The Prognostic Importance of Leucocytosis, Thrombocytosis and Serum Vascular Endothelial Growth Factor (VEGF) Level in Inoperable Non-Small Cell Lung Cancer (NSCLC): A Match-Pair Analysis

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ABSTRACT

The aim of the study was to evaluate the relationship among leucocytosis, thrombocytosis and serum vascular endothelial growth factor (VEGF) levels and their prognostic value in patients with NSCLC. Fifty-five patients with histopathological and/or cytopathological diagnosed NSCLC were enrolled into the study. The patients were grouped as patients with leucocytosis (>10.000/mm^3) and/or thrombocytosis (>400.000/mm^3) (group 1), and others with none of them as a control group (group 2). Serum VEGF levels were measured by ELISA. Group 1 had three subgroups: patients with leucocytosis (group 1a), patients with thrombocytosis (group 1b) and patients with both of them (group 1c). Survival of the patients were analysed by Kaplan-Meier method. There was no survival difference between group 1 and 2, although there was a trend in favour of control group. The patients with higher levels of serum VEGF had a shorter survival than others who had lower levels, when the mean VEGF level of control group (206 pg/ml) was defined as cut-off value in group 1. The patients with leucocytosis and thrombocytosis had significantly higher VEGF levels when compared with control group (p= 0.022). No survival difference was observed for groups 1a, 1b and 1c when compared with control group. In conclusion, serum VEGF levels were significantly higher in patients who had leucocytosis and/or thrombocytosis although leucocytosis and/or thrombocytosis could not have been shown as a prognostic factor in patients with NSCLC. The association between serum VEGF and leucocytosis/thrombocytosis could not have been concluded as a cause or result.

Keywords: Leucocytosis, Thrombocytosis, non-Small cell lung cancer, Prognostic factor, Serum VEGF

ÖZET

Inoperabl Küçük Hücreli Dışı Akciğer Kanserinde Lökositoz, Trombositoz ve Serum Vasküler Endotelyal Büyüme Faktörü (VEGF) Seviyesinin Prognostik Önemi: Vaka-Kontrol Çalışması

Çalışmanın amacı; küçük hücreli dışı akciğer kanserinde (KHDAK) lökositoz, trombositoz ve serum vasküler endotelyal büyüme faktörü (VEGF) seviyesi arasındaki ilişkiyi ve bunların prognostik önemini araştırmaktır. Histopatolojik ve/veya sitolojik olarak KHDAK tanısı alan 55 hasta çalışmaya alınmıştır. Lökositozu (>10.000/mm^3) ve/veya trombositozu (>400.000/mm^3) olanlar grup 1, olmayanlar ise grup 2 olarak gruplandırılmıştır. Serum VEGF seviyeleri ELISA yöntemiyle çalışılmıştır. Grup 1; löositozu olanlar (grup 1a), tombositozu olanlar (grup 1b), her ikisi birlikte olanlar (grup 1c) olarak subgruplara ayrılmıştır. Sağkalım analizleri "Kaplan-Meier" yöntemine gore yapılmıştır. Grup 1 ve 2 arasında sağkalım farkı izlenmezken control grubunun prognozu daha iyi olma eğilimi göstermiştir. Kontrol grubunun ortalama serum VEGF düzeyi (206 pg/ml) grup 1 için sınır olarak alındığında, yüksek serum VEGF seviyesi olanların sağkalımı düşük olanlara göre daha kısa bulunmuştur. Lökositoz ve trombositozu olanlarda serum VEGF düzeyleri kontrol grubuna göre anlamlı olarak daha yüksek bulunmuştur (p= 0.022). Grup 1a, 1b ve 1c ile control grubu arasında sağkalım farkı izlenmemiştir. Sonuç olarak, KHDAK'de lökositoz ve/veya trombositoz prognostik faktör olarak gösterilememesine rağmen lökositoz ve/veya trombositozu olanlarda serum VEGF düzeyleri daha yüksek bulunmuştur. Serum VEGF düzeyi ile lökositoz/trombositoz arasındaki ilişkinin sebep mi yoksa sonuç mu olduğu netleştirilememiştir.

