

## RESEARCH COMMUNICATION

# Granulocyte Colony Stimulating Factor (G-CSF) and Macrophage Colony Stimulating Factor (M-CSF) as Potential Tumor Markers in Non Small Cell Lung Cancer Diagnosis

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### Abstract

Non small cell lung cancer (NSCLC) is an important subject threatening public health. There is not a marker for early detection of this cancer type in practice yet. When studies on this subject were searched, cytokines show promise in diagnosis and guiding treatment. The reason for selecting serum M-CSF and G-CSF levels is observing tumor related leukocytosis in most of the NSCLC patients. In our study, using enzyme-linked immunosorbent assay, we compared serum M-CSF and G-CSF levels of 24 newly diagnosed NSCLC cases with 30 healthy individuals. Our data suggests that patients' serum G-CSF levels were significantly higher than controls ( $103.2 \pm 52.4$  pg/mL for patients and  $25.0 \pm 4.5$  pg/mL for controls,  $p < 0.001$ ) but not significantly different in M-CSF levels ( $250.7 \pm 102.9$  pg/mL for patients and  $211.6 \pm 57.8$  pg/mL for controls,  $p > 0.05$ ). Our patients' neutrophil count was higher than the normal range (mean  $7.7 \times 10^9/L$ ,  $N = 2.5 - 7.5 \times 10^9/L$ ). Increased serum G-CSF levels with high neutrophil counts in patients suggests tumor related increase of this cytokine but there was no relationship between serum cytokines and tumor type. There are different results in the literature about cytokine levels in NSCLC patients but the main conclusion, with which we concur, is that ongoing studies will provide consistent results of value to patients.

**Keywords:** NSCLC - tumor markers - G-CSF - M-CSF

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### Introduction

Lung cancer is uncontrolled cell growth in lung tissue and can cause metastasis to adjacent and far tissues. Although anti-smoking campaigns and high taxes are applied to tobacco products, according to World Health Organization (WHO) data lung cancer causes 1.3 million deaths per year worldwide (World Health Organization, 2009). As for other cancer types, carcinogens, ionizing radiation and viral infections are main causes of lung cancer.

Non-small cell lung cancer (NSCLC) covers almost 80% of lung cancers and differs from small cell lung cancer (SCLC) for treatment and prognosis. Three major types of NSCLC are adenocarcinoma, squamous cell carcinoma and large cell carcinoma. Only a small numbers of NSCLC patients can present with early-stage disease and can live longer with surgical resection and lymph node dissection compared to late-stage patients (Lu C et al., 2004). 5-year survival rate drops from 57-85% to 38-65% when patient stage increases from I to II (Jemal et al., 2007). As in all diseases, early detection of lung cancer is very crucial.

Biomarker studies focus on early detection, treatment

and follow up of diseases especially cancers. During the clinical course of many cancers, especially lung cancers, paraneoplastic syndromes are frequently seen (Thomson et al., 1986). One of the paraneoplastic syndromes is observing high counts of white blood cells (WBC) without underlying infection, bone marrow metastasis or corticosteroid administration (Ascensao et al., 1987; Slungaard et al., 1983). There are a number of publications showing tumor related secretion of various cytokines such as granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF) and interleukin-6 (IL-6) and showing correlation with WBC counts (Kimura et al., 1978; Okabe et al., 1984; Shijubo et al., 1992; Inoue et al., 1997).

G-CSF, also known as colony-stimulating factor 3, is a cytokine which stimulates production of granulocytes and then regulates their life with pathways such as Janus kinase via signal transducer and activator of transcription and Ras via mitogen-activated protein kinase and phosphatidylinositol 3-kinase via protein kinase B. G-CSF is used in oncology and hematology as an agent to help recover from neutropenia. M-CSF, also known as colony-stimulating factor 1, is a cytokine which

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**Table 1. Clinicopathological Characteristics of Patients (n=24)**

Sex	number
Male	20 (%83,3)
Female	4 (%16,7)
<b>Staging</b>	
I	2 (%8)
II	1 (%4)
III	11 (%46)
IV	10 (%42)
<b>Histological type</b>	
Adenocarcinoma	7 (%29,1)
Squamous-cell carcinoma	6 (%25)
Undetermined	11 (%45,9)

regulates production of macrophages with pathways such as phosphatidylinositol 3-kinase via protein kinase B.

