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Study on the Changes of Immune Factors in Different Stages of Non-Small Cell Lung Cancer Chemotherapy

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Abstract

Objective: To analyze various immune cytokines (NKG2D, IL-12, IL-15, IL-18, DC cells, TNF-a, IFN-r) and peripheral blood of patients with non-small cell lung cancer (NSCLC) at different times after chemotherapy. Changes in CD4+, CD8+, Th17 and IgG, IgM, and IgA levels. **Methods:** A total of 118 NSCLC patients who attended the Oncology Department of the Affiliated Hospital of Chengde Medical College from September 2018 to September 2021 were selected as the research objects, and the patients were analyzed at different time points (before chemotherapy, after the first chemotherapy, and after the second chemotherapy). The effects of NKG2D, IL-12, IL-15, IL-18, DC cells, TNF-A, IFN-r, CD4+, CD8+ Th17, IgG, IgM and IgA levels in peripheral blood at different time points (before chemotherapy, after the first chemotherapy and after the second chemotherapy) were analyzed. The changes of NKG2D, IL-12, IL-15, IL-18, DC cells, TNF-A, IFN-r and the levels of CD4+, CD8+ Th17, IgG, IgM and IgA in peripheral blood were compared at each time point. **Results:** NKG2D, IL-12, IL-15, IL-18, TNF-a, IFN-r gradually decreased before chemotherapy, one week after chemotherapy, and two weeks after chemotherapy, the difference was statistically significant, but DC cells were not significant Variety. CD4+ and CD8+ both increased significantly, and the levels of Th17, IgG, IgM, and IgA gradually decreased. **Conclusion:** In the course of chemotherapy, all immune factors except DC cells were significantly decreased compared with those before chemotherapy, and the decrease of immune factors except DC cells was positively correlated with the length of chemotherapy cycle. If additional immunotherapy is needed, it should be carried out in the early stage of chemotherapy.

Keywords

Non-Small Cell Lung Cancer, Chemotherapy, NKG2D, IL-12, IL-15, IL-18,

DC Cells, TNF-a, IFN-r, Immune Cytokines

1. Introduction

In recent years, the incidence of malignant tumors has gradually increased, which may be directly related to the development of medical testing and the improvement of people's living standards [1]. At the same time, the disease spectrum of malignant tumors is also changing, and the incidence of lung cancer is increasing year by year compared with other malignant tumors.

Once lung cancer is diagnosed, the guarantee of health and life is immediately threatened. Therefore, the occurrence, development and treatment of tumors are currently more popular researches. A large number of literature reports [2] [3] [4], in the process of tumor progression, most malignant tumors will cause the body's own immune mechanism to be affected. The reason may be that immunosuppressive factors are released during tumor progression, and the immune mechanism is affected. Existence is the main direction of current anti-tumor therapy, so the research on immune mechanism is particularly important.

For the treatment of tumors, chemotherapy is one of the main methods. In the course of chemotherapy, tumor cells will be killed, and the tumor's immunosuppressive effect will also be destroyed, thereby releasing or restoring the body's autoimmunity. For this, we treat non-small cell lung cancer (NSCLC) patients with chemotherapy. The expression of immune factors before and after has been studied in order to provide effective evidence support for immunosuppressive therapy against tumors. The report is as follows:

2. Object and Method

2.1. Basic Information

We selected 118 NSCLC patients who were hospitalized in the Department of Medical Oncology from September 2018 to September 2021 in the Affiliated Hospital of Chengde Medical College as the subjects of this study. Basic information is shown in **Table 1**.

Inclusion criteria: 1) Patients who were clearly diagnosed as NSCLC by pathology. 2) Patients between 18 - 80 years old. 3) Patients who have lost the

Table 1. Basic information of research objects.

