Original Article

Clinical significance of detection Th1 and Th2 cytokines in serum of patients with non small cell lung cancer

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ABSTRACT **Objective:** To explore the clinical significance of peripheral blood Th1 and Th2 cytokine in non small cell lung cancer patients.

Method: Cytometric Bead Array was employed to detect six cytokines (IFN-γ, TNF-α, IL-2, IL-4, IL-5, IL-10) in peripheral blood serum of 99 patients with non small cell lung cancer, then comparison and analysis can be achieved combining with clinical data.

Results: There was no close relationship between the expressions of six cytokines and clinical data (such as age, gender, histologic types, disease stage and smoking history) ($P > 0.05$), but the expression between each cytokine is closely related. Stratified analysis shows expression of IL-2 and IL-4 are closely related to the prognosis to NSCLC of mediuan and advanced stage. Exclude the influencing factors such as age, gender, disease staging and smoking history, fatality of patients with high IL-2 expression is 0.46 times to that of patients with low IL-2 expression (CI: 0.23-0.92, $P < 0.05$); on the contrary, fatality of patients with high IL-4 expression is 2.86 times to that of patients with low IL-4 expression (CI: 1.20-6.82, $P < 0.05$)

Conclusion: Detection of Th cytokine can reflect the tumor immunity of organisms, which indirectly shows patients' condition and prognosis. IL-2 cytokine is a good independent factor affecting prognosis for patients in medium and advanced stage, while IL-4 is a poor prognostic factor.

KeyWords: Non small cell lung cancer, serum, cytokine, Cytometric Bead Array

The occurrence organism of lung cancer is rather complex. Dropout of extro-regulatory factor and intra-cellular information system would lead to mutation and malignant generation. Adjuvant T lymph nodes (Th) subgroup is a commonly used comprehensive index for evaluation of organism's immunologic balance status. Under different antigenic stimulation, Th can subdivide into Th1 and Th2 two subgroups, in which, Th1 cell secretes IL-2, IFN-γ, TNF-α, etc, cell-mediated immune response; while Th2 cell excretes IL-4, IL-5, IL-10, etc, mediated humoral immune response. The two inhibit and transform mutually in a balance. The break of the balance between the Th1 and Th2 may cause immunosuppression, and even the occurrence and development of tumor.

Cytometric Bead Array (CBA) is a kind of technique with flow cytometry, through which, the matter-to-be-survived in liquid can be captured in a status of tiny scattered particles (1). Comparing with the traditional detecting cytokine ways of ELISA and RT-PCR, it owns advantages such as high sensitivity, high specificity and detection of many cytokines in a time, etc. CBA is applied to detect the count of IFN-γ, TNF-α, IL-2, IL-4, IL-5 and IL-10 in serum of 99 primary NSCLC (non-small cell lung cancer) cases, with an aim of analyzing the clinical significance of Th1 and Th2 cytokines in patients with NSCLC, and especially employed to explore IL-2 and IL-4's influence on survival rate.

1 Patients and Methods

1.1 Patients

All of the 99 cases with primary NSCLC admitted to our hospital during March, 2001 and June, 2005 have been pathologically approved. Their ages differences from 33 to 87, and the average age is 60.0.There are 80 male cases and 19 female cases. 42 cases are with squamous cell carcinoma, 34 are with adenocarcinoma,

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and 23 cases are with adenosquamous carcinoma and other types of non small cell carcinoma. 75 are smokers, and 24 non-smokers. According to the sixth version of UICC lung cancer TNM staging standard, 6 cases are of stage I, 7 are of stage II, and there are 47 stage III cases and 39 stage IV cases. After accurate diagnosis was made, all of the 99 cases received chemotherapy, radiotherapy or radiotherapy+chemotherapy. Therapeutic effect evaluation after treatment is as follows: 71 cases are with response (CR+PR), 21 cases have no response (SD+PD), and there are 7 cases lack of evaluation (because patient haven't received CT scan and other examinations, so the evaluation can't be performed).

