Exp Oncol 2012
34, 4, 348–353

Experimental ONCOLOGY

NOVEL CYTOKINES: IL-27, IL-29, IL-31 AND IL-33. CAN THEY BE USEFUL IN CLINICAL PRACTICE AT THE TIME DIAGNOSIS OF LUNG CANCER?

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There are several antiproliferative and angiogenic factors, recently have been discovered (IL-27, IL-29, IL-31 and IL-33), but they have not been tested yet in lung cancer patients. ***The aim*** **of this pilot study was to assess the clinical usefulness of determination of IL-27, IL-29, IL-31 and IL-33 in advanced stages of lung cancer.** ***Patients and Methods*****: The study included 45 patients (38 males; mean age 62 years; 45 with advanced NSCLC). Serum and BALF cytokine concentrations were evaluated by ELISA method before chemotherapy. The comparative groups consisted of patients with sarcoidosis (BBS, n = 15), hypersensivity pneumonitis (HP, n = 8) and healthy subjects (n = 15).** ***Results*****: The serum IL-29 levels were higher in NSCLC patients than in the sarcoidosis group. However, serum IL-27, IL-31 and IL-33 did not differ markedly between: NSCLC, BBS, HP and the control group. Concentrations of IL-29 and IL-31 in BALF did not differ significantly between investigated groups. In all groups levels of IL-27 and IL-29 are significantly higher in serum than in BALF. Concentrations of IL-31 in BBS, HP and control groups tended to higher in BALF than in serum. These differences were significantly in NSCLC patients. Patients in stage IIIB of NSCLC had higher serum levels of IL-29 than these in stage IV. Lung cancer patients with partial remission (PR) after chemotherapy had significantly higher concentration of IL-27 in BALF than patients with SD. However, patients with SD had higher levels of IL-29 in BALF than patients with PD. A negative correlation was found between serum IL-31 levels before therapy and time to progression of NSCLC.** ***Conclusion*****: Determination of IL-27, IL-29 and IL-31 in serum and BALF can be useful in clinical practice, but their practical significance needs further studies.**
Key Words**: lung cancer, interleukin 27, interleukin 29, interleukin 31, interleukin 33, bronchoalveolar lavage fluid.**

Inflammation and angiogenesis are closely associated, and pathologic angiogenesis has been implicated on the development of chronic inflammatory diseases and several cancers. Interplay between inflammation and angiogenesis is mediated largely by growth factors and cytokines, particularly interleukins [1].

Neoangiogenesis plays a vital role in the tumor growth and the development of metastasis [2]. Recently there have been discovered several antiproliferative and angiogenic factors, but they have not been tested yet in lung cancer patients. Some of them are: IL-27, IL-29, IL-31 and IL-33.

IL-27 has been associated mainly with the differentiation of Th1 lymphocytes, enhancement of the cellular type immune response and the reciprocal inhibition of Th2 humoral immune reactions [3]. The IL-27 acts as an inhibitor of angiogenesis, stimulates the expression of IFN-gamma-inducing protein (IP-10/CXCL10) and a monokine induced by IFN-gamma (MIG/CXCL9). Both molecules were reported to inhibit tumor growth and metastasis. Only a small percentage of vascular endothelial cells in normal tissues is positive for CXCR3 (CXC-Chemokine receptor 3), which is a receptor for IL-27-induced CXCL10, whereas the frequency of CXCR3-positive cells in neoplastic tissues is much higher than in normal ones [4].

IL-29, designated as a type III interferon (IFN), has been recently identified the class II cytokine receptor ligands that are distantly related to members of the IL-10 family and the type I IFN family [5]. Interferons are a large family of proteins having a wide variety of biological properties [6]. These include inhibition of cell growth, activation of T cell and natural killer (NK) cell cytotoxicity, promotion of T helper type I responses and inhibition of angiogenesis [7]. The crucial to the antiproliferative effect of IL-29 on non-small cell lung cancer (NSCLC) is the upregulation of p21 expression and induction of apoptosis [8]. Actually, p21 expression in NSCLC tumors has been reported to be a predictor for a favorable prognosis [9].