Basic information	Gender		Age	Types of lung cancer			TNM staging	
	Male	Female		Lung Squamous Cell Carcinoma	Lung Adenocarcinoma	Lung Adenosquamous Carcinoma	Stage III	Stage IV
Number of cases (n)/numerical value	67	51	37 - 75 years old (58.27 ± 7.65)	27	81	10	31	87

opportunity for surgery after the assessment of the expertise in surgery, imaging, and pathology. 4) KPS score ≥ 60 points. 5) Patients whose survival period exceeds 3 months. 6) No chemotherapy contraindications.

Exclusion criteria: 1) Patients with surgical indications requiring surgery. 2) Combined with serious basic diseases, such as immune system, severe dysfunction of head, heart, lungs and abdominal organs, severe infection, blood system diseases, etc.

All research subjects were approved by the ethics committee of the Affiliated Hospital of Chengde Medical College and were enrolled and signed an informed consent form.

2.2. Research Methods

1) The chemotherapy regimen is cisplatin/carboplatin + pemetrexed/gemcitabine/paclitaxel. Dosage: pemetrexed 500 mg/m^2, gemcitabine 1250 mg/m^2, paclitaxel 175 - 200 mg/m^2, cisplatin 75 mg/m^2, carboplatin AUC = 6, intravenous drip administration, 3 weeks as a cycle.

2) All patients included in the study were given a fasting vein in the morning on the day of chemotherapy (defined as the control group), 3 weeks after the first chemotherapy (defined as the experimental group A), and 3 weeks after the second chemotherapy (defined as the experimental group B). 30 ml of blood, after drawing the blood, let it stand at room temperature, after 2 hours, after 1000 rpm/centrifugation for 20 minutes (centrifugal radius 14 cm), take the supernatant and put it in the refrigerator at −80˚C for later use. The enzyme-linked immunosorbent assay was used to detect the levels of NKG2D, IL-12, IL-15, IL-18, DC cells, TNF-a, IFN-r in serum, and each factor detection kit was purchased from Kangtai Heyuan Biotechnology Co. Ltd. Company (Beijing). Use flow cytometry to detect the ratio of CD4+ and CD8+ in CD4+, calculate CD4/CD8, and test kit (Shanghai Ruifan Biotech Co. Ltd.). Use immunoturbidimetric method to detect IgG, IgM, IgA immunoglobulin levels, kit (Shanghai Baiye Biotechnology Center).

2.3. Statistical Analysis

The statistical analysis of the study was performed using SPSS20.0 software. Count test uses t test, measurement data uses χ^2 test, test standard $\alpha = 0.05$, $P < \alpha$ has statistical significance.

3. Results

1) The expression of NKG2D, IL-12, IL-15, IL-18, DC cells, TNF-a, IFN-r before chemotherapy, one week after chemotherapy, and two weeks after chemotherapy. Comparison between the experimental group and the control group, except for DC cells. Significant changes, the other factors gradually decreased, and the difference was statistically significant. See **Table 2** for details.

2) The expression of NKG2D, IL-12, IL-15, IL-18, DC cells, TNF-a, IFN-r

before chemotherapy, one week after chemotherapy, and two weeks after chemotherapy. Comparison of the experimental group. Except DC cells, the difference of other factors was statistically significant. See Table 3 for details.

3) Comparing the levels of CD4+, CD8+ and Th17 before chemotherapy, after 1 cycle of chemotherapy and after 2 cycles of chemotherapy, CD4+ and CD8+ all increased significantly, while Th17 decreased. See Table 4 for details.

4) The levels of IgG, IgM and IgA decreased gradually before chemotherapy, one week after chemotherapy, and two weeks after chemotherapy. See Table 5 for details.

Table 2. Comparison of the expression of each factor between the experimental group and the control group.