1.2 Cytokine Detection

Before treatment, non-anticoagulant peripheral blood 2ml is taken from each patient. After kept in 37℃ water bath for two hours, serum 50μl was absorbed and gathered with microspheres mixed fluid and fluorescent dye phycoerythrin of six cytokines (IFN-γ, TNF-α, IL-2, IL-4, IL-5 and IL-10) respectively 50μl; then after full blending, it would be kept in room temperature but away from light for 3h, then put 1ml lotion in each tube, and centrifugate for 5 min (2000r/min), then supernatant are discarded; next, another 120μl lotion is added and well mixed. Three or five minutes later, collect data through computer, and standard curve is automatically drawn by CBA analyzing software, besides, each cytokine count in blood plasma would be calculated. Standard substance of the six cytokines is prepared in accordance with instruction of CBA kit and a standard curve can be got. The flow cytometry, CBA kit and analyzing software are all from BD (Becton, Dickinson and Company), US.

1.3 Follow Up

Clinic, letter and telephone follow-up are adopted. There are 90 cases of the whole group got follow up, and 9 cases lost follow up. Follow-up deadline: Aug, 2008. Follow-up duration: 1 to 73 months, the average follow-up duration is 13 months.

1.4 Statistical methods

SPSS11.0 statistical software is applied for analyzing obtained data. The cytokine data is in skewed distribution with median (M)

Table 1

Interrelationship between expression of IFN-γ, TNF-α, IL-10, IL-5, IL-4, IL-2 and clinical pathological features of patients with NSCLC (non-parametric statistics analysis)

		IFN-γ	TNF-α	IL-10	IL-5	IL-4	IL-2
	n	Median(5th-95th)	Median(5th-95th)	Median(5th-95th)	Median(5th-95th)	Median(5th-95th)	Median(5th-95th)
Gender							
Female	19	17.40(3.79-121.10)	6.50(0.00-134.50)	3.20(0.00-21.10)	2.36(1.24-10.00)	4.43(0.00-12.70)	4.20(0.00-13.40)
Male	80	18.20(3.33-87.00)	4.65(0.00-84.71)	3.45(1.51-9.15)	2.74(1.407.25-)6	4.35(1.51-12.62)	3.66(0.00-7.04)
P		0.943	0.253	0.780	0.505	0.831	0.458
Disease Staging							
I-II	13	20.00(0.00-234.40)	3.84(0.00-25.00)	3.30(1.51-8.24)	3.50(1.000-4.79)	4.60(1.00-10.49)	2.71(1.10-7.64)
III	47	17.40(1.29-108.46)	4.17(0.44-71.53)	3.50(0.00-20.06)	2.72(1.52-8.92)	4.40(1.80-13.00)	3.91(0.00-7.69)
IV	39	17.09(6.01-47.20)	5.80(0.00-134.50)	3.22(1.51-9.20)	2.50(1.40-5.80)	4.30(1.70-12.70)	3.70(0.00-7.10)
P		0.915	0.346	0.999	0.694	0.929	0.906
Histologic Type							
Squamous Cell Carcinoam	42	18.27(0.48-116.05)	5.25(0.17-37.93)	3.55(0.23-20.19)	2.86(1.59-5.77)	4.55(1.50-12.70)	3.85(0.00-6.93)
Adenocarcinoma	34	16.93(5.45-56.20)	4.65(0.00-156.48)	3.34(1.07-10.33)	2.50(1.18-8.62)	4.38(0.75-11.15)	3.36(0.00-7.75)
Others	23	20.02(1.28-196.96)	3.20(0.26-188.16)	3.00(1.59-8.82)	2.68(1.40-24.99)	3.60(1.64-15.81)	3.40(0.00-7.82)
P		0.653	0.644	0.788	0.377	0.482	0.642
Smoking History							
Non-Smokers	24	21.81(4.49-113.20)	3.89(0.38-113.26)	3.69(0.00-20.45)	2.50 (1.40-9.33)	4.47(0.38-15.25)	3.66(0.00-12.08)
Smokers	75	17.09(2.58-80.92)	4.88(0.00-92.85)	3.25(1.51-8.43)	2.80(1.40 -6.10)	4.30(1.58-11.35)	3.70(0.00-7.08)
P		0.216	0.945	0.958	0.772	0.964	0.695
Therapeutic Effect							
Poor	21	15.43(0.92-42.47)	4.40(1.12-202.76)	3.84(1.67-29.62)	2.80(1.23-4.74)	4.50(1.52-10.95)	3.70(0.11-5.97)
Good	71	19.70(4.52-102.14)	5.80(0.00-99.50)	3.40(0.90-11.52)	2.65(1.46-8.38)	4.40(1.50-12.90)	3.80(0.00-7.78)
P		0.364	0.173	0.319	0.955	0.922	0.860