IL-31 is a recently discovered cytokine, belonging to the gp130/IL-6 cytokine family that includes IL-6, viral IL-6, IL-11, IL-27 [10]. IL-31 is an immunoregulatory cytokine that is mainly produced by activated Th cells [11]. IL-31 acts through the heterodimeric receptors of IL-31 (IL-31R) and oncostatin receptor (OSMR) which are expressed on IL-31 activated monocytes and expressed on epithelial cells [11]. Bronchial and alveolar epithelial cells, pulmonary fibroblasts and macrophages are the primary targets of IL-31 in the lung [12]. IL-31 can significantly elevate the gene and protein expressions of epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF) [13]. A recent study demonstrated that IL-31 is highly effective in suppressing the prolifeation of lung epithelial cells by up-regulating p27Kip1 (cyclin-dependent

Received: June 3, 2012.
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Abbreviations used: BBS – Besnier-Boeck-Schaumann disease, sarcoidosis; BALF – bronchoalveolar lavage fluid; HP – hypersensivity pneumonitis; IL – interleukin; NSCLC – non-small cell lung cancer; PD – progressive disease; PR – partial remission; SD – stable disease, stabilization.

kinase inhibitor p27 Kip1) and down-regulating cyclin B1, cdc2, cdk6, mcm4 and Rb [14].

IL-33 is a newly identified cytokine of the IL-1 family, which also includes the inflammatory cytokines IL-1α, IL-1β, and IL-18 [15]. It has been shown to signal via ST2 receptor. IL-33 expression is broadly detected in various tissues, including stomach, lung, spinal cord, brain, and skin, as well as in cells, including smooth muscle cells and epithelial cells lining bronchus [16]. On the basis of current reports very little is known about cellular sources of IL-33. Recent publications imply that vascular endothelial cells are the dominant IL-33-expressing cell population *in vivo* [17]. Choi et al. [18] have shown that IL-33 promotes angiogenesis and endothelial permeability in an endothelium-derived NO-dependent manner.

In our study, we measured the serum and BALF (bronchoalveolar lavage fluid) levels of IL-27, IL-29, IL-31 and IL-33 in lung cancer patients and assessed their inter-relationship and clinical significance. We also measured levels these cytokines (comparative groups) in patients with sarcoidosis, hypersensivity pneumonitis (HP) and healthy persons. Our study provides new insight into the mechanism of function new angiogenic cytokines in lung cancer.

MATERIALS AND METHODS

Patients. The study involved 45 patients diagnosed with the histological diagnosis of NSCLC and treated in the Department of Lung Diseases and Tuberculosis in Bialystok (38 male patients). The mean age of patients was 61.9 ± 4 years. At the beginning of the study patients had neither been treated with any anticancer medication nor undergone radiotherapy. Squamous cell carcinoma (SCC) comprised 48.8% (22 individuals) of patients with NSCLC, adenocarcinoma was revealed in 20% (9 patients), whereas NSCLC was diagnosed in 31.1% (14 patients). After four cycles of chemotherapy partial remission (PR) was reported in 19 patients (42.2%), stabilization (SD) in 11 patients (24.4%), and progression of disease (PD) in 15 patients (33.3%). Serum samples obtained from the whole blood of patients with lung cancer taken before chemotherapy were used. To exclude the possible interference of chemotherapy, subsequent blood samples were obtained at least 28 days after the last administration of cytotoxic drugs. Blood serum was stored at -80 °C until the assay. At the first stage, blood samples were taken to assess IL-27, IL-29, IL-31 and IL-33 after complete diagnostics of lung cancer had been made, including X-ray and CT of the chest, bronchofiberscopy with H-P test, lung transbronchial biopsy (TBB), or transbronchial needle aspiration biopsy (TBNA). The clinical analysis comprised the evaluation of clinical staging of NSCLC (TNM, IASLC), and the performance stage according to Zubrod. The response to therapy was estimated after four cycles of chemotherapy according to the RECIST (Response Evaluation Criteria in Solid Tumors) criteria [19]. All patients underwent basic laboratory tests and accessory investigations (ultrasonography of the abdominal cavity, if necessary, of the chest, EEG, and CT of the central nervous system).

The comparative groups in study consisted of 15 patients with pulmonary sarcoidosis (BBS, Besnier-Boeck-Schaumann disease) and 8 with HP (extrinsic allergic alveolitis). BBS patients were in the second stage of the disease (confirmed by X-Ray of the chest, High Resolution Computed Tomography of the lung and histological examination) [20]. Patients with HP were in subacute stage, confirmed by HRCT of the chest, alveolitis in BAL, exposure history of known offending antigen. BBS and HP patients were lifetime nonsmokers and did not experience any acute respiratory illness during the 6 weeks prior to the study. They had neither been treated with glucocorticoid medication.

