CHANGES AND SIGNIFICANCE OF LYMPHOCYTE SUBSETS IN PATIENTS WITH ADVANCED NSCLC BEFORE AND AFTER ENDOSTAR COMBINED CHEMOTHERAPY

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ABSTRACT

Objective*: To investigate the changes in lymphocyte subsets in patients with advanced non-small-cell lung cancer (NSCLC) before and after the treatment using endostar combined with chemotherapy.*

Methods: *Eighty-six patients with advanced NSCLC who were treated in the oncology department of our hospital from June 2018 to February 2019 were randomly selected and divided into a control group and study group using the random-number expression method, with 43 patients in each group. The control group was treated using chemotherapy (docetaxel + carboplatin). The patients in the study group were treated with endostar on the basis of the control group, and both groups were treated for 6 weeks. The clinical efficacy of the two groups was evaluated. The levels of vascular endothelial growth factor (VEGF), matrix metalloproteinase-2 (MMP-2), and matrix metalloproteinase-9 (MMP-9) were measured using ELISA. The lymphocyte subsets (CD_3^+, CD_4^+, and CD_4^+/CD_8^+) and natural killer cells (NK) were measured by flow cytometry. The adverse reactions of the two groups were compared.*

Results: *After treatment, the objective effective rate and disease-control rate of the two groups were 65.12% and 81.39%; 83.72% and 95.35%, respectively, which were significantly higher in the study group than in the control group ($P<0.05$). After treatment, the levels of VEGF, MMP-2, and MMP-9 in the two groups were significantly lower than those before treatment, and the indexes in the study group were significantly lower ($P<0.05$). After treatment, the levels of NK, CD_3^+, CD_4^+, and CD_4^+/CD_8^+ in the two groups were significantly higher than those before treatment, the levels of CD_8^+ in the study group were significantly higher than those in the control group, and the levels of CD_8^+ in the study group were significantly lower than those in the control group ($P<0.05$). The incidence of adverse reactions was 25.58% in the study group and 48.84% in the control group. The incidences of adverse reactions in the study group were significantly lower than those in the control group ($P<0.05$).*

Conclusion: *Endostar combined with chemotherapy has a significant clinical effect in the treatment of advanced NSCLC. It can reduce the levels of VEGF, MMP-2, and MMP-9 in patients with advanced NSCLC; inhibit tumour invasion and metastasis; and improve the immune function of the body in a safe and reliable manner.*

Keywords: *Advanced NSCLC, endostar, chemotherapy, lymphocyte subsets.*

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Introduction

For many years, malignant tumours have been harmful to human life and health. At present, the worldwide incidence and mortality rate of lung cancer is the highest among all types of cancer. According to statistics, about 1.6 million people suffer from lung cancer every year, and more than 1.35 million people die of lung cancer every year worldwide. According to the different pathological types, biological characteristics, and treatment methods of lung cancer, it can be divided into non-small-cell lung cancer (NSCLC) and small-cell lung cancer. About 85% of cases are NSCLC[1-2]. NSCLC is a type of malignant tumour with high metastasis, invasion, and recurrence rate. Most patients are in the late stages at the time of diagnosis and miss the best time for operation. The treatment of advanced NSCLC is primarily chemotherapy. A combination of docetaxel and carboplatin is a commonly used clinical treatment, which can effectively kill tumour cells in the body, but it also damages immune cells, resulting in immune-system disorders[3]. Endostar is a kind of recombinant human endostatin, which has the effect

of anti-tumour proliferation and anti-angiogenesis. It can improve the local concentration inside the tumour and improve its sensitivity to chemotherapy[4].

It has been found that lymphocyte subsets are closely related to maintaining immune-system balance, killing tumour cells, and conducting immune surveillance[5]. This continuous monitoring of the immune system can maintain the normal immune balance of the body. Various immune cells in the body play an important role in killing tumour cells and controlling tumour growth[6]. The purpose of this study is to explore the changes in lymphocyte subsets and their significance before and after treatment.

