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Leptin serum levels in cachectic and non-cachectic lung cancer patients

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Abstract

Introduction: Leptin, protein taking part in body mass regulation, might play a role in cancer cachexia development. The aim of the study was to measure leptin serum levels in cachectic, non-cachectic lung cancer patients, healthy controls and to correlate leptin concentration with nutritional status markers.

Material and methods: 40 lung cancer patients were enrolled into the study: 20 with cachexia, 20 without cachexia, and 10 healthy controls. Leptin serum concentration, body mass, BMI, arm circumference and skin triceps fold thickness were measured in each subject.

Results: Serum leptin level in cachectic cancer patients was significantly lower than in non-cachectic and healthy controls. Leptin concentration correlated with body mass, arm circumference and skin triceps fold thickness.

Conclusions: Cachectic lung cancer patients have significantly lower serum leptin concentrations than non-cachectic patients and healthy controls which may suggest, that leptin does not play an important role in cancer cachexia development. Leptin levels positively correlate with good nutritional status markers. Non-cachectic lung cancer patients have similar leptin serum levels as healthy controls.

Key words: lung cancer, cancer cachexia, leptin

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Introduction

Cancer anorexia-cachexia syndrome (CACS) is one of the causes of health deterioration and a negative predictive factor of treatment response. It is also a negative prognostic factor in cancer patients. At the moment of death the CACS syndrome is found in 80% of cancer patients [1]. The similarity of biochemical processes taking place in patients with CACS to those of certain infectious diseases focused the attention of scientists on the inflammatory reaction and the role of multiple proteins (hormones, cytokines and others) in the CACS pathogenesis [2].

Leptin, discovered in 1994, is a protein released from adipocytes, produced by the obesity

gene (*ob*), built of 167 aminoacids [3]. Research on mice has shown that leptin has a strong potential to inhibit appetite and to stimulate the processes of energy consumption. That is why leptin given to mice causes weight loss [4]. Such an effect has not been observed in people: obese patients have high leptin concentration [5], which is attributed to leptin resistance [6]. Leptin has been a subject of many investigations, but its role in controlling body mass remains unclear [7]. Some of these studies concern the pathogenesis of cancer anorexia-cachexia syndrome [2, 8]. Until now serum leptin concentrations in cachectic and non-cachectic lung cancer patients have not been compared. The aim of this study was to evaluate serum leptin concentrations in the gro-

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ups of lung cancer patients with and without cachexia when compared to healthy controls, and to explore the correlations between serum leptin concentration level and the antropometric indicators of cancer cachexia: body mass, arm circumference and skin triceps fold thickness.

Material and methods

Forty lung cancer patients were enrolled into the study (25 males, 15 females); 50–75 years old (mean 61): 20 with cachexia, 20 without cachexia (Table 1). The diagnosis of lung cancer was made using histopathology. The patients with weight loss $\geq 10\%$ in 3 months before the diagnosis were classified as cachectic. Nutrition status was estimated by: 1. Body mass index (BMI), 2. Antropometric indicators: arm circumference (normal value in male > 23 cm, in female > 22 cm) and skin triceps fold thickness (normal value in male > 10 mm, in female > 13 mm). For every patient TNM classification and performance status according to the WHO were established. Fifteen healthy persons formed the control group (5 males, 10 females), age 28–77 years, mean 44 (Table 1 and 2).

Fasting blood for leptin serum concentration was drawn from every patient at about 7.30 AM, always before the beginning of the oncologic treatment as far as the lung cancer patients were concerned. 9 ml of blood was collected to a Saerstedt monovette system, which was then centrifugated for 10 minutes in 4°C by 2000 g. Obtained serum was kept in -80°C until further estimation. Leptin serum concentrations were measured with the use of Human Leptin Elisa set (BioVentor — Laboratorni medicina a.s. Czechs). The statistical analysis was performed with the use of computer program Statistica™. Mean, median, standard deviation (SD), standard error of measurement (SEM) were calculated for each group. In case of the parametric distribution of variables Student t-test was used for independent trials or for pairs. For groups with the non-parametric distribution, Wilcoxon test was used in case of dependent variables comparison, whereas U Mann Whitney's test in case of the independent variables comparison. In the pool analysis Chi² test with Yates correction was employed because of a low number of patients enrolled into the analyzed groups. The differences between analyzed groups were assumed as stati-