Bronchoscopy and BALF analysis in all patients and control group processing were performed as a part of routine clinical management, according to the recommended guidelines [21]. Subjects underwent bronchoscopy by flexible fiberoptic bronchoscope (Pentax FB 18 V; Pentax Corporation, Tokyo, Japan). Subjects received atropine (0.5 mg, i.m.), and midazolam (5 mg, i.m.). Local anesthesia was performed by inhalation of an aerosol solution of 22 ml of 2% lidocaine 15 min before bronchoscopy. The bronchoscope was inserted and wedged in the right middle lobe, and three 50 ml aliquots of sterile saline solution, warmed to 37 °C, were instilled into the subsegmental bronchus. Fluid was gently aspirated immediately after each aliquot was introduced, and collected in a sterile container. A second 50 ml aliquot of recovered fluid was labeled as BALF. BALF samples were analyzed for total and differential cell counts, flow cytometry to measure $CD4^+$, and $CD8^+$ lymphocyte counts, and for IL-27, IL-29, IL-31 and IL-33 levels detected by ELISA.

During bronchoscopy, oxygen saturation and ECG tracings were continuously monitored. One aliquot was reserved for a total cell number using Nageotte's chamber and these results were expressed as 10^5 cells/ml. The remaining fluid was immediately centrifuged at 800 rpm for 10 min at 4 °C. The cell pellet was washed twice with phosphate-buffered saline solution (without Ca^{2++} and Mg^{2++}). Cytocentrifugates were stained by the May-Grunwald Giemsa method. The differential cell count of macrophages, neutrophils, lymphocytes, eosinophils, and epithelial cells was made using light microscopy (magnification x 1000) by counting at least 400 cells. Two observers blinded to the patients' characteristics counted 400 cells to determine the differential cell count.

In accordance with the declaration of Helsinki, the study protocol was approved by the local ethics committee and written informed consent was obtained from all participants.

Control subjects. The control group for serum and BALF analysis consisted of 15 healthy volunteers (13 men, 2 women) without any acute or chronic inflammatory conditions. The mean age of controls at the time of sampling was 60.1 ± 5. There were

no significant differences in age and sex between patients and controls.

NSCLC patients' therapy. Chemotherapy was carried out in a 21-day cycle using cisplatin at a dose of 30 mg/m^2 on days 1, 2, and 3 and gemcytabine at a dose of 1000 mg/m^2 on days 1 and 8 of the cycle. All patients received four cycles of chemotherapy. Some of the patients underwent later radiotherapy or next cycles of chemotherapy.

Serum and BALF IL-27, IL-29, IL-31 and IL-33 analysis. IL-27, IL-29, IL-31 and IL-33 concentrations were determined by means of enzyme-linked immunosorbent assay (ELISA) method according to the manufacturer's instructions.

All specimens were assayed twice and the average of the two measurements was used in the data analysis. Values were considered positive if they were twice that of the background with a responding coefficient of variation of less than 10%.

The minimum detectable dose (MDD) of IL-27 (US-CNK Life Science Inc.), IL-29 (eBioscience®), IL-31 (eBioscience®) and IL-33 (eBioscience®) was respectively 6.1 pg/ml, 2.0 pg/ml, 1.0 pg/ml and 0.2 pg/ml.

Statistical analysis. Statistical analysis was performed using Statistical 10.0 software. Data distribution was analysed with Shapiro Wilk W test. Student's t test for depended or independed data was used to compare the respective groups and pairs to compare features in two time intervals. Mann — Whitney's test and Wilcoxon's test were used for the features inconsistent with this distribution. Correlations between the parameters were calculated by the Spearman's rank test. Survival curves were generated using the Kaplan — Meier's method, and the significance of the difference in survival rates was determined by the log-rank test. Multivariate analysis was performed using a Cox's proportional hazards model. All patients with lung cancer were divided into two groups according to their IL-27, IL-29, IL-31 and IL-33 serum levels. The cut-off point was set at 32.85 pg/ml for serum IL-27, 70.17 pg/ml for serum IL-29, 12.29 pg/ml for serum IL-31 and 5.13 pg/ml for serum IL-33 concentrations. Receiver-operating characteristics (ROC) curves were applied to find the cut-off level of IL-27, IL-29, IL-31 and IL-33.