Immune cytokine	control group	experimental group A	experimental group B	t value	P value
IFN-r	1.24 ± 0.314	0.85 ± 0.20		7.209	0.001
IFN-r	1.24 ± 0.314		0.39 ± 0.192	16.011	0.001
IL-12	10.17 ± 1.259	8.52 ± 1.078		6.899	0.001
IL-12	10.17 ± 1.259		6.28 ± 1.183	15.604	0.001
IL-15	4.40 ± 1.081	3.29 ± 1.170		4.834	0.001
IL-15	4.40 ± 1.081		1.97 ± 0.886	12.064	0.001
IL-18	136.27 ± 9.265	92.08 ± 7.960		25.068	0.001
IL-18	136.27 ± 9.265		57.58 ± 6.742	47.583	0.001
DC cell	1.57 ± 0.239	1.51 ± 0.214		1.231	0.222
DC cell	1.57 ± 0.239		1.55 ± 0.212	0.387	0.700
TNF-a	8.04 ± 0.848	5.57 ± 0.644		16.035	0.001
TNF-a	8.04 ± 0.848		3.33 ± 0.646	30.566	0.001
NKG2D	2.01 ± 0.262	1.65 ± 0.127		8.727	0.001
NKG2D	2.01 ± 0.262		1.45 ± 0.136	13.197	0.001

Table 3. Comparison of expression of each factor among experimental groups.

Immune cytokin	experimental group A	experimental group B	t value	P value
IFN-r	0.85 ± 0.20	0.39 ± 0.192	11.547	0.001
IL-12	8.52 ± 1.078	6.28 ± 1.183	9.703	0.001
IL-15	3.29 ± 1.170	1.97 ± 0.886	6.244	0.001
IL-18	92.08 ± 7.960	57.58 ± 6.742	22.913	0.001
DC cell	1.51 ± 0.214	1.55 ± 0.212	−0.930	0.355
TNF-a	5.57 ± 0.644	3.33 ± 0.646	79.007	0.001
NKG2D	1.65 ± 0.127	1.45 ± 0.136	7.279	0.001

Table 4. Comparison of CD4+, CD8+, Th17 levels in different time periods.

Grouping	CD4+ %	CD8+ %	TH17
Before chemotherapy	27.12 ± 2.32	25.23 ± 2.51	3.52 ± 0.36
After 1 cycle of chemotherapy	28.98 ± 2.67	30.89 ± 3.58	3.18 ± 0.47
After 2 cycles of chemotherapy	33.86 ± 2.81	32.34 ± 4.24	2.23 ± 0.39

Table 5. Comparison of IgG, IgM, IgA levels in different time periods.

Grouping	IgG	IgM	IgA
Before chemotherapy	12.01 ± 1.32	1.43 ± 0.21	1.32 ± 0.15
After 1 cycle of chemotherapy	11.01 ± 1.12	1.21 ± 0.14	1.06 ± 0.12
After 2 cycles of chemotherapy	10.78 ± 1.35	0.95 ± 0.13	0.91 ± 0.13

4. Discussion

Tumors in the thoracic and abdominal viscera are generally not easy to find. When clinical symptoms appear, the malignant tumors found in the examination are generally in the middle and late stages, and some of them have reached the terminal stage with rapid progress. Most of the malignant tumors discovered in this way have lost the opportunity for surgery, and NSCLC is a relatively large malignant tumor that is difficult to detect early [5]. NSCLC is a relatively common tumor in lung cancer, with an early incidence rate accounting for about 80% of the total number of lung cancers, and the lack of early specific screening methods for NSCLC. At the same time, the occurrence and development of NSCLC are relatively insidious compared with other types of lung cancer. When NSCLC was discovered, patients were already in the advanced or terminal stage of tumors. Among them, about 70% of NSCLC patients had lost the best time for treatment, and there was no opportunity for surgery [6] [7].

The current treatment for patients with advanced NSCLC is mainly chemotherapy, which can prolong part of their survival time. However, due to the strong side effects of chemotherapy, the effect of chemotherapy is not very satisfactory [8]. Therefore, it is particularly important to improve the side effects of chemotherapy or to improve the body's resistance to side effects.

At present, some literature reports that the anti-tumor effect of chemotherapy combined with immunotherapy is better than that of chemotherapy alone [9]. Some scholars believe that [10] chemotherapy can effectively increase the serum level of immunosuppressive factors to reduce the damage of malignant tumors to the immune mechanism, but there is still no report on the specific mechanism.