Table 1. Patients and healthy controls characteristics

Patients	Without cachexia	With cachexia	Healthy
Sex			
Men (M)	10 (50%)	15 (75%)	5 (33%)
Women (K)	10 (50%)	5 (25%)	10 (67%)
Diagnosis			
NSCLC			
IIIA	3 (19%)	3 (18%)	
IIIB	5 (31%)	8 (47%)	
IV	8 (50%)	6 (35%)	
SCLC			
ED	1 (25%)	1 (33%)	
LD	3 (75%)	2 (67%)	

NSCLC — non-small cell lung cancer; SCLC — small cell lung cancer; ED — extensive disease; LD — limited disease

Table 2. Healthy controls

	n	Mean	Median	Minimum	Maximum	SD	SEM
Age (years)	15	44	36	28	77	16.5	4.3
Mass [kg]	15	73	76	55	100	13.9	3.6
BMI [kg/m ²]	15	24	23	19	31	4.0	1.0
Arm [cm]	15	28	28	24	35	2.9	0.8
Fold [cm]	15	2.0	1.9	1.5	2.5	0.3	0.1
Leptin [ng/ml]	15	10	8	1	23	7.6	2.0

BMI — body mass index; SD — standard deviation; SEM — standard error mean

stically significant if p-value was less than 0.05. Dependences among the analyzed data were estimated with the help of the linear correlation ratio. The linear correlation ratios (R) were assumed as statistically significant if p-value was less than 0.05.

This trial was approved by the Bioethical Committee of Wrocław Medical University.

Results

In the non-cachectic group there were: 10 males and 10 females; 16 patients with non-small cell lung cancer (NSCLC) and 4 patients with small cell lung cancer (SCLC). Body mass in this group, 3 months before establishing the diagnosis was 55–107 kg (mean 77), BMI 20–36 (mean 27) and at the time of diagnosis: 55–107 kg (mean 77), BMI 20–36 kg (mean 27). In the group of NSCLC: 3 patients had the stage IIIA, 5 — stage IIIB, 8 — stage IV. In the group of SCLC, 3 patients had a limited disease and 1 patient — an extensive disease (Table 1). The performance status according to WHO was 0 in one patient, 1 in nine patients, 2 in seven patients and 3 in three patients. Arm circumferences and skin triceps fold thicknesses are shown in Table 3.

In cachectic group there were: 15 males and 5 females; 17 patients with NSCLC and 3 patients SCLC. Body mass in this group 3 months before establishing the diagnosis was 50–119 kg (mean 76), BMI 18–39 (mean 27). At the time of diagnosis: 43–107 kg (mean 64), BMI 16–33 kg (mean 22).

In the group of NSCLC: 3 patients had the stage IIIA, 8 — IIIB, 6 — IV. In the group of SCLC 1 patient had a limited disease and 2 patients — an extensive disease (Table 1). Performance status according to WHO was 0 in one patient, 1 in nine patients, 2 in five patients and 3 in five patients. Arm circumferences and skin triceps fold thicknesses are shown in Table 4.

The characteristics of healthy controls (age, body mass, BMI, arm circumferences and skin triceps fold thicknesses) are shown in Table 2.

Leptin serum concentrations in both groups and healthy controls are shown in Tables 2–4.

Leptin concentration was significantly decreased in cachectic patients in comparison to both non-cachectic patients ($p = 0.012$, χ^2 in Yates' correction: 6.24) and healthy controls ($p < 0.001$). In 75% patients with cancer cachexia serum leptin concentration was below median value observed in healthy persons, whereas in cancer patients without cachexia it was found in only 30% (Fig. 1, $p = 0.01$, χ^2 in Yates' correction: 6.42).

We examined correlation between leptin serum concentration and nutrition status indicators like: arm circumference, skin triceps fold thickness and body mass. Though there was no difference in the initial body mass between groups of patients with and without cachexia ($77 \text{ kg} \pm 3 \text{ SEM}$ vs. $76 \text{ kg} \pm 4 \text{ SEM}$; Table 3, 4) — serum leptin concentration showed significant correlation with body mass 3 months before the diagnosis of lung cancer (Fig. 2A, $R = 0.4$, $p = 0.008$).