Time to progression (TTP) was defined as the time from randomization (diagnosing) until objective tumor progression (www.cancer.gov, www.fda.gov). A value of $p < 0.05$ was considered to be the level of statistical significance.

RESULTS

None of the prognostic parameters for NSCLC patients analyzed in the study was correlated significantly with the serum IL-27, IL-29, IL-31 and IL-33 levels (Table 1). The levels of IL-27, IL-29, IL-31 and IL-33 in serum and BALF are shown in Table 2. The baseline serum IL-29 levels were significantly higher in patients with advanced NSCLC than in the BBS group (p = 0.043). However, serum IL-27, IL-31 and IL-33 did not differ markedly between: NSCLC, BBS, HP and the control group (Table 2).

Table 1. Patients' characteristics

Parameter	NSCLC	BBS	HP	Control
No. of patients	45	15	8	15
Age, year mean ± SD	61.9±4	58.3±3	59.5±2	60.1±5
Gender, male/female	38/7	13/2	7/1	13/2
Weight loss (10%), yes/no	19/26	0	0	0
Performance status, 0–1/2	30/15	15	15	15
Histology, squamous cell/other	22/23	15 histology confirmed BBS	0	0
Stage, IIIB/IV/other	18/27	stage II of BBS	subacute HP	/-/
Hemoglobin (12 g/dl), low/normal	13/32	0/15	0/8	0/15
Albumin (3.5 g/dl), low/normal	9/36	0/15	0/8	0/15
LDH (450 U/L), normal/elevated	28/17	15/0	8/0	15/0
Response to chemotherapy, yes/no	30/15	/-/	/-/	/-/

Abbreviation: No = number; numerical data are shown as mean ± standard deviation

Table 2. Distribution of serum IL-27, IL-29, IL-31 and IL-33 values in patients and controls

	Concentration	Median Range			
		NSCLC (n=45)	BBS (n=15)	HP (n=8)	Control (n=15)
SERUM	IL-27 (pg/ml)	17.69	17.41	17.69	16.85
	IL-29 (pg/ml)	16.0–193.6[a] 43.2	16.0–273.3[b] 35.22	16.5–21.7[c] 43.14	16.0–284.1[d] 45.17
	IL-31 (pg/ml)	14.5–154.8[e] 10.0	18.8–81.6[f] 9.59	18.8–378.5[g] 8.72	5.7–111.3[h] 10.09
	IL-33 (pg/ml)	5.1–18.0[i] 2.83	6.8–17.3 2.77	6.3–10.5 8.05	7.3–14.8 2.23
BALF	IL-27 (pg/ml)	0.4–14.0 14.99	0.5–27.7 14.29	1.1–16.1 14.62	0.7–9.6 13.84
	IL-29 (pg/ml)	14.7–86.8[j] 20.49	14.1–15.2[k] 20.40	14.5–15.1l 23.36	13.7–15.0[m] 18.53
	IL-31 (pg/ml)	8.2–37.2[n] 11.56	12.4–31.8[o] 11.73	16.5–31.8[p] 11.75	12.4–29.9[r] 11.17
	IL-33 (pg/ml)	6.3–13.9[s] 2.92	10.2–12.7 4.32	5.0–12.9 5.65	5.8–13.7 3.38 0.6–56.9
		1.1–84.5[t]	2.6–6.2[u]	0.1–12.5	
	[a]vs[j] p=0.00001	[d]vs[m] p = 0.0009	[f]vso p = 0.001	[i]vs[s] p = 0.02	[l]vs[m] p = 0.014
	[b]vs[k] p = 0.001	[e]vs[f] p = 0.043	[g]vs[p] p = 0.035	[j]vs[m] p = 0.016	[l]vs[u] p = 0.015
	[c]vs[l] p = 0.011	[e]vs[n] p = 0.0002	[h]vs[r] p = 0.002	[k]vs[m] p = 0.005	

Abbreviations: HP – hypersensitivity pneumonitis, extrinsic allergic alveolitis; n – number; IL – interleukin.

Patients with NSCLC, BBS and HP had the concentrations of IL-27 in BALF at the same level. However, these concentrations were significantly higher than in controls. NSCLC patients also had markedly lower levels of IL-33 in BALF than patients with BBS (p = 0.015). Concentrations of IL-29 and IL-31 in BALF did not differ significantly between investigated groups. In all groups levels of IL-27 and IL-29 are significantly higher in serum than in BALF. Concentrations of IL-31 in BBS, HP and control groups tended to higher in BALF than in serum. These differences were significant in NSCLC patients (p = 0.02) (Table 2).