Anahtar Kelimeler: Lökositoz, Trombositoz, Küçük hücreli dışı akciğer kanseri, Prognostik faktör, Serum VEGF

INTRODUCTION

Non-small cell lung cancer (NSCLC) is the most common malignancy and the leading cause of cancer deaths in developed countries. Patients with advanced stage NSCLC have a poor prognosis. Therapeutic improvement with new generation of cytotoxic agents seems to have reached a plateau.[1,2] The most important prognostic factors in NSCLC are stage and performance status.[2,3]

The biological tumor characteristics might serve as prognostic factors.[1-3] The factors affecting tumor growth such as angiogenesis have prognostic role in many cancers.[3] Vascular endothelial cell growth factor (VEGF) plays an important role in the process of angiogenesis.[4-6] It has been demonstrated that VEGF is overexpressed in 50-95% of patients with NSCLC and reported to be associated with poor prognosis.[7-9] However, the prognostic role of serum VEGF concentration in NSCLC is still controversial.[10-12] We reported higher levels of VEGF in NSCLC patients in our previous study. However, there was no statistically significant difference in terms of stage of the disease.[13] It has been reported that leucocytosis and thrombocytosis might have been associated with poor prognosis in NSCLC.[14,15] However, it is not known whether there is a relationship between the leucocytosis/thrombocytosis and serum VEGF levels in NSCLC.

In this study, we evaluated the prognostic role of leucocytosis and thrombocytosis, and their relationship with serum VEGF levels in patients with advanced NSCLC.

PATIENTS AND METHODS

Patients younger than 70 years old with histologically and/or cytologically confirmed diagnosis of locally advanced or metastatic NSCLC and without previous treatment were included. 'Eastern Cooperative Oncology Group (ECOG)' performance status was < 3. All of the patients have given informed consent. The patients who had leucocytosis due to infections or corticosteroid use were excluded.

Complete blood counts were performed before the first cycle of the treatment. Serum samples for VEGF were kept at -20^{0}C until assayed.

The patients were divided into two groups according to the existence of leucocytosis and/or thrombocytosis. Group 1 consisted of patients with leucocytosis and/or thrombocytosis and group 2 consisted of patients with none of them. In addition, group 1 was divided into three subgroups: Patients in group 1a had only leucocytosis, group 1b had only thrombocytosis and group 1c had both leucocytosis and thrombocytosis.

Serum VEGF levels measurements are done by ELISA (CytElisaTM human VEGF, Cytimmune Inc.).

Statistical Analysis

Quantitative variables were described as the mean ± standard error. Student's t-test, Mann Whitney U test, chi-square, and one-way ANOVA were used for the statistical analysis. Tukey post-hoc comparisons were done between groups. Survival rates of the patients were analyzed by Log-rank test, and the curves were prepared according to the Kaplan-Meier method.

RESULTS

Patient Characteristics

Fifty-five patients with inoperable advanced NSCLC were enrolled to this study between February 2001 and January 2004. Twenty-six patients with leucocytosis and/or thrombocytosis were included in study group (group 1), and 29 patients without leucocytosis or thrombocytosis were defined as a control group (group 2). There was no difference between two groups in terms of gender, age, performance status, stage of disease and treatment modality. The patient characteristics are shown in Table 1.

Serum VEGF Levels

Group 1 had significantly higher mean serum VEGF levels when compared with group 2 (269.96±43.26 pg/mL and 144.17±25.97 pg/mL, respectively; p= 0.016) (Table 1).

According to histological subtypes of NSCLC, there was no significant difference between groups in terms of serum VEGF levels, platelet and leucocyte counts (Table 2). Serum VEGF levels were significantly higher in group 1b (with only thrombocytosis) and group 1c (with leucocytosis and thrombocytosis) when compared to group 2 (p= 0.022 and p= 0.001, respectively, Table 3).

Survival

There was no difference in terms of the median survival time between two groups (214±20 days in group 1 and 247±47 days in group 2; p= 0.137). The Kaplan-Meier curves for overall survival of both groups were given in figure 1. There was also no difference for survival rates between patients who had above or below the median level of 206 pg/mL of all cohort (213±45 days vs 243±12 days in group 1 and 2, respectively, p= 0.820).