Table 3. Patients without cachexia. Measure I of body mass and BMI: 3 months before diagnosis. Measure II of body mass and BMI and measure of skin triceps fold thickness and arm circumference: at the time of diagnosis

	n	Mean	Median	Minimum	Maximum	SD	SEM
Age (years)	20	61	59	50	75	8	2
Mass [kg]							
Measure I	20	77	73	55	107	13	3
Measure II	20	74	70	55	107	14	3
Change (%)*	20	96	97	91	100	3	1
BMI [kg/m ²]							
Measure I	20	27	28	20	36	4	1
Measure II	20	26	27	20	36	4	1
Change (%)*	20	96	97	92	100	3	1
Arm [cm]	20	29	29	23	39	4	1
Fold [cm]	20	2	2	1	3	1	0
Leptin [ng/ml]	20	12	12	1	33	9	2

*Change (%) — percentage change of body mass: measure II comparing to measure I; BMI — body mass index; SD — standard deviation; SEM — standard error mean

Table 4. Patients with cachexia. Measure I of body mass and BMI: 3 months before diagnosis. Measure II of body mass and BMI and measure of skin triceps fold thickness and arm circumference: at the time of diagnosis

	n	Mean	Median	Minimum	Maximum	SD	SEM
Age (years)	20	57	56	42	83	11.5	2.5
Mass [kg]							
Measure I	20	76	68	50	119	20.2	4.4
Measure II	20	64	57	43	107	19.2	4.
Change (%)*	20	84	86	65	90	6.6	1.4
BMI [kg/m ²]							
Measure I	20	27	25	18	39	5.5	1.2
Measure II	20	22	21	16	33	4.5	1.0
Change (%)*	20	84	86	65	90	6.6	1.4
Arm [cm]	20	24	23	19	33	3.7	0.8
Fold [cm]	20	1.3	1.2	0.7	2.4	0.5	0.1
Leptin [ng/ml]	20	6.0	1.6	0.1	27.2	7.8	1.7

Change (%) — percentage change of body mass: measure II comparing to measure I; BMI — body mass index; SD — standard deviation; SEM — standard error mean

