IL-6 and VEGF in Small Cell Lung Cancer Patients

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Abstract. *Recent data suggest a link between chronic inflammation, angiogenesis, and the development of cancer. The aim of this study was the evaluation of serum IL-6 and VEGF in comparison with the tumor markers NSE and ProGRP, with respect to the prognosis of small cell lung cancer patients. The study of IL-6, VEGF, NSE, ProGRP and platelet count was performed in a group of 72 patients with previously untreated small cell lung cancer at different stages of disease: 40 with limited and 32 with extensive disease. Significantly higher IL-6 and VEGF concentrations and platelet count, as well as NSE and ProGRP levels, were found in patients with small cell lung cancer in comparison with the reference group. Patients with extensive cancer had significantly higher levels of IL-6, VEGF, NSE and ProGRP than those with limited cancer. Elevated VEGF levels, with no significant differences in frequency of elevated NSE and ProGRP concentrations, were often observed in patients with IL-6 levels higher than 5.1 ng/l. Univariate analysis confirmed a significant relationship not only between overall survival and stage of disease or gender, but also with VEGF, IL-6, NSE and ProGRP levels. Moreover, multivariate analysis revealed that only the extent of the disease and IL-6 may be independent prognostic factors in the group of small cell lung cancer patients under investigation. However, simultaneous determinations of ProGRP and IL-6, as well as ProGRP and VEGF, in addition to the extent of the disease, may serve as additional, independent prognostic factors in small cell lung cancer.*

Interleukin 6 (IL-6), a multifunctional cytokine that was initially recognized as a regulator of immune and inflammatory responses, is also known to be a major growth modulators of many tumor types. Moreover, it has been suggested that IL-6 may play a significant role in carcinogenesis, and in the interaction between cancer and host organism as a potential mediator in the development of cancer cachexia (1, 2). Multiple studies have confirmed elevated IL-6 levels in the serum of patients with certain neoplastic diseases, including lung cancer, and in particular non-small cell lung cancer, and revealed the relationship between elevated levels of this cytokine and poor clinical prognosis (3-5).

On the other hand, angiogenesis is one of the processes recognized as being essential for tumor growth and metastasis (6). Various growth factors have been shown to stimulate angiogenesis in physiological and pathological conditions, including neoplastic disease (7, 8). Vascular endothelial growth factor A (VEGF-A), a member of the vascular permeability family, is known to be one of the major factors in angiogenesis, and therefore appears to play a crucial role in the proliferation and migration of endothelial cells (9, 10).

The aim of this study was the evaluation of serum IL-6 and VEGF in comparison with the tumor markers neuron-specific enolase (NSE) and pro-gastrin releasing peptide (ProGRP), with respect to the prognosis of small cell lung cancer patients.

Patients and Methods

The study of IL-6, VEGF, NSE, ProGRP and platelet count was performed in a group of 72 patients with previously untreated small cell lung cancer (SCLC) at different stages of disease: 40 with limited and 32 with extensive disease. The patients were, treated in the Oncology Center, M. Skłodowska-Curie Memorial Institute, Cracow Division between 2000 and 2006. For all patients, the diagnosis of SCLC was confirmed by the histological examination of biopsy and cytological specimens taken during bronchoscopy. In order to establish the disease stage, the following investigations were applied to each patient: physical examination, chest radiography, computed tomography of chest, upper abdomen and brain, bone scintigraphy. The study was also performed in a reference group consisting of 36 gender- and age-matched healthy persons.

Venous blood samples were drawn, and centrifuged at 3000 rpm for 10 minutes. The serum samples were then stored at –80˚C until required for testing.

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Key Words: IL-6, VEGF, NSE, ProGR, prognosis of SCLC patients.

Determinations of IL-6, VEGF subunit A and ProGRP levels were carried out using an enzyme-linked immunosorbent assay (ELISA) and reagent kits manufactured by Bender MedSystems (Germany), and ALSI (Japan) respectively. NSE was determined using electrochemiluminescence method and reagent kits as well as Elecsys 2010 analyzer (Roche Diagnostics GmbH, Germany). Platelet number was measured in Cell Dyn 3700 hematological analyzer (Abbott Diagnostics Division, Germany).