The serum levels of IL-29 were higher in group IIIB NSCLC than in group IV NSCLC (p = 0.034) (Table 3). We also observed a tendency to lower concentrations of IL-29 in BALF, but differences were not significant. Patients in group IIIB had the same concentrations of IL-27, IL-31 and IL-33 as patients in group IV (Table 4).

Table 3. The serum and BALF levels of IL-27, IL-29, IL-31 and IL-33 before chemotherapy in group IIIB and group IV NSCLC patients

Concentration	III B NSCLC (n = 18)		IV NSCLC (n = 27)	
	Serum	BALF	Serum	BALF
IL-27 (pg/ml)	18.26	14.91	17.13	14.99
	16.5–75.9	14.6–186.8	16.0–193.6	14.7–102.4
IL-29 (pg/ml)	62.66	18.53	43.20	20.49
	18.8–154.8[a]	10.3–29.9	14.5–92.8[b]	8.2–37.2
IL-31 (pg/ml)	9.59	11.36	10.34	11.56
	5.1–14.3	6.9–12.9	6.6–18.0	6.3–13.9
IL-33 (pg/ml)	3.77	2.69 1.1–6.7	2.57 0.7–9.3	3.15
	0.4–14.0			1.8–84.5

Abbreviations: [a]vs[b] p = 0.034.

Table 4. The serum levels of IL-27, IL-29, IL-31 and IL-33 before chemotherapy in relation to response to treatment

Concentration in serum	Response to chemotherapy		
	PR (n = 19)	SD (n = 11)	PD (n= 15)
IL-27 (pg/ml)	17.69 (16.5–31.8)	17.13 (16.0–37.2)	17.69 (16.5–193.6)
IL-29 (pg/ml)	43.2 (18.8–154.8)	39.23 (14.5–85.4)	47.14 (27.1–104.0)
IL-31 (pg/ml)	9.34 (5.1–14.3)	10.59 (8.3–18.0)	10.34 (8.8–16.3)
IL-33 (pg/ml)	2.83 (0.7–14.0)	4.31 (1.6–13.1)	2.0 (0.4–9.3)

Abbreviations: PR – partial response; SD – stable disease; PD – progressive disease.

In the group of NSCLC patients, PR was reported in 19 patients (42.2%), SD in 11 patients (24,4%), and PD in 15 patients (33.3%). No correlation was found between the serum concentrations of IL-27, IL-29, IL-31 and IL-33 before therapy and the effect of chemotherapy (Table 5). Lung cancer patients with PR after chemotherapy had significantly higher concentration of IL-27 in BALF (before treatment) than patients with SD (p = 0.006). However, patients with SD had higher levels of IL-29 in BALF than patients with progressive disease ($p = 0.048$). No correlation was found between the BALF concentrations of IL-31 and IL-33 and the effect of chemotherapy (Table 6).

Table 5. BALF levels of IL-27, IL-29, IL-31 and IL-33 before chemotherapy in relation to treatment response

Concentration in BALF	Response to chemotherapy		
	PR (n = 19)	SD (n = 11)	PD (n= 15)
IL-27 (pg/ml)	16.06 (14.8–86.8[a])	14.92 (14.6–19.6[b])	14.95 (14.7–102.4
IL-29 (pg/ml)	20.49 (8.2–37.2)	22.42 (14.5–28.1[c])	19.51 (10.3–26.2[d])
IL-31 (pg/ml)	11.95 (6.9–13.9)	11.17 (10.5–12.7)	11.36 (6.3–12.1)
IL-33 (pg/ml)	3.15 (1.1–84,5)	3.15 (1.8–8.3)	2.92 (2.0–11.7)

Abbreviations: PR – partial response; SD – stable disease; PD – progressive disease; [a]vs[b] p = 0.006; [c]vs[d] p = 0.048.

Table 6. Influence of statistically significant prognostic factors and serum parameters on survival in patients with advanced NSCLC

Prognostic factor	*p* value
Age	0.025
Weight loss	0.002
Performance status	0.001
Serum IL-27	0.657
Serum IL-29	0.765
Serum IL-31	0.456
Serum IL-33	0.732
BALF IL-27	0.345
BALF IL-29	0.451
BALF IL-31	0.421
BALF IL-33	0.527

A negative correlation was found between serum IL-31 levels before therapy and TTP of NSCLC (R = -0.45; $p = 0.038$) (Figure).

