacteria, agglutination and phagocytosis, playing an important role in the early defense^{16,17}. The results of this study showed that there were no significant differences in IgG, IgA and IgM levels between patients with lung cancer and diffuse ground-glass nodules, but they were significantly lower than those of patients with solitary ground-glass nodules. This is because with the progress of lung disease, especially when developed into lung cancer, lung tissue damage will cause inflammatory response of the body and lead to slight infection, the immune imbalance will decrease the IgG, IgA and IgM levels, reducing the body's immune resistance. CD3⁺ in the T lymphocyte subsets is the antigen presentation on the surface of mature T cells and can assist in the identification of T cell antigen receptors. CD4⁺ is a helper T cell with immunoregulatory function; CD8⁺ T cells are mainly the cytotoxic T cells that can lead to immune dysfunction^{18,19}. The results of this study showed that CD3⁺, CD4⁺, CD8⁺, CD4⁺/CD8⁺ levels in patients with lung cancer and diffuse ground-glass nodules were not significantly different, but they were significantly lower than those of patients with solitary ground-glass nodules. This is because the level of T lymphocyte subsets is closely related to the occurrence, development and metastasis of lung cancer, and patients often suffer from immune imbalance and immune dysfunction²⁰. CD3⁺, CD4⁺ and CD8⁺ are in a balanced state in the lung microenvironment, which can normally regulate the body's cellular immune response, influenced by a variety of inflammatory cytokines, and its expression level will be changed; the more severe the inflammation is, the more greatly the balance will be damaged, and significant immunosuppression will be produced, reducing its number. With the continuous progression of lung disease, CD4⁺/CD8⁺ ratio is decreased significantly, and the induction and restriction will be imbalanced, causing the pathophysiological changes in lung tissues.

Conclusions

Patients with lung cancer and pulmonary ground-glass attenuation suffer from inflammatory response and immune dysfunction, and the inflammatory factor levels in patients with lung cancer and diffuse ground-glass nodules were significantly higher than those in patients with

solitary ground-glass nodules, and the immune function was lower. Imaging combined with serological examination can provide a better basis for the differential diagnosis of lung cancer and realize the early intervention in lung cancer lesions, which has important clinical significance.

Conflict of interest

The authors declare no conflicts of interest.

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