Through Tab.1, we can see that the expression of cytokine has no interrelationship with gender, disease staging, histological type, smoking history and therapeutic effect (P>0.05).

expression. According to the median expression of each cytokine, low expressed group and high expressed group are gotten. Non-parameter test is applied for expressing each cytokine's interrelationship with gender, disease staging, histological type, smoking history and therapeutic effect; Pearson analysis is adopted for analyzing each cytokine's correlation with age, gender, histological type, disease staging, and smoking history, and so it is with correlation between each cytokine; COX regression is employed for analyzing the relationship between each cytokine and overall survival (OS) of NSCLC in median and advanced stage.

2 Result

2.1 Each cytokine's interrelationship with gender, disease staging, histological type, smoking history and therapeutic effect. (Tab.1)

2.2 Correlation exists between cytokine expressions

IL-4 has obvious correlation with IFN-γ , IL-2, IL-5 and IL-10, and correlation coefficients are respectively 0.28, 0.52, 0.29 and 0.45 ($P < 0.05$); IL-10 has obvious correlation with IFN-γ and IL-2, and correlation coefficients are respectively 0.30 and 0.33 ($P < 0.05$); IFN-γ and IL-5 have obvious correlation with IL-2, and the correlation coefficients are 0.21 and 0.26 ($P < 0.05$); one exception is that TNF-α has no correlation with other cytokines (IFN-γ , IL-4, IL-2, IL-5, IL-10) ($P > 0.05$) (please refer to Tab. 2).

2.3 The Correlation of IL-2 and IL-4 Expression with NSCLC Patient's Survival

The expression of each cytokine has no correlation with NSCLC patient's survival; however, stratified analysis shows that the IL-2 and IL-4 expression relates to the prognosis of NSCLC patients in median and advanced stage. No matter modulation to age, gender, disease staging and smoking history is done or not, IL-2 and IL-4 would have statistical significance on overall survival to patients with NSCLC of median and advanced stage. In approximate survival analysis before IL-2 modulation, the death risk of high expressed IL-2 cases is 0.47 times to that of cases with low expressed IL-2 cases (CI: 0.24-0.91, $P < 0.05$), and this difference has statistical significance; in survival analysis after IL-2 modulation, the death risk of high expressed IL-2 cases is 0.46 times to that of cases with low expressed IL-2 cases (CI: 0.23-0.92, $P < 0.05$), and the difference has statistical significance as well; it is indicated that IL-2 is a factor of good prognosis for median and advanced NSCLC cases. On the contrary, in approximate survival analysis before IL-4 modulation, the death risk of high expressed IL-4 cases is 2.49 times to that of cases with low expressed IL-4 cases (CI: 1.10-5.61, $P < 0.05$); in survival analysis after IL-4 modulation, the death risk of high expressed IL-4 cases is 2.86 times to that of cases with low expressed IL-4 cases (CI: 1.20-6.82, $P < 0.05$), and the difference also has statistical significance; it is showed that IL-4 is a factor of bad prognosis for median and advanced NSCLC cases. (Tab.3)

3. Discussion

The existence of lung cancer can induce tumor immunological reaction of organism, which includes T cell mediated cellular immunity and B cell mediated humoral immunity. Cellular immunity leads the main position among tumor immunities. According to functional status, human Th can be subdivided to Th0, Th1 and Th2 cells. Under antigenic stimulation, prosoma of Th cell would differentiate to Th0 cell of intermediate stage, and Th0 cell would selectively differentiate to Th1 or Th2 cell under the influence such as cytokine and so on. Th1 cell is mainly induced by IL-2 and

Table 2
Pearson Analysis to Correlation between Expression of IFN-γ , TNF-α , IL-10, IL-5, IL-4 and IL-2 and NSCLC Clinical Pathological Signs and to Interrelationship Between Each Cytokine