Table 1. Characteristics of the Patients

Characteristics	Group 1	Group 2	p value
Gender, n (%)			1.000
Male	25 (96.2)	27 (93.1)	
Female	1 (3.8)	2 (6.9)	
Age, years (mean ±standard error)	57.6± 2.0	59.9±1.8	0.408
Performance status (ECOG), n (%)			0.926
1	19 (73)	22 (76)	
2	6 (23)	6 (20)	
3	1 (4)	1 (4)	
Leucocytes, $x10^3/mm^3$ (mean±standard error)	12.8±9.72	7.1±0.31	<0.001
Platelets $x10^3/mm^3$ (mean±standard error)	430±26.24	251.1±9.45	<0.001
Stages of disease, n (%)			0.701
III-b	13 (50)	13 (44.8)	
IV	13 (50)	16 (55.2)	
Histological types, n (%)			0.985
Squamous carcinoma	19 (73)	19 (66)	
Adeno carcinoma	7 (27)	10 (34)	
Chemotherapy, n (%)			
CG	9 (35)	9 (31)	0.577
MVC	15 (65)	15 (51)	
Radiotherapy, n (%)			0.522
Curative intent	10 (39)	15 (52)	
Palliative intent	4 (16)	7 (25)	

CG= cisplatin plus gemcitabine, MVC= mitomycine C plus vinblastine plus cisplatin

Table 2. The mean serum VEGF levels and counts of leucocytes/ platelets according to histo-cytological diagnoses

	Squamous cell carcinoma (n: 38)	**Adeno carcinoma** (n: 17)	**p value**
Mean serum VEGF levels (pg/mL) (±std.error)	208.80±32.74	187.05±48.95	0.713
Mean Leucocyte Counts (x 10^3/mm^3) (±std.error)	9.70±0.63	9.98±1.11	0.810
Mean Platelet Counts (x 10^3/mm^3) (±std.error)	340.13±23.08	325.88±27.62	0.718

No survival difference was found in group 1a, 1b and 1c when compared to group 2, (Figures 1, 2).

DISCUSSION

Tumor cells and platelets are considered as the main sources of serum VEGF.[7,10-19] In addition, leucocytes may also be another source for serum VEGF (18). Therefore, it could be expected that serum VEGF levels might have been higher in patients with leucocytosis and/or thrombocytosis. Serum VEGF levels were significantly higher in the study group (group 1) than control group (group 2). There was no difference in serum VEGF levels in group 1a or group 1b when compared to group 2. However, VEGF levels in group 1c were significantly higher then group 2. Most of our patients had mild-to-moderate high levels of thrombocytosis and/or leucocytosis. We consider that including these patients might have prevented a correlation between serum VEGF and thrombocytosis/leucocytosis. Choi et al., have recently shown that there was a significant relationship among serum VEGF level, platelet and leucocyte counts in patients with NSCLC.[19]

Our results indicate that existence of leucocytosis and/or thrombocytosis did not effect survival in patients with advanced NSCLC. Brattstrom et al., have shown that thrombocytosis was not a poor prognostic factor in patients with locally advanced NSCLC, which is similar to our results.[11] However, many studies have shown that prognosis was poor in NSCLC patients with thrombocytosis[6,7,12,14], and leucocytosis.[9] Our negative results may be due to limited number of patient and mild-to-moderate high level of cell counts. A certain cut-off value for thrombocytosis and leucocytosis has not been reported to show the association with prognosis.