In this study, we measured WBC counts, serum G-CSF and M-CSF levels in patients with untreated primary NSCLC then investigated the relationship with these factors and clinicopathologic manifestations.

## Materials and Methods

### Patients

This study included 24 patients with previously untreated primary NSCLC (20 males, 4 females; age: 47-81 years, mean: 60,6 years) between July 2006 and June 2007 who were referred to Gazi University Faculty of Medicine Department of Oncology. Diagnosis of all patients was confirmed by histologic or cytologic examination from specimens either obtained by fiber optic bronchoscopy or percutaneous needle biopsy, computed tomography (CT)-guided biopsy, thoracotomy or sputum or washing cytology. The staging procedure included clinical examination, chest radiography, bronchoscopy, chest and brain CT, abdominal ultrasonography and radioisotopic bone scans. In addition, each patient's WBC count was determined using SYSMEX XT2000i (Abbott, USA). The main clinicopathological characteristics of patients are summarized in Table 1.

For control purposes, samples from 30 normal volunteers (15 males, 15 females) were collected. All of the patients and controls provided informed consent in accordance with institutional guidelines.

### Collection and Storage of Serum Specimens

Peripheral blood was collected from each patient immediately before treatment. Using red-top Vacutainer ten milliliter of blood was drawn and let for coagulation at room temperature for 30 minutes and sera collected after centrifugation. Specimens then stored at  $-80^{\circ}\text{C}$ .

### Serum Cytokines

Concentrations of granulocyte colony-stimulating factor (G-CSF) and macrophage CSF (M-CSF) were measured by enzyme-linked immunosorbent assay (ELISA) with the use of commercially available kits (Biosource, Camarillo, CA, USA). The tests were

performed according to the manufacturer's instructions. The lower detection limit for serum G-CSF and M-CSF were 20pg/mL and 5pg/mL respectively.

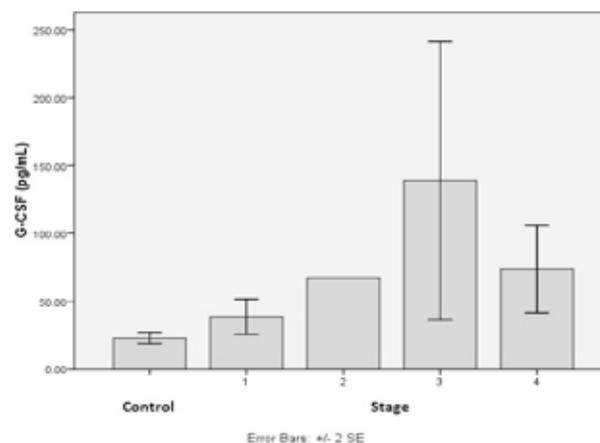
### Statistical Analysis

Statistical Package for the Social Sciences (SPSS) 16 and Microsoft Office 2007 Excel are used for statistical analysis. To analyze the associations between variables Mann-Whitney U test, Kruskal-Wallis one-way analysis of variance and Spearman correlation coefficients were employed. To assess the diagnostic sensitivity and specificity of cytokine and tumor marker measurements, receiver-operating characteristic (ROC) curves were applied, and differences between areas under the curves (AUCs) were estimated by the Wilcoxon test. Differences at a p value  $<0.05$  were considered significant. Values are presented as mean $\pm$ SE.

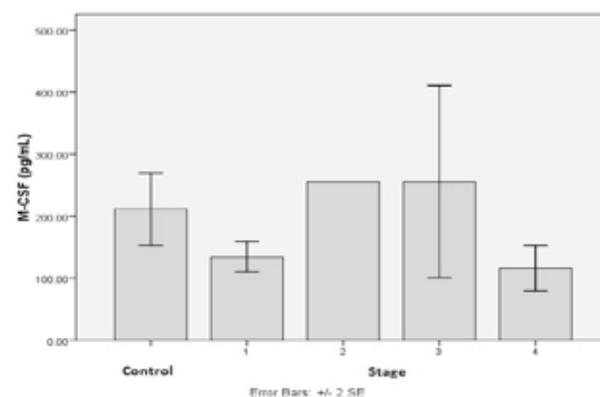
## Results

### Serum Cytokine Concentrations in Patients versus Controls

The mean G-CSF level of patients with NSCLC was 103.2pg/mL ( $\pm$ 52.4pg/mL) and that of the controls was 25.0pg/mL ( $\pm$ 4.54pg/mL). The mean of M-CSF level of patients with NSCLC was 250.7pg/mL ( $\pm$ 102.9pg/mL) and that of the controls was 211,56pg/mL ( $\pm$ 57.8pg/mL). Statistical analysis showed that serum G-CSF levels were significantly higher than controls (Mann-Whitney U,  $p<0.001$ ) (Figure 1). No such significance was revealed for serum M-CSF levels (Mann-Whitney U,  $p>0.05$ ) (Figure