	IFN-γ , correlation coefficient (p)	TNF-α , correlation coefficient (p)	IL-10, correlation coefficient (p)	IL-5, correlation coefficient (p)	IL-4, correlation coefficient (p)	IL-2, correlation coefficient (p)
Age(n=99)	-0.07(0.482)	-0.01(0.951)	-0.12(0.247)	-0.06 (0.587)	-0.18 (0.074)	-0.14(0.154)
Gender(n=99)	-0.02(0.829)	0.03(0.769)	-0.01(0.958)	-0.05(0.607)	-0.06(0.574)	0.13(0.184)
HistologicType (n=99)	0.03(0.803)	0.10(0.320)	-0.11(0.267)	0.09(0.370)	-0.11(0.259)	-0.03(0.784)
TNM Staging (n=99)	-0.09(0.362)	0.14(0.165)	-0.01(0.918)	0.02(0.867)	0.05(0.625)	-0.00(0.994)
Smoking or Not (n=99)	-0.04(0.703)	0.01(0.905)	-0.04(0.667)	0.03(0.757)	0.01(0.921)	-0.09(0.382)
IFN-γ (n=99)	1.00	0.04(0.674)	0.30(0.003)	0.08(0.405)	0.28(0.005)	0.21(0.037)
TNF-α (n=99)	0.04(0.674)	1.00	0.05(0.622)	-0.01(0.895)	0.09(0.377)	0.03(0.774)
IL-10(n=99)	0.30(0.003)	0.05(0.622)	1.00	0.14(0.152)	0.45(0.000)	0.33(0.001)
IL-5(n=99)	0.08(0.405)	-0.01(0.895)	0.14(0.152)	1.00	0.29(0.003)	0.26(0.009)
IL-4(n=99)	0.28(0.005)	0.09(0.377)	0.45(0.000)	0.29(0.003)	1.00	0.52(0.000)
IL-2(n=99)	0.21(0.037)	0.03(0.774)	0.33(0.001)	0.26(0.009)	0.52(0.000)	1.00

Table 3
The Correlation between Expression of IFN-γ , TNF-α , IL-10, IL-5, IL-4, IL-2 and Survival of Cases with Advanced stage NSCLC

	Risk Rate Before Modulation (95% CI.)	P	Risk Rate After Modulation (95% CI.)*	P
IFN-γ Expression				
Low	1.00		1.00	
High	1.07(0.51-2.24)	0.868	0.95(0.48-2.17)	0.955
TNF-α Expression				
Low	1.00		1.00	
High	1.34(0.71-2.53)	0.362	1.06(0.55-2.03)	0.864
IL-10 Expression				
Low	1.00		1.00	
High	0.81(0.43-1.51)	0.504	0.84(0.44-1.58)	0.585
IL-5 Expression				
Low	1.00		1.00	
High	0.99(0.53-1.84)	0.966	1.10(0.58-2.10)	0.774
IL-4 Expression				
Low	1.00		1.00	
High	2.49(1.10-5.61)	0.028	2.86(1.20-6.82)	0.018
IL-2 Expression				
Low	1.00		1.00	
High	0.47(0.24-0.91)	0.026	0.46(0.23-0.92)	0.028

* The age, gender, TNM staging and smoking history have been modulated.

IFN-γ . Th1 cell excretes IL-2, IFN-γ , TNF-α , etc, and the mediate cell immunological reaction. Th2 cell excretes IL-4, IL-5, IL-10, etc, and the mediate humoral immunological reaction. IL-4 helps Th0 cell to differentiate into Th2 cell. In high IL-4 density, Th0 cells can mostly differentiate into Th2 cells, keeping Th0 away from differentiating into Th1 cells. The IFN-γ secreted by Th1 can suppress the proliferation of Th2, while IL-4 and IL-10 secreted by Th2 cell can suppress the generation of IFN-γ . IFN-γ would induce Th1 cell differentiation and suppress Th2 cell proliferation; IL-4 and IL-10can induce Th2 cell differentiation, and can also suppress Th1 cell proliferation; IL-2 would cause both Th1 and Th2 cell proliferation (2). Through these, it can be seen that intricate relationship exists between each cytokine, which forms a huge cytokine net, some promote mutually, while still some suppress mutually. Our research result also indicates that the intimate relationship between cytokines. That is to say, but for the exception of TNF-α , which has no correlation with other cytokines, intimate correlation exists between IFN-γ , IL-4, IL-2, IL-5 and IL-10.