An association between poor prognosis and NSCLC patients with high serum VEGF levels has been reported in some of the studies.[7,11,20] But, it could not have been confirmed by other studies.[19-21-23] No correlation between serum VEGF levels and tumor tissue VEGF expression has been reported. However, it has been recently demonstrated that the elevated VEGF levels in tumor tissue was associated with poor prognosis in operable NSCLC.[21,24,25] Imoto et al., found that tumor tissue VEGF levels were associated with poor prognosis, whereas serum levels were not.[21] These results suggest that se-

Table 3. Serum VEGF levels in group 1a (with only leucocytosis), group 1b (with only thrombocytosis) and group 1c (with leucocytosis and thrombocytosis)

	Group 1a	**Group 1b**	**Group 1c**
The mean VEGF levels pg/mL (mean±std. error)	142.60±44.93	222.45±54.27	351.69±73.39
p value*	0.84	0.022	0.001

* p value: as compared with group 2

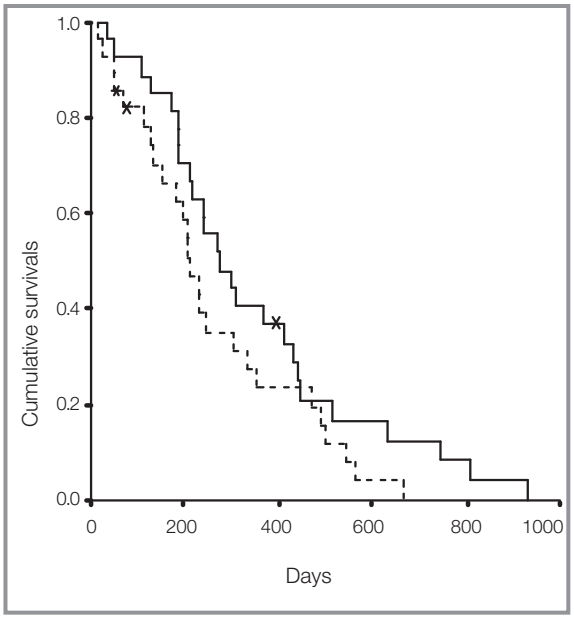

Figure 1. Kaplan-Meier Estimates of Overall Survival in both groups (---: group 1, ——: group 2).

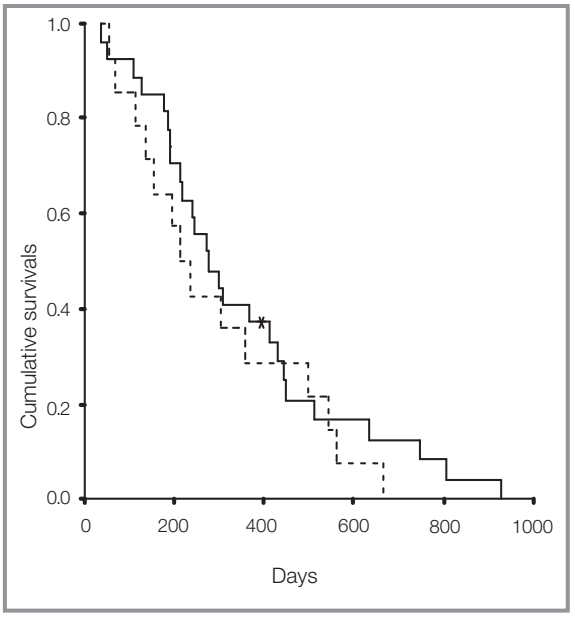

Figure 2. Kaplan-Meier Estimates of Overall Survival in group1c and group 2 (- - - : group 1c, ——: group 2).

rum level of VEGF is not as valuable as tumor-tissue VEGF level for predicting prognosis in NSCLC.

There is no known cut-off value for serum VEGF level in cancer patients. We hypothesized to use the median serum VEGF level of control group as a threshold value to detect a survival difference. But, the survival difference between patients with low and high VEGF levels was not significant. Serum VEGF levels displayed in wide ranges with high standard deviation in the literature, which was similar to ours. It seems to be a major problem to use serum VEGF level as a biological marker. In addition, it should be emphasized that there was no difference between serum VEGF levels according to histologic subtypes, and it is consistent with previous studies.[11,19,21]

In conclusion, this study shows that VEGF levels were significantly higher in patients who had leucocytosis and/or thrombocytosis. However, no prognostic relationship has been determined in terms of leucocytosis/thrombocytosis or serum VEGF levels. It is unlikely that serum VEGF levels could be accepted as a tumor marker, because of contradictive results published so far.

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