Statistical software Statistica 8.0 (StatSoft, Poland) was used for all analysis. The Mann-Whitney *U*-test and Chi-square test were applied to compare different groups for continuous variables and for categorical variables, respectively. Spearman's correlation method was used to evaluate relationships between the parameters. Overall survival was calculated using the Kaplan-Meier method. To assess the effects of the variables on survival, the log-rank test was applied, while multivariable regression model was performed using the Cox's model.

Results

In SCLC patients, in comparison with the reference group, significantly higher IL-6, VEGF concentrations and platelet count were found, as well as NSE and ProGRP levels (Table I). Elevated levels of IL-6 above 5.1 ng/l, VEGF higher than 401.5 ng/l, platelet count higher than 390 $\times 10^3$/μl, NSE above 19.0 μg/l and ProGRP above 48.0 ng/l were observed in 51.4%, 45.8%, 19.4%, 72.2% and 73.8% of patients, respectively.

In the SCLC group, positive correlations were found between serum VEGF and IL-6 (r_s=0.274, p=0.020), NSE and IL-6 (r_s=0.317, p=0.007), VEGF and platelet count (r_s=0.353, p=0.002) as well as between NSE and ProGRP (r_s=0.377; p=0.001). However, there was no correlation between VEGF and tumour markers as well as between IL-6 and ProGRP levels.

Significant differences were observed between groups separated in respect to stage of disease. Patients with extensive disease had significantly higher levels of IL-6, VEGF, NSE and ProGRP than those with limited cancer. No significant differences in platelet counts between the two groups were observed (Table II). Moreover, in the group with extensive disease, more frequent elevated levels of these factors were found (Figure 1).

The analysis of the receiver operating characteristic (ROC) curves drawn for the group of patients with extensive against limited disease and comparison of the areas under the curve (AUC) revealed that IL-6 correlated slightly better with tumor stage in SCLC than did VEGF, NSE and ProGRP (Figure 2).

In the subgroup of patients with serum IL-6 levels higher than 5.1 ng/l, elevated VEGF levels often occurred with a lack of significant differences in the frequency of elevated NSE and ProGRP concentrations (Figure 3).

Univariate analysis confirmed a significant relationship not only between overall survival and stage of disease or gender, but also with VEGF, IL-6, NSE and ProGRP levels (Table III). Patients with elevated levels of these latter factors had significantly shorter survival times. The multivariate analysis revealed that only the extent of the disease and IL-6 may be independent prognostic factors in the group of SCLC patients under investigation.

However, when we analyzed tumor markers complementary to determinations of VEGF and IL-6, it was shown that relative risk of death in SCLC was two times lower in patients with limited disease, ProGRP lower than 670 ng/l, IL-6 lower than 6.0 ng/l and VEGF lower than 425 ng/l (Table IV, and Figures 4 and 5).

Table I. *Results of the investigated parameters in SCLC patients and in the reference group.*

Parameter		Reference group	SCLC	*p*-Value
IL-6	Median	2.51	5.73	0.000
(ng/l)	Range	0.00-15.55	0.00-150.42	
NSE	Median	9.40	35.94	0.000
(μg/l)	Range	4.85-19.64	3.05-323.30	
ProGRP	Median	18.55	333.93	0.000
(ng/l)	Range	3.80-72.89	9.46-7555.90	
VEGF	Median	125.48	364.65	0.000
(ng/l)	Range	45.00-525.79	15.31-1255.08	
PLT	Median	242.0	294.0	0.000
($\times 10^3$/μl)	Range	197.0-392.0	77.0-769.0	

Table II. *Results of the investigated parameters according to the extent of disease.*

Parameters		SCLC-LD	SCLC-ED	*p*-Value
IL-6	Median	2.90	8.70	0.0014
(ng/l)	Range	0.00-150.42	0.21-50.00	
NSE	Median	23.62	44.24	0.0018
(μg/l)	Range	3.05-270.70	9.45-323.30	
ProGRP	Median	250.95	727.75	0.0135
(ng/l)	Range	9.46-2889.40	20.05-7555.90	
VEGF	Median	253.55	517.76	0.0041
(ng/l)	Range	15.31-1166.36	15.31-1255.08	
PLT	Median	272.0	316.5	N.S.
($\times 10^3$/μl)	Range	161.0-571.0	178.0-749.0	

SCLC-LD: SCLC patients with limited disease; SCLC-ED: SCLC patients with extensive disease; N.S.: non-significant.