NKG2D, IL-12, IL-15, IL-18, DC cells, TNF-a, IFN-r factors are all important factors related to the body's immunity. The main role of NKG2D is a receptor that mediates the killing activity of NK cells. It is a member of the NKG2 series. It is also the main target for adaptability and inhibition between NK cells and tumor cells. The above effects indicate that the active state of NKG2D is the

main connection basis for NK cells to kill tumor cells, and it is also one of the foundations for maintaining the normal immune activity of the human body [11] [12]. Studies have shown that [13] the activity of NKG2D is directly and positively related to the immune function of the body, and it is also closely related to the occurrence and development of tumors. IL-12 is a cytokine secreted by antigen-presenting cells such as monocytes, macrophages and dendritic cells. It belongs to the class I cytokine receptor series. Its role is mainly to stimulate T cells and NK cells. Cell activity is enhanced to achieve effective immune killing effect. IL-15 is a cytokine secreted by macrophages. Its function is similar to IL-12. It can activate and enhance the activity of NK cells to achieve immune killing effect. Studies have found that [14] [15] when IL-12 and IL-15 are significantly increased, the activity of NK cells is significantly increased, and the expression of NKG2D is also significantly increased. IL-18 is also a member of the IL-1 family, which can induce TH1 cells to produce. It produces IFN-*γ*, which has a synergistic effect with IL-12 and IL-15. DC cells are the most powerful antigen-presenting cells, which can effectively extract, process and present antigens step by step. Immature DC cells have extremely active migration ability, and they will immediately transform into mature cells after receiving external stimuli. Mature DC cells can present antigen to the corresponding stimulating factors. It can be said that DC cells are at the center of the body's immunity. At the same time, DC cells can secrete IL-12, indicating that DC cells are closely related to the body's immune mechanism [16] [17]. TNF-a is one of the tumor necrosis factors. Current studies have shown that it is a factor that can directly kill tumor cells without affecting normal tissue cells. This factor is particularly important in anti-tumor therapy. IFN-r is an interferon secreted by T lymphocytes. It has both highly effective antiviral biological activity and extensive immunomodulatory effects. At the same time, there are literatures that [18] [19] IFN-r can effectively regulate NK cells and the communication between DC cells has a significant connection effect on the combined immunomodulatory effects of the two kinds of cells. In summary, the various factors are related to immunity and anti-tumor, and some of them have cross-effects. The expression of these factors has a greater relationship with the body's anti-toxic side effects during chemotherapy.

Our research results show that all factors other than DC cells are significantly reduced after chemotherapy, and the decline of immune factors other than DC cells is positively correlated with the length of the chemotherapy cycle, indicating that chemotherapy has significantly destroyed the body's immune mechanism, resulting in a significant decline in the body's immunity. As a result, the corresponding chemotherapy response occurred. Compared with CD4+, CD8+, Th17, CD4+, CD8+, all increased significantly, Th17 decreased, and IgG, IgM, and IgA levels decreased. This is also a manifestation of impaired immune function in patients after chemotherapy. For this situation, immunotherapy should be added in the early stage of chemotherapy, but the corresponding treatment methods need to be further studied. At the same time, the study found that DC

cells did not decrease significantly. It is possible that after chemotherapy stimulates the body, the body needs more DC cells for antigen presentation to enhance part of the body's immunity. This is the direction of our next research.

5. Conclusion

During chemotherapy, all immune factors except DC cells were significantly decreased compared with those before chemotherapy, and the decrease of immune factors other than DC cells was positively correlated with the length of the chemotherapy cycle. If immunotherapy needs to be increased, it should be carried out in the early stage of chemotherapy. Before chemotherapy, after 1 cycle of chemotherapy, and after 2 cycles of chemotherapy, CD4+, CD8+, and Th17 were significantly increased, Th17 decreased, and IgG, IgM, and IgA levels decreased. It is also a manifestation of impaired immune function of patients after chemotherapy.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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