**Figure 1. Serum G-CSF Levels**



**Figure 2. Serum M-CSF Levels**

2). There was no statistical relationship between serum G-CSF and M-CSF levels (Spearman,  $p > 0,05$ ).

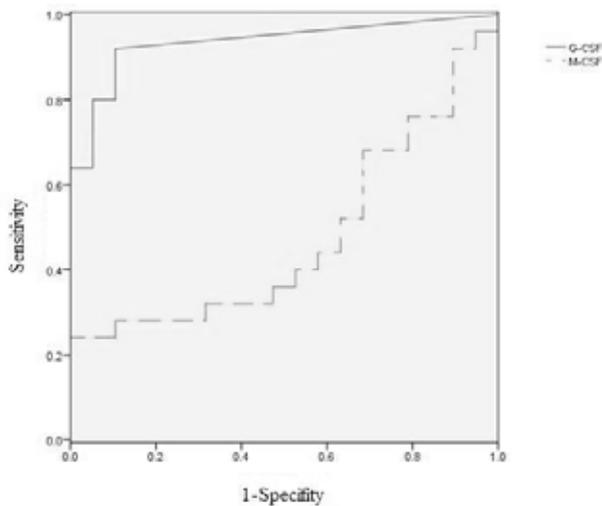
Non-parametric analyses of ROC curves of serum G-CSF and M-CSF present AUCs of 0,935 and 0,476 respectively. Wilcoxon test showed  $p < 0,001$  for serum G-CSF (Figure 3).

*Relationship Between Cytokine Levels and White Blood Cell Count*

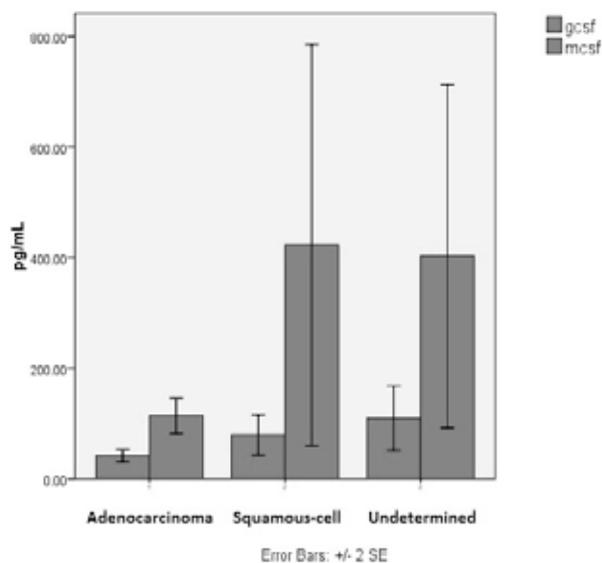
Pretreatment mean blood leukocyte count was  $10,4 \times 10^9/L$ . Although this value was within our reference range of  $4-11 \times 10^9/L$ , it was close to the upper margin. On the other hand, mean neutrophil count was  $7,7 \times 10^9/L$  which was higher than our range of  $2,5-7,5 \times 10^9/L$ . Patients' mean percent neutrophil count was also higher than our reference range of 54-62% with a value of 77%. Statistical analysis showed no correlation between given cytokines and white blood cell numbers (Spearman correlation coefficient,  $p > 0,05$ ).

*Relationship Between Cytokine Levels and Patients' Characteristics*

Patients' characteristics are shown in Table 1.



**Figure 3. ROC Curves for Serum G-CSF and M-CSF in Patients with NSCLC**



**Figure 4. Tumor Types and Cytokine Levels**

Statistical analysis showed no significance between stage and serum cytokine levels (Kruskal-Wallis,  $p > 0,05$ ) but patients with any stage have significantly higher serum G-CSF levels compared to controls (Mann-Whitney U test,  $p < 0,05$ ) (Figure 1). No such difference was found for serum M-CSF levels (Mann-Whitney U test,  $p > 0,05$ ) (Figure 2).