Materials and methods

General information

After approval was obtained from the hospital ethics committee, 86 patients with advanced NSCLC who were treated in the oncology department of our hospital from June 2018 to February 2019 were randomly selected for the study. According to the random-number expression method, they were randomly divided into a control group and study group, with 43 patients in each group. The inclusion criteria were as follows: all patients met the World Health Organization's diagnostic criteria for advanced NSCLC[7], which was confirmed by laboratory pathology examination; patients' card score was greater than 75 points; patients and their families had provided informed consent; and patients' TNM stage was stage III–IV. The exclusion criteria were – the patient has a serious history of drug allergies; the patient withdraws for any reason; their liver, kidney, and heart function are not complete; and pregnant or lactating patients. In the study group, there were 25 males and 18 females, aged 57-72 years, with an average age of 66.57±3.62 years.

There were 12 patients in TNM stage III and 21 patients in stage IV; in the control group, there were 27 males and 16 females, aged 55-73 years, with an average age of 65.77±4.62 years. Ten of them were in TNM stage III and 23 in stage IV. There was no significant difference in age, gender, and TNM stage between the two groups (P>0.05).

Research methods

The control group was treated with chemotherapy (docetaxel [Zhejiang Haizheng Pharmaceutical Co., Ltd., production batch number: 2018092, specification: 0.5 ml: 20 mg/s] 60 mg/m^2, intravenous drip for 1 h, repeat every 3 weeks) + (carboplatin [Qilu Pharmaceutical Co., Ltd., production batch number: 20180181, specification: 10 mg/ml] 10 mg/ml was dissolved in 500 ml 5% glucose solution, 400 mg/m2 every time, repeated every 3 weeks) treatment of advanced NSCLC. The patients in the study group were treated with chemotherapy combined with endu (Shandong Xiansheng Medejin Biopharmaceutical Co., Ltd., production batch number: 20170088, specification: 15mg×3 ml). The drug was added to 500 ml normal saline, and 7.5 mg/m^2 was given once a day for 1–14 days of the treatment cycle. Both groups were treated for 6 weeks.

Observation indicators

Before and after the treatment, 4 ml of blood was taken from the elbow median vein in the morning. After centrifugation, the supernatant was taken and stored at –80°C.

The clinical effects of the two groups were evaluated, including complete remission, partial remission, stability, and progress. Progress is tumour increase in size of the tumour or the appearance of a new focus in comparison to before treatment; stability is that the tumour reduces by less than 40% in comparison to before treatment; partial remission is that the tumour size reduces by 40% in comparison to before treatment; and complete remission is that the focus disappears completely and the laboratory examination index returns to a normal value. Objective effective rate = (complete remission + partial remission) ÷ n × 100%, disease control rate = (complete remission + partial remission + stability) ÷ n × 100%.

The levels of vascular endothelial growth factor (VEGF), matrix metalloproteinase-2 (MMP-2), and matrix metalloproteinase-9 (MMP-9) were measured using ELISA. The VEGF test kit was purchased from Shanghai Kanglang Biotechnology Co., Ltd. (product number: KLC108.96); the MMP-2 test kit was purchased from Shanghai Jingkang Bioengineering Co., Ltd. (product number: JK1065); and the MMP-9 test kit was purchased from Shanghai Zeye Biotechnology Co., Ltd. (product number: H-EL-MMP-9).

Determination of lymphocyte subsets: the levels of T lymphocyte subsets (CD_3^+, CD_4^+, CD_8^+, and CD_4^+/CD_8^+) and natural killer cells (NK) were measured by flow cytometry (Beckman Kurt Trade (China) Co., Ltd., model: cytoflex).

Adverse reactions: the main adverse reactions were leukopenia, thrombocytopenia, gastrointestinal reactions, and myelosuppression. The adverse reactions of the two groups were compared.

Statistical methods

In this study, the measurement data were compared using the independent sample t-test, expressed in ($\bar{x}\pm s$). The χ^2 test was used to compare the counting data. The levels of VEGF, MMP-2, and MMP-9 were measured by ELISA. The lymphocyte subsets of the two groups were detected by flow cytometry. If the experimental result is P<0.05, it implies statistical significance. The SPSS17.0 software package was used for statistical data analysis.

Results

Comparison of clinical effects between the control group and the study group

After treatment, the objective effective rate and disease control rate of the two groups were 65.12% and 81.39%; and 83.72% and 95.35%, respectively (see Table 1), showing that values were significantly higher in the study group than in the control group (P<0.05).