Discussion

SCLC, one of the subtypes of lung cancer, is an aggressive and rapidly growing neoplasm with metastatic lesions in regional lymph nodes or distant organs at the time of diagnosis. Nevertheless, SCLC is highly sensitive to

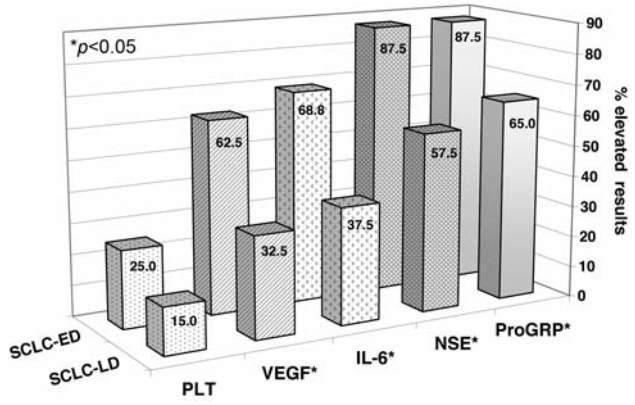

Figure 1. *Frequency of elevated results of studied parameters depending on the extent of disease (limited disease, LD, and extensive disease, ED).*

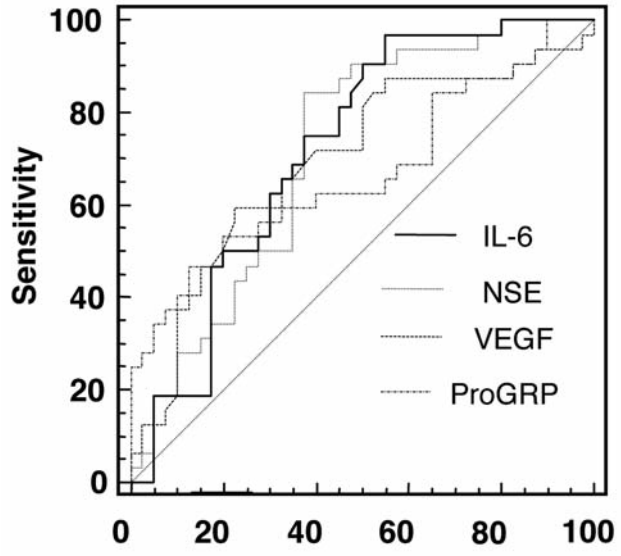

Figure 2. *ROC curves for studied parameters in SCLC patients (extensive disease versus limited disease). Area under ROC curves (AUC) were for IL6 0.721±0.06, NSE 0.716±0.06, VEGF 0.696±0.06, and for ProGRP 0.670±0.07.*

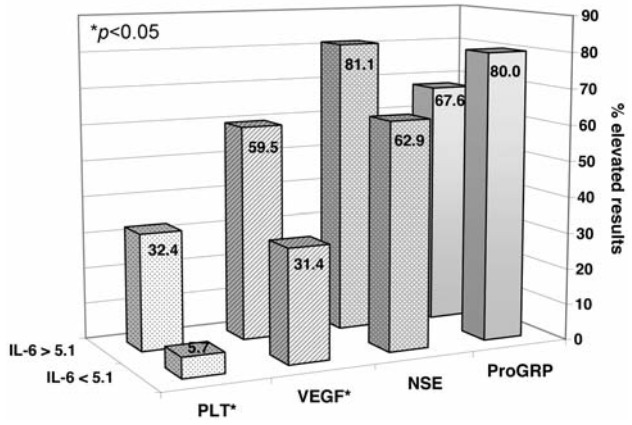

Figure 3. *Frequency of elevated results of studied parameters depending on IL-6 level (<or>5.1 ng/l).*