When all patients with lung cancer were divided into high and low groups using cut-off serum and BALF IL-27, IL-29, IL-31 and IL-33 concentrations, the prognoses of high and low groups were not different. The cut-off serum concentrations were 30.85 pg/ml (IL-27), 70.17 pg/ml (IL-29), 12.29 pg/ml (IL-31) and 5.13 pg/ml (IL-33). The cut-off BALF concentrations were 14.93 pg/ml (IL-27), 12.67 pg/ml (IL-29), 12.97 pg/ml (IL-31) and 4.78 pg/ml (IL-33). The median survival of patients from all groups was 10.7 months.

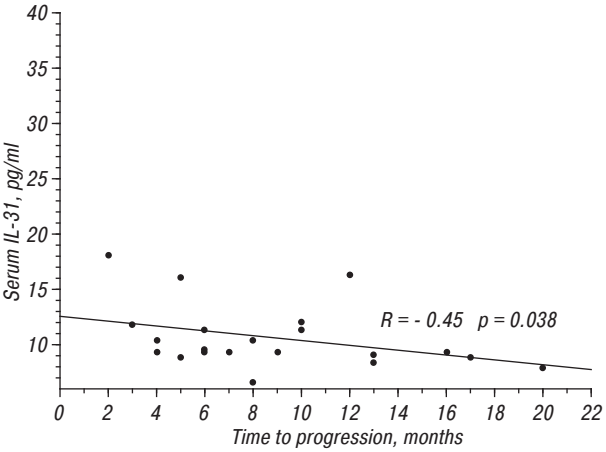

Figure. Serum IL-31 levels in relation to time to NSCLC progression

Older age, weight loss, and performance status yielded prognostic value (Table 6). Conversely, neither IL-27, IL-29, IL-31 nor IL-33 levels (serum and BALF) have proven to be significant for survival.

In NSCLC group a negative correlation was found between serum levels of IL-33 and levels of IL-27 in BALF (R = -0.44, $p = 0.005$).

We didn't found any correlation between the levels of the same cytokine in serum and BALF. The differential cell profiles in BALF are shown in Table 7. Patients with NSCLC had the highest percentage and number of macrophages ($p < 0.05$). Patients with BBS had the highest percentage and number of lymphocytes.

Table 7. Differential cell profiles in BALF

	Median Range				p, value
	NSCLC (n=45)	BBS (n=15)	HP (n=8)	Control (n=15)	
Total cell:	65.0	70.0	95.0	55.0	p<0.05
10^4 cell/ml	(25–200)	(40–120)	(50–170)	(40–155)	
Macrophages, %	80.97	65.0	33.0	80.0	p<0.05
	(15–95)	(42–94)	(17–45)	(22–90)	
n x 10^4/ml	52.0	45.5	31.35	44.0	p<0.05
	(9.7–61.7)	(29.4–65.8	(16.1–42.7)	(12.1–49.5)	
Lymphocytes, %	9.0 (2–49)	33.0	63.0	18.0	p<0.05
		(4–56)	(53–82)	(8–38)	
n x 10^4/ml	5.85	23.1	59.85	9.9	p<0.05
	(1.3–31.8)	(2.8–39.2)	(50.3–77.9)	(4.4–20.9)	
Neutrophils, %	1.0	1.0	1.0 (0–3)	0	/-/
	(0–760)	(0–1.0)			
n x 10^4/ml	0.65	0.7	0.95	0	/-/
	(0–49.4)	(0–0.7)	(0–2.85)		
Eosinophils, %	1.0 (0–6)	1.0 (0–1)	1.0 (0–2)	0	/-/
n x 10^4/ml	0.65	0.7	0.95	0	/-/
	(0–3.9)	(0–0.7)	(0–1.9)		

DISCUSSION

Tumor growth and metastases formation is controlled by several pathological processes and are mediated by inhibitors and stimulators. In NSCLC group the negative correlations between serum levels of IL-33 and levels of IL-27 in BALF, obtained in our study, prove mutual inter-relations between factors mentioned above.

To our knowledge, this is the first report on serum and BALF levels of IL-27, IL-29, IL-31 and IL-33 in NSCLC determined by ELISA technique.