Group	n	Complete remission	Partial remission	Stability	Progress	Objective effective rate	Disease control rate
Study group	43	21(48.84)	15(34.88)	5(11.63)	2(4.65)	36(83.72)	41(95.35)
Control group	43	16(37.21)	12(27.91)	7(16.28)	8(18.61)	28(65.12)	35(81.39)
χ^2						3.909	4.074
P						0.048	0.044

Table 1: Comparison of the clinical effects between the control group and study group cases (%).

Comparison of VEGF, MMP-2, and MMP-9 levels between the control group and the study group

After treatment, the levels of VEGF, MMP-2, and MMP-9 in the two groups were significantly lower than those before treatment (see Table 2), and the indexes in the study group were significantly lower (P<0.05).

Group	n	Time	VEGF (ng/L)	MMP-2 (pg/mL)	MMP-9 (ng/mL)
Study group	43	Before treatment	574.28±78.67	597.69±101.28	194.28±26.67
		After treatment	73.19±9.54^{ab}	72.48±14.93^{ab}	52.63±7.92^{ab}
Control group	43	Before treatment	568.46±75.33	589.78±99.05	192.65±24.49
		After treatment	138.67±22.55^{a}	123.32±20.61^{a}	119.28±16.48^{a}

Table 2: Comparison of VEGF, MMP-2, and MMP-9 levels between the control group and the study group ($\bar{x}\pm s$).
Note: a indicates that compared with the same group before treatment, $^{a}P<0.05$; b indicates that compared with the control group after treatment, $^{b}P<0.05$.

Changes in lymphocyte subsets in the control group and study group

After treatment, the levels of NK, CD_3^+, CD_4^+, and CD_4^+/CD_8^+ in the study group and the control group were significantly higher than those before treatment, and the levels of CD_8^+ in the study group were significantly lower than those in the control group (P<0.05). This can be clearly seen in Table 3.

Group	n	Time	NK (%)	CD_8^+(%)	CD_3^+(%)	CD_4^+/CD_8^+(%)	CD_4^+(%)
Study group	43	Before treatment	8.82±0.87	35.86±5.74	67.78±5.87	1.38±0.68	39.78±5.57
		After treatment	12.14±1.48^{ab}	27.67±3.58^{ab}	72.72±5.46^{ab}	1.62±0.76^{ab}	47.54±4.77^{ab}
Control group	43	Before treatment	8.86±0.76	34.49±3.52	68.56±6.82	1.37±0.47	39.51±4.97
		After treatment	10.17±1.95^{a}	31.28±3.26^{a}	64.89±7.68^{a}	1.51±0.62^{a}	44.68±3.86^{a}

Table 3: Changes in lymphocyte subsets in the control and study groups ($\bar{x}\pm s$).
Note: a indicates that compared with the same group before treatment, $^{a}P<0.05$; b indicates that compared with the control group after treatment, $^{b}P<0.05$.

Comparison of adverse reactions between the control group and the study group

The results showed that there were gastrointestinal reactions in four cases (9.31%), myelosuppression in two cases (4.65%), leukopenia in three cases (6.98%), thrombocytopenia in two cases (4.65%) in the study group, which gives 25.58% of adverse reactions. Further, in the control group, gastrointestinal reactions occurred in seven cases (16.28%), myelosuppression in three cases (6.98%), leukopenia in six cases (13.95%), and thrombocytopenia in five cases (11.63%), which gives 48.84% of adverse reactions. This shows that the rate of adverse reactions was significantly lower in the study group than in the control group (P<0.05). See Table 4 for more details.

Group	n	Gastrointestinal reactions	Myelosuppression	Leukopenia	Thrombocytopenia	Total
Study group	43	4 (9.31)	2 (4.65)	3 (6.98)	2 (4.65)	11 (25.58)
Control group	43	7 (16.28)	3 (6.98)	6 (13.95)	5 (11.63)	21 (48.84)
χ^2						4.977
P						0.027

Table 4: Comparison of adverse reactions between the control group and the study group cases (%).