chemotherapy and radiotherapy. Despite major advances in systemic therapy, the prognosis of patients with SCLC still remains poor. It may be ascribed not only to the advancement of disease at the time of diagnosis, but also to its biological aggressiveness and relatively limited utility of diagnostic methods for early detection, especially in respect to new treatment modalities. Accordingly, sensitive and reliable tumor markers may provide useful information for treatment strategies of SCLC. Although NSE has been used as a tumor marker of SCLC, clinical experience has revealed several disadvantages including low positive rates in patients with limited disease and relatively high frequency of elevated levels in patients with advanced non-small cell cancer (11, 12). Recently, ProGRP has appeared as a valuable tumor marker for SCLC. In the opinion of many investigators, determination of this marker has come to play an important role in the diagnosis and monitoring of treatment as well as in the detection of relapses in patients with SCLC (11, 13). However, the prognostic value of NSE, as well as ProGRP, remains a subject of controversy (11, 12). IL-6, a pleiotropic cytokine with a wide range of biological roles in immune regulation, hematopoiesis, inflammation, and carcinogenesis, is produced by various types of normal and transformed human cells involved in regulation of immune response, acute phase reaction, cell differentiation and cell proliferation (1, 5). There are several reports suggesting that IL-6 contributes to tumor progression, directly and indirectly, through inhibition of the anti-tumor response by host cells (14, 15). However, the mechanism responsible for the elevated levels of serum IL-6 in cancer patients remains unclear. It could be the effect of abnormal production by tumor cells as well as the result of host organism immune system response (2). In this study, the significantly higher frequency of elevated IL-6 levels observed in patients with extensive disease as well as the correlation between NSE and IL-6, seemed to confirm the role of tumor cells in the production of this cytokine by cancer cells. However, this relationship could also be associated with the suggested role of IL-6 in the promotion of neuroendocrine differentiation (16). Unfortunately, data regarding IL-6 in SCLC are exceptionally rare.

In recent years, close relationships have been established between cancer cachexia, hypermetabolism, acute phase response and cytokine levels, especially IL-6 which belongs, besides TNF-α, IFN-γ and IL-1, to the so-called "classic" cachectic cytokines (17). The pathomechanism of cancer

Table III. *Parameters influencing survival of SCLC patients - results of univariate and multivariate analysis.*

Parameter	Variant	N	Median survival (months)	*p*-Value	RR	CI	*p*-Value
Extent	SCLC-LD	40	17.7	0.0012	1	1.29-3.77	0.004
of disease	SCLC-ED	32	9.0		2.21		
Gender	Female	24	15.0	0.0216	-	-	
	Male	48	11.0				
Age (years)	<60	42	12.0	N.S	-	-	
	>60	30	11.0				
IL-6	<6.0	37	14.0	0.0024	1	1.21-3.69	0.008
(ng/l)	>6.0	35	11.0		2.11		
NSE	<32.0	31	15.0	0.0043	-	-	
(μg/l)	>32.0	41	11.0				
ProGRP	<670.0	45	14.5	0.048	-	-	
(ng/l)	>670.0	27	11.0				
VEGF	<420.0	41	14.0	0.043	-	-	
(ng/l)	>420.0	31	9.0				
PLT	<395.0	59	14.0	0.043	-	-	-
$\times 10^3/\mu l$	>395.0	13			9.0		

SCLC-LD: SCLC patients with limited disease; SCLC-ED: SCLC patients with extensive disease; N.S.: non-significant; N: number of patients; RR: relative risk; CI: 95% confidence interval.

cachexia is multifactorial and involves a variety of proinflammatory cytokines, participating both in the promotion and the progression of cachectic process (3). Apart from weight loss and reduced food intake, systemic inflammation has recently been proposed as a third factor defining cachectic cancer patients (14). Weight loss is the most evident symptom of cancer cachexia, but can appear relatively late, mainly in patients with advanced stages of disease. Deficit of energetic and structural substrates can have direct or indirect character as the result of intensive demand. It is well recognized now that up-regulated synthesis of acute phase response proteins is co-responsible for the tissue wasting observed in cachexia and that IL-6 and IL-8 have been involved in the initial phase of this process (15). IL-6 has relatively the long biological half-time life, which may explain why this cytokine and not TNF-α or IL-1, is frequently found to be elevated in cancer and associated with weight loss (3, 15).