In our study, concentrations of antyproliferative and antyangiogenic factors such as IL-27, IL29, were lower in BALF than in serum. We observed a tendency for the highest concentrations of IL-27 and IL-29 (serum and BALF) in NSCLC group. This reflects a weaker local (BALF) than systemic (in serum) immune/antyproliferative response. The high levels of IL-27, IL-29 in NSCLC provide a particularly weakened defense mechanism in cancer patients. Moreover, it is well known that cancer patients have the most severe angiogenesis. Higher levels of IL-27 in serum than in BALF may be due to the origin of this cytokine. It may be released from endothelial cells, hematopoietic cells, Th17 lymphocytes, B lymphocytes, and NK cells [22–24]. This can be also the likely reason for higher BALF concentrations of IL-27 in BBS than healthy individuals. Increased expression of IL-27 in sarcoidosis patients was observed by Larousserie et al. [25]. It is known about the increased angiogenic activity in patients with sarcoidosis [26]. Sekiya et al. [26] concluded that serum VEGF values in patients with sarcoidosis are a predictive factor in determining extrathoracic organ involvement and as a parameter that determines the treatment with corticosteroid. Serum VEGF might be a useful prognostic marker in sarcoidosis. The results of our study are in accordance with the findings of Sekiya et al. [26] because BALF levels of IL-33, proangiogenic factor, were higher in BBS patients than in NSCLC. The results of our study in NSCLC group are in accordance with the findings of other authors based on TNF-alfa (tumor necrosis factor-alpha) [27].

ROC curve analysis did not confirm the usefulness of IL-27, IL-29, IL-31 and IL-33 concentrations in serum and BALF for lung cancer diagnostics.

In our study, the serum levels of IL-29 were higher in group IIIB NSCLC than in group IV NSCLC. These findings are in conformity with the studies of Singhal S et al. [28]. The crucial to the antiproliferative effect of IL-29 on NSCLC is upregulation of p21 expression which has been reported to be a predictor for a favorable prognosis [28] Patients in stage IIIB have better prognosis than in stage IV (patients with distant metastases).

We showed that BALF IL-27 concentration before treatment was markedly higher in patients with PR after chemotherapy than in those with SD. Moreover, patients with SD had higher BALF levels of IL-29 than patients with PD. We have shown that higher concentrations of inhibitors of tumor growth in BALF may contribute to response to chemotherapy. The results of our study are in accordance with the data of Shimizu et al. [9]. This study [9] has shown that malignant melanoma cells transfected with IL-27 genes had lower VEGF levels and reduced vasculature in lung metastasis compared with animals cells that did not express IL-27. Therefore, IL-27 can inhibit tumor progression by several mechanisms simultaneously, regardless of tumor immunogenicity, potentially making it a valuable therapeutic tool in poorly immunogenic tumors. Importantly, IL-27 inhibits the growth of both primary tumors and metastases [29]. Moreover, IL-27 may also influence other cells, including Th7 lymphocytes, B lymphocytes, and NK cells [22–24].

Newly identified cytokine, IL-29 is considered to belong to the IFN family and designed as type III IFN. In addition to its primarily reported antiviral activity, type III IFN has also been shown to have direct antitumor action against several human tumor lines including colon cancer [30] and esophageal carcinoma [31]. There are no data about serum, BALF concentrations of IL-27 and IL-29 in NSCLC patients.

In our study, we found a negative correlation between serum IL-31 levels before therapy and time to NSCLC progression (after chemotherapy). We confirm the results of study of Ip et al. [13] which have shown that IL-31 can significantly elevate the gene and protein expressions of EGF and VEGF [13].

In conclusion, patients in stage IIIB of NSCLC had higher serum levels of IL-29 than in stage IV. Higher BALF levels of IL-27 or IL-29 before therapy may suggest a better response to chemotherapy. Higher serum levels of IL-31 before therapy may suggest shorter TTP. Our results require confirmation in a larger group of patients. Determination of IL-27, IL-29 and IL-31 in serum and BALF can be useful in clinical practice, but their practical significance needs further studies.

CONFLICTS OF INTEREST

The authors had no conflicts of interest to declare in relation to this article.

ACKNOWLEDGMENTS

We are indebted to Robert Mroz for his help with the ELISA equipment. This work was granted by Polish Ministry of Science and Higher Education (NN 401 230134; 2301/B/P01/2008/34).

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