In the cytokine expressions of various malignant tumor tissues already been found, Th2 cytokine predominates. Th1/Th2 balance of organism immunological reaction migrates to Th2, and anti-tumor immunity is suppressed, which leads to the occurrence and development of tumor. In Th cytokines, IL-2 is a cytokine with anti-tumor activity character, through integration with NK cell, LAK cell and B lymphocytes and other lymphocytic membrane IL-2 receptors, immunocyte proliferation could be regulated, so that effect of anti-tumor could be achieved.

Neuner et al, found that the secretion of IL-2 within NSCLC cases is inhibited, prognosis of NSCLC can be declared through the IL-2 expression level, besides, IL-2 is a factor of good prognosis (3). Szopinski et al have done research on 42 SCLC cases, in their opinion, the count of IL-2 and IFN-γ before treatment has a lower tendency, comparing with that in widespread stage and limited stage (4). It's known that SCLC case in widespread stage is later than the case in limited stage, and its prognosis is much worse than case in limited stage, so it can be inferred that IL-2 can indirectly indicate patient's condition and prognosis, that is to say, there is a later disease state and poorer prognosis tendency to the SCLC cases who are with low IL-2 expression before treatment. However, the difference has no statistically significance since the amount of sample is only a few. Fischer et al, consider that long survival can be observed in the cases who are with high IL-2 expression when been diagnosed with SCLC and can get complete remission after chemotherapy; besides, IL-2 can be a prognostic factor independently to other factors (5). Our research result also shows that in stratified analysis, IL-2 expression has some correlation with prognosis of median and advanced NSCLC. No matter we do modulation to age, gender, disease staging and smoking history or not, IL-2 will have statistically significance to the overall survival of median and advanced NSCLC cases. Death risk of high IL-2 ex-

pression cases is 0.47 times to low IL-2 expression cases, and it is indicated that IL-2 is a factor of good prognosis for median and advanced stage NSCLC cases.

IL-4 can suppress anti-tumor immunological reaction, induce Th1 cell proliferating and differentiating to Th2 cell, and suppress LAK function stimulated by lymphocyte. The research by Francipane et al. shows the prevention of IL-4 expression helps the apoptosis of CSCs (colon stem cells),in this way, the efficacy of cytotoxic drug treatment for organism would be enhanced (6). Through up-regulating anti-apoptosis proteinum, IL-4 can protect tumor away from attack of CD-95 (Fas/Apo-1) and chemotherapeutics, which does harm to tumor immunity (7). The research made by Cui et al. shows that in tumor microenviroment of NSCLC, IL-4 can inhibit COX-2 expression, and suppress anti-tumor immunological reaction, and it is a factor of bad prognosis (8). Our research indicates: the death risk of high IL-4 expression cases is 2.86 times to that of low IL-4 expression cases, which shows IL-4 is a factor of bad prognosis for median and advanced NSCLC cases.

It's known that if want to keep the organism in a good anti-tumor immunity, Th1 cytokine secretion should predominate. Improving blood plasma IL-2 level artificially can effectively promote T cell proliferation and activation. IL-2 is a valid immunologic stimulant, which has been widely applied in anti-tumor and treating HIV infection. IL-2 monoclonal antibody (murine IL-2 mAb S4B6) and IL-2 united together can enhance anti-tumor effect (9). IL-2 and melatonin uniting together shows good clinical effect as well. There is also domestic report shows that taking IL-2 gene into DC cell so that DC vaccine can be obtained, after patients receiving immunotherapy with the vaccine, their immunologic function would be improved obviously; besides, relapse and metastases of tumor can be suppressed, through this way, patients' survival time can be prolonged, and the survival rate can be elevated. Employing the combination of IL-2, TNF-α and IFN-γ these three kinds of cytokines secreted by Th1 cell to tumor clinical has an obvious synergism, which can directly melt and kill tumor cells.

The poor prognosis of NSCLC cases in median and advanced stage has close correlation with immunological status change of the tumor itself. The improvement to patients' prognosis with traditional integrated treatment of operation, chemotherapy and radiotherapy combination is not ideal. In clinical, cytokines secreted by Th are tried to detect anti-tumor immunity of organism, which can indirectly indicate patients' condition and prognosis. Meanwhile, cytokines secreted by Th are used for instructing immunotherapy in clinical, and for retrieving tumor unbalanced tumor immune. The application of immunotherapy into integrated therapy of median and advanced NSCLC serves a way to improve patient's recovery rate and survival rate.

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