Chronic inflammation has become a recognized risk factor for epithelial-derived malignancies (17) Chronic inflammation can be the effect of infection, autoimmune disease, malignant and benign tumors, or other pathologies, and results in the infiltration of inflammatory cells at specific sites in the body. Inflammation is thought to contribute to the development and progression of various types of cancer, including lung, breast, gastrointestinal, ovarian, prostate skin and liver cancer (14, 17).

Lung cancer patients show high prevalence of malnutrition, which is related to worse prognosis. A few studies in lung cancer patients have shown resting energy expenditure about 20% higher than expected (5). Systemic IL-6 can be considered a mediator of malnutrition in lung cancer patients. Yanagawa *et al*. reported raised IL-6 serum levels in lung cancer patients in relation with the intensity of acute phase response (18). Expression of G-CSF and IL-6 genes has been demonstrated in lung cancer cell line and the production of multiple cytokines in lung cancer patients may be related to their poor prognosis (3, 5). This observation may be confirmed by the values of IL-6 presented in this study, which, in addition to the stage of disease, is an independent prognostic factor in patients with SCLC.

Angiogenesis is the process responsible for the formation of new blood vessels from existing vasculature. Whereas in healthy adults angiogenesis occurs only during female reproductive cycles, in chronic inflammatory diseases and tumors, it is necessary to supply energetic and structural substrates in amounts adequate to the increased metabolic needs (9, 19). One of the angiogenic polypeptides identified in tumor tissue is the vascular endothelial growth factor (VEGF). VEGF has been shown to be hypoxia inducible *in vitro* and *in vivo* (9, 20, 21). VEGF-mediated angiogenesis is considered to play an essential role in cancer progression and in the metastatic process (22-24). VEGF not only stimulates the proliferation of tumor blood vessels, but also increases vascular permeability, possibly contributing to tumour-cell extravasation and metastatic formation (25). Elevated serum VEGF has been found in various types of disseminated cancer, including breast, colorectal and lung cancer (7, 26-29). In the SCLC patients investigated in this study, VEGF higher than 401.5 ng/l was observed in 45.8% of them, significantly more often in patients with extensive disease. The observed relationship between VEGF and platelet counts may confirm the opinion of Salven *et al*. who suggested that platelets contain VEGF, which can be released when blood samples are subjected to prolonged incubation (23). Moreover in this study, significant relationships were found not only between VEGF and platelet count, but also between VEGF and IL-6. This is in agreement with the current opinion regarding the relationship between VEGF and inflammatory mediators (19). In addition, contrary to the opinion of Hasegawa *et al.,* in the group of SCLC patients in this study, a significant relationship between VEGF and NSE (29) was observed. Patients with serum VEGF levels higher than 420 ng/l had significantly shorter overall survival than those with lower levels of this growth factor. Salven *et al*. found that high pretreatment serum VEGF was not only associated with poor response to therapy and shorter survival, but also had independent prognostic value in SCLC patients (23). This observation could not be confirmed when multivariate

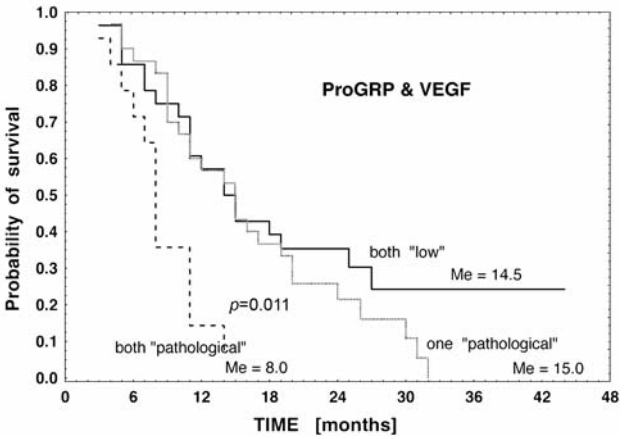

Figure 4. *Probability of SCLC patient survival depending on the both ProGRP and VEGF levels. "low": VEGF ≤420 ng/l; ProGRP ≤670 ng/l; "pathological": VEGF >420 ng/l; ProGRP >670 ng/l; Me, median survival in month.*