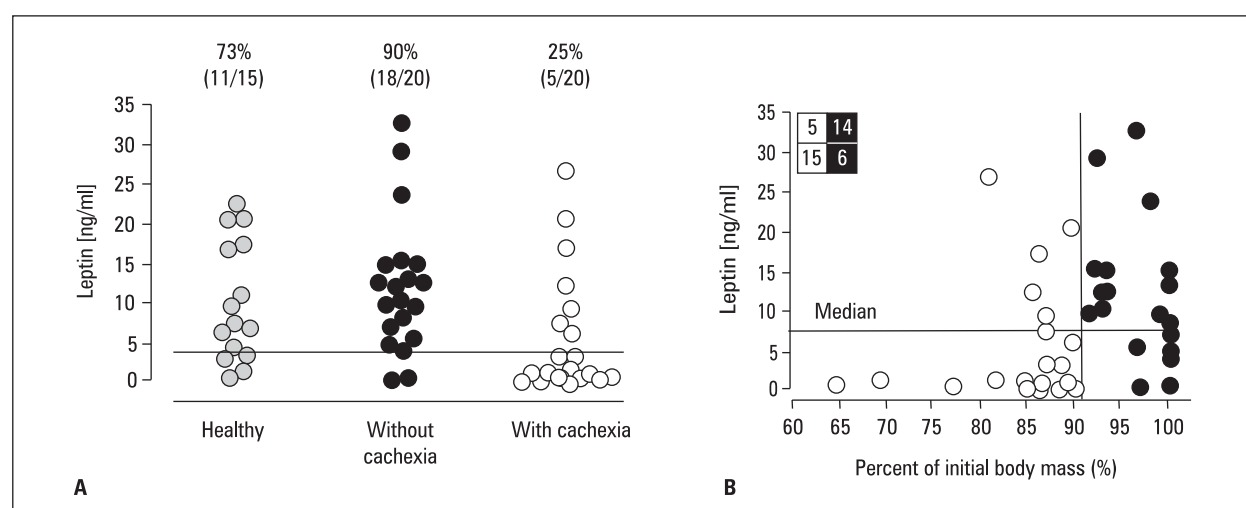


Figure 1. Leptin concentrations in lung cancer patients and in healthy controls. **A.** Comparison of leptin serum concentration in healthy controls, lung cancer patients without cachexia and lung cancer patients with cachexia. Values above each group express the percentage of high leptin concentrations (above the line defining the median of leptin serum concentrations in healthy controls) ($p < 0.02$ Yates cor.: < 6.2). **B.** Leptin serum concentrations in relationship to the change of body mass in %. Patients with cancer cachexia: white points

This correlation became even more pronounced and its significance increased to $p \leq 0.001$ ($R = 0.7$, Fig. 2B) at the time of diagnosis.

We have shown significant correlations between serum leptin concentrations and arm circumferences and skin triceps fold thicknesses as well (Fig. 2C and D).

Discussion

The trial included patients with lung cancer, which is in Poland the most frequent neoplasm in men and the second one after breast cancer in women [9, 10].

In this research two lung cancer patients groups were assessed: with and without cachexia syndrome. These groups were well balanced according to age, histopathologic type of lung cancer and stage of disease, however in the cachexia group men were in a majority. Moreover a comparison of leptin concentration between cancer patients and healthy controls was performed.

The authors realize that men predominance in the group of cachectic lung cancer patients decreases the value of performed analyses, especially due to well known dependence between leptin serum concentration, gender and age [11]. Never-