Discussion

Lung cancer is one of the most common causes of malignant tumours in the world. Recently, the incidence and mortality rates of lung cancer are increasing year on year. It has become the malignant

tumour with the highest mortality rate in the world, seriously affecting people's lives and health[8]. Since most NSCLC patients are in the middle and late stages of the disease when they are diagnosed and the tumours cannot be removed surgically, the first choice of treatment for advanced NSCLC is chemotherapy; the main chemotherapy scheme is based on platinum combined with vinorelbine. However, owing to the lack of oxygen cells resistant to radiation in NSCLC tumour tissue, it is difficult for conventional radiotherapy and chemotherapy to improve the therapeutic effects of current anti-tumour measures; therefore, it is the focus of current research to find new anti-tumour treatment methods. Endostar is the first new endostatin drug in China, which has a broad-spectrum inhibitory effect on tumour angiogenesis. It can affect the VEGF conduction pathways and the activity of proteolytic enzymes, induce endothelial cell apoptosis, inhibit the formation of tumours' vascular networks, and subsequently inhibit the growth and migration of the tumours[9].

The results showed that the objective effective rate and disease control rate of the two groups were 65.12% and 81.39%; 83.72% and 95.35%, respectively, which were significantly higher than those of the control group ($P<0.05$). The incidence of adverse reactions in the study group was 25.58%, significantly lower than that in the control group (48.84%) ($P<0.05$). It has been suggested that endostar, combined with chemotherapy for advanced NSCLC, has a good clinical effect and is safe and reliable. VEGF is a type of glycoprotein, which is one of the most powerful angiogenic factors[10].

It has been found that most tumour cells can secrete VEGF, which is closely related to tumour invasion and metastasis[11]. VEGF can selectively and directly act on the receptors on the endothelial cell membrane, stimulate the occurrence and growth of blood vessels, and promote the permeability of tumour blood vessels. Tumour invasion and metastasis is a process in which tumour cells and tumour matrix components participate together. The basement membrane is a natural barrier to the process of tumour cell invasion and diffusion. Matrix metalloproteinases (MMPs) can specifically degrade basement membrane and promote the invasion and metastasis of malignant tumour cells[12].

MMP-2 and MMP-9 are the most important enzymes to degrade type IV collagen, which also play an important role in the process of tumour vascularization, tumour cell infiltration, and metastasis[13]. In this study, after treatment, the levels of VEGF, MMP-2, and MMP-9 in the two groups were significantly lower than those before treatment, and the indexes of the study group were also significantly lower ($P<0.05$). It is suggested that endostar combined with chemotherapy can significantly reduce the levels of VEGF, MMP-2, and MMP-9 in patients with advanced NSCLC, and inhibit angiogenesis, tumour invasion, and metastasis.

It has been found that metastasis and the invasion of malignant tumours are closely related to immune function[14]. T-lymphocyte subsets and NK cells are important indicators to test the immune function of the body and play an important role in ascertaining the recovery and prognosis of the body[15]. NK cells are important immune cells, which are closely related to anti-tumour characteristics, anti-viral capabilities, and immune regulation. Activated NK cells can synthesize and secrete a variety of cytokines that play a role in regulating immunity and killing target cells directly. CD_8^+, also known as cytotoxic T-cells, can be combined with MHC-1 molecules to produce a negative regulatory effect[16]. The constant ratio of CD_4^+/CD_8^+ plays an important role in maintaining the cellular immune balance of the body. If the ratio of CD_4^+/CD_8^+ decreases, this indicates that the immune function of the body is inhibited. CD_4^+ can help B-cells secrete antibodies and regulate other T-cell immune responses, effectively enhancing the immune function of the body. In the study results, the levels of NK, CD_3^+, CD_4^+, and CD_4^+/CD_8^+ in the study group and the control group were significantly increased after treatment (in comparison to before treatment), and the levels of CD_8^+ were significantly reduced. Further, the levels of NK, CD_3^+, CD_4^+, and CD_4^+/CD_8^+ in the study group were significantly higher than those in the control group, and the levels of CD_8^+ were significantly lower than those in the control group ($P<0.05$). It is suggested that endostar combined with chemotherapy can improve the immune function of patients with advanced NSCLC.

It can be concluded that endostar combined with chemotherapy can reduce the levels of VEGF, MMP-2, and MMP-9 in patients with advanced NSCLC, inhibit tumour invasion and metastasis, and improve the immune function of the body. The clinical effect is clear, safe, and reliable.

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