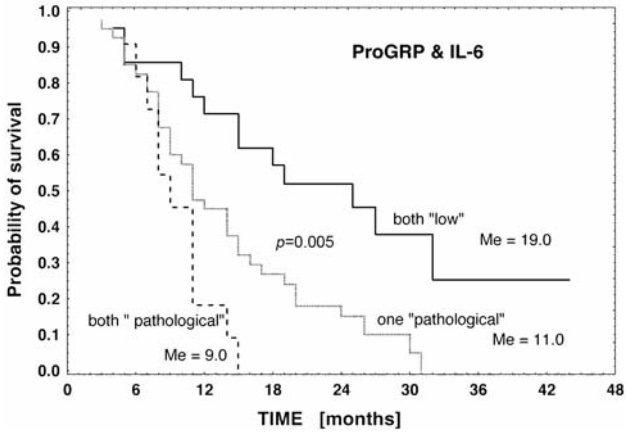

Figure 5. *Probability of SCLC patient survival depending on the both ProGRP and IL-6 levels. "low": IL-6 ≤6.0 ng/l; ProGRP ≤670 ng/l; "pathological": IL-6 >6.0 ng/l; ProGRP >670 ng/l; Me, median survival in month.*

Table IV. *Results of univariate and multivariate analysis with the use of complementary variables.*

Parameter	Variant	N	Median survival (months)	*p*-Value	RR	CI	*p*-Value
Extent	SCLC-LD	40	17.7	0.0012	1		0.009
of disease	SCLC-ED	32	9.0		2.05	1.19-3.53	
Gender	Female	24	15.0	0.0216	-	-	
	Male	48	11.0				
Age (years)	<60	42	12.0	N.S	-	-	
	>60	30	11.0				
NSE & IL-6	Both "low"	19	19.0	0.0007	-	-	
	one/both "pathological"	53	11.0				
ProGRP & IL-6	Both "low"	21	20.8	0.0002	1		0.011
	one/both "pathological"	51	11.0		2.44	1.23-4.84	
VEGF & Il-6	Both "low"	26	18.0	0.0016	-	-	
	one/both "pathological"	46	11.0				
VEGF & NSE	Both "low"	20	18.0	0.0001	-	-	
	one/both "pathological"	52	11.0				
VEGF & ProGRP	One/ both "low"	58	15.0	0.010	1		0.026
	both "pathological"	14	8.0		2.18	1.10-4.31	

SCLC-LD: SCLC-LD: SCLC patients with limited disease; SCLC-ED: SCLC patients with extensive disease; N.S.: non-significant; N: number of patients; RR: relative risk; CI: 95% confidence interval. "low": IL-6 <6.0 ng/l; VEGF <420 ng/l; NSE <32.0 μg/l; ProGRP <670 ng/l. "pathological": IL-6 >6.0 ng/l; VEGF >420 ng/l; NSE >32.0 μg/l; ProGRP >670 ng/l.

analysis was used in the SCLC patients investigated in this study, (29, 30). However, multivariate analysis of tumor markers, VEGF and IL-6, revealed that the relative risk of death in SCLC was two times lower in patients with limited disease as well as with low levels of ProGRP and IL-6 or low levels of ProGRP and VEGF.

In conclusion, in the group of SCLC patients under study there were observed relationships between levels of IL-6, VEGF, NSE as well as ProGRP and stage of disease. The elevated levels of these four biomarkers as well as stage of disease are associated with pour outcome of patients. It was revealed that stage of disease and IL-6 are independent prognostic factors in small cell lung cancer. Moreover simultaneous determinations of ProGRP and IL-6 as well as ProGRP and VEGF, besides extensive disease may serve as additional, independent prognostic factors. The correlation between IL-6 and NSE may indicate the postulated role of this proinflammatory cytokine in neuroendocrine differentiation.

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