The relationship between serum G-CSF and M-CSF levels and histopathological tumor type was assessed in adenocarcinoma, squamous cell carcinoma and undetermined type NSCLC. Kruskal-Wallis analysis showed no statistical significance with a  $p > 0,05$  (Figure 4).

**Discussion**

Although there are improvements in lung cancer treatment, non small cell lung cancer is still a public health problem. Advances in scientific assay development helped us measure hematopoietic cytokine levels. In the literature, there are a number of publications showing that cytokines can help in screening, diagnosing and guiding treatment of various cancer types (Kimura et al., 1978; Katsumata et al., 1996; Mroczko and Szmitkowski, 2005; Mroczko et al., 2005).

Asano et al (1977) were the first to publish G-CSF secreting lung cancer in 1977. Suda et al (1980) also reported G-CSF producing lung carcinoma. Increased white blood cell count is a common finding at lung cancer patients (Thomson et al., 1986). Shijubo et al (1992) reported a case of large cell lung cancer patient with severe granulocytosis and established a cell line and then showed production of G-CSF which was in correlation with white blood cell count. Our patients had borderline high WBC counts with neutrophil dominance. A study by Kasuga et al (2001) on 227 lung cancer patients, during a 7-year period, showed 14,5% of patients had tumor related leukocytosis and researchers also reported the positive correlation of serum G-CSF levels and tumor growth. These findings, along with ours, contribute to the thesis that lung cancer cells produce growth factors.

Contrary to our results, Katsumata et al (1996) previously reported a significant difference for serum M-CSF levels for 183 lung cancer patients but not for serum G-CSF or IL-6 levels. Just like our study Katsumata et al (1996) could not find significance for stage of the disease. Katsumata et al (1996) showed increased pretreatment WBC and neutrophil counts which were in correlation with serum G-CSF and M-CSF levels in their study. Our patients, on the other hand, had borderline increased WBC numbers with grossly increased neutrophil numbers but those two cytokines were not in correlation with cell numbers.

A study by Kaminska et al (2006) based on 103 NSCLC patients compared to controls showed significant difference for serum M-CSF levels and correlation with staging of the disease. Both Katsumata et al (1996) and Kaminska et al (2006) showed prognostic value for serum M-CSF levels. Kaminska et al (2006) could not find any significant difference for G-CSF levels between patients and controls and researchers also reported that serum G-CSF levels and staging of the disease were not

correlated.

A study based on 15 parameters which were MIP-1, SCF, CEA, TNF-RI, TNF- $\alpha$ , IFN- $\gamma$ , M-CSF, G-CSF, TNF-RII, sICAM-1, MMP-2, CRP, IL-2R, osteopontin ve IL-1ra for the establishment of a multi-analyte serum biomarker panel to identify lymph node metastases in non-small cell lung cancer by Borgia JA et al., (2009) showed G-CSF and M-CSF to exhibit promising results with AUCs of 0.657 and 0.679 respectively. Our study showed a higher value for G-CSF with an AUC of 0.935 but a lower value for M-CSF with an AUC of 0.476.

It is unclear why tumors secrete cytokines but a study by Pei et al (1999) suggests that M-CSF, GM-CSF and G-CSF promote tumor cell invasion by increasing productions of gelatinases (matrix metalloproteinase 2 and matrix metalloproteinase 9) and urokinase plasminogen activator. Researchers demonstrated productions of these substances by zymographs of conditioned media derived from human lung adenocarcinoma cell line and contributed that enhanced production of extracellular matrix-degrading proteinases by cancer cells in response to CSFs may promote invasive behavior of the cancer cells (Pei et al., 1999).

In conclusion, in the present study, we measured serum G-CSF and M-CSF levels in newly diagnosed non small cell lung cancer patients using ELISA. The reason for choosing serum M-CSF and G-CSF levels are observing tumor related leukocytosis in most of the NSCLC patients. The serum G-CSF levels were significantly higher in NSCLC patients compared to healthy subjects. There was no significance for serum M-CSF levels.

Our main obstacle was small number of patients. Patient follow up is still ongoing so no survival analysis could be performed. Similar studies had more patients and longer patient follow ups. There are different results in the literature about cytokine levels in NSCLC patients but the common sense is, ongoing studies will show consistent results and will be valuable for patients.

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