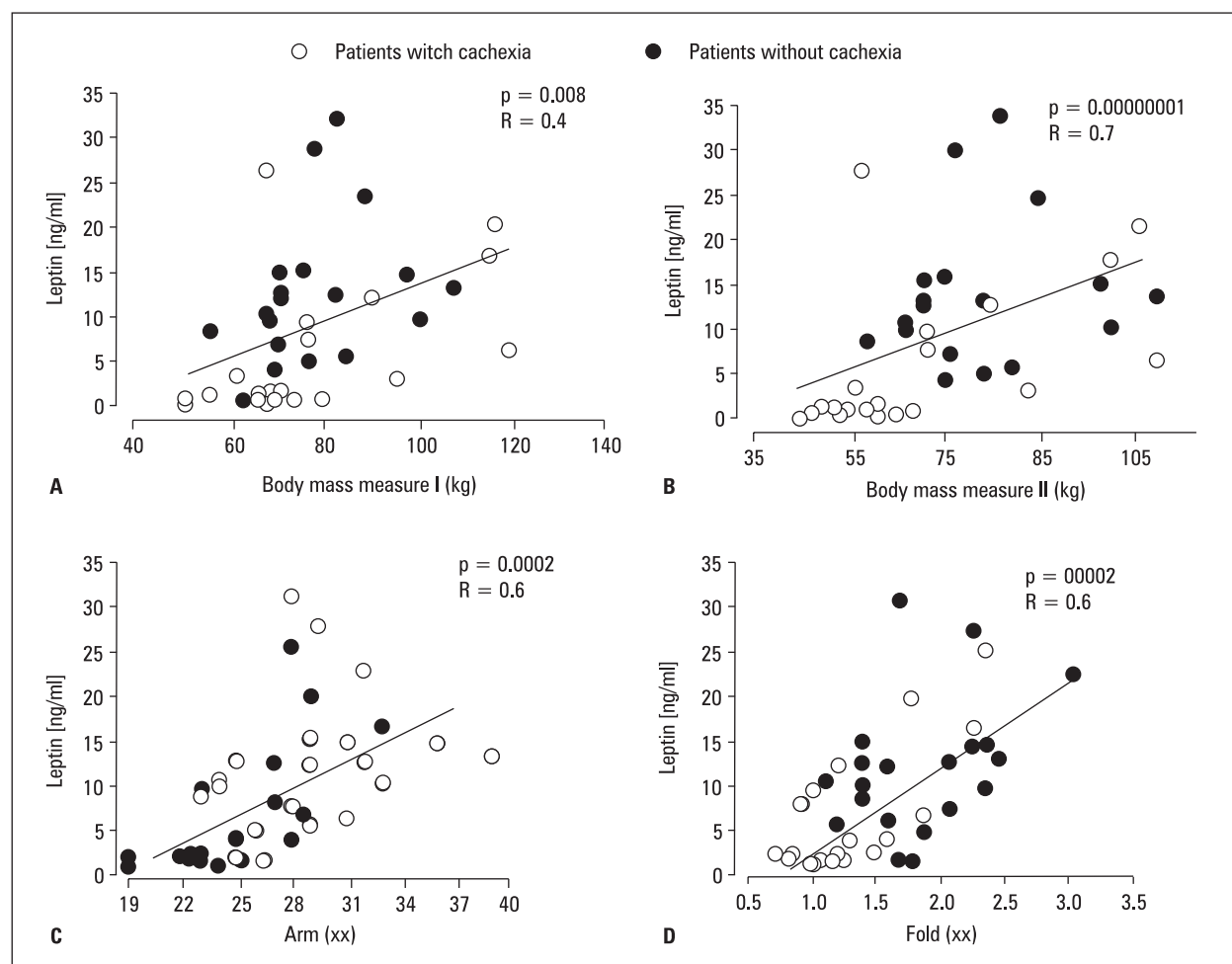


Figure 2. Correlations of leptin concentrations. Correlations between leptin serum concentration and: body mass measured 3 months before diagnosis (**A**), body mass in the time of diagnosis (**B**), arm circumference (**C**) and skin fold (**D**) ($p < 0.05$)

theless, despite these limitations, other investigators confirm our results [12].

In our study serum leptin concentration has been shown to be significantly lower in the cachectic cancer patients when compared to the non-cachectic ones (1.6 ng/ml vs. 12 ng/ml).

Aleman et al. and Tas et al. had examined leptin serum concentration in NSCLC patients and found that it was lower than in healthy controls (7.11 ng/ml vs. 18.50 ng/ml and 6.2 vs. 12.5 ng/ml) [13, 14]. They had not divided cancer patients into the cachectic and the non-cachectic group. We have done it in our study and found that patients with lung cancer have significantly lower serum leptin concentration compared with healthy persons but only when they are cachectic. In our study the non-cachectic patients with lung cancer and healthy controls had comparable leptin concentrations. In both Aleman's and our study higher serum leptin concentration was combined with better nutrition. Aleman et al. found a positive leptin correlation with such factors as BMI and body fat mass [13]. We confirmed the positive

leptin correlation with body mass, arm circumference and skin triceps fold thickness.

Other two groups of researchers estimated serum leptin concentration in cachectic patients with lung cancer finding its lower level in comparison with the group of healthy controls [12, 15]. However in these studies the non-cachectic patients were not included.

The etiology of cancer anorexia-cachexia syndrome (CACS) is not well known. Its origin is influenced by many factors produced by cancer cells and also host cells as an answer to growth of tumor (cytokines, hormones and others). Moreover the changes of leptin serum concentration are a part of many disorders observed in this syndrome. Some published data suggest that changes of serum leptin concentration should rather be regarded as a result of cachexia and not the cause of it, because its concentration depends on the total body fat mass [13]. In patients with extensive cancer disease, high cytokines concentration may imitate the inhibiting leptin influence on subthalamus, stimulating the feeling of fullness [16]. More-

over the changes in leptin secretion in cachectic cancer patients might be associated with subthalamus feedback disorder [12].

Studies with antagonists of leptin performed on mice led to the increase of appetite and body mass. This opens new perspectives for application of leptin antagonists in the future treatment of cancer anorexia-cachexia syndrome [17].

Conclusions

1. Cachectic lung cancer patients have significantly lower serum leptin concentration as compared with non-cachectic ones and healthy controls.
2. Serum leptin concentration in lung cancer patients, like in healthy persons, depends on nutrition status.
3. The role of neuropeptides and hormones regulating nutrition status in lung cancer patients needs further studies.

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