

# Expression of Fas receptor on peripheral blood lymphocytes from patients with non-small cell lung cancer

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**Abstract:** In recent years many data indicate that lymphocytes from cancer patients undergo increased apoptosis. The objective of this study was to evaluate the expression of Fas receptor on lymphocytes obtained from patients with lung cancer. Eighteen patients with non-small cell lung cancer and 18 healthy volunteers were investigated. Expression of Fas (CD95) on CD4+ and CD8+ blood lymphocytes was evaluated by flow cytometry. The proportion of blood Fas+ lymphocytes was significantly higher in lung cancer patients when compared with healthy individuals and in smokers when compared with nonsmokers.

**Key words:** Lung cancer - Lymphocytes - Apoptosis - Smoking - Fas

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## Introduction

The role of lymphocytes accumulated in the tumor environment in the host defense against lung cancer is well documented. Nevertheless, these defense mechanisms are impaired and many recent data indicate that lymphocytes from patients with malignancies undergo apoptosis [11]. One way of apoptosis signaling is the Fas-Fas ligand (FasL) interaction [6]. It has been demonstrated that many neoplastic cells express FasL [11] and can directly induce death of activated Fas-positive lymphocytes [7]. The role of cytotoxic CD8+ lymphocytes in immunology of lung cancer was reported before [2]. An elevated proportion of these cells in the bronchoalveolar lavage (BAL) of patients with lung cancer was found, while the proportion of these cells in the blood was lower [2]. The involvement of apoptosis is possible in such changes of lymphocyte proportions in patients. The aim of this study was to evaluate the expression of Fas receptor on CD4+ and CD8+ lymphocytes obtained from peripheral blood of patients with non-small cell lung cancer.

## Materials and methods

**Subjects.** Eighteen patients with lung cancer (4 female and 14 male) and 18 healthy volunteers (10 female and 8 male) were investigated. The age of patients ranged from 37 to 81 yrs and of healthy persons:

from 30 to 76 yrs. All cancer patients were smokers (mean number of pack-years - number of cigarette packs smoked per day multiplied by the number of years of smoking - was  $48\pm36$ ) and there were 10 smokers (mean number of pack-years  $32\pm36$ ) and 8 nonsmokers in the control group.

In all patients, histological examination confirmed non-small cell lung cancer - in 4 patients squamous cell type, in 1 - adenocarcinoma, and in the others undifferentiated. There was 1 patient at stage 1, 3 were at stage 2, 6 at stage 3 and 8 at stage 4 of lung cancer. The study was approved by the Ethics Committee of the Warsaw Medical School. All the healthy volunteers signed an informed consent form. Subjects with any infection or chronic pulmonary disease were excluded from the study. The persons examined were not undergoing corticosteroid or immunosuppressive therapy one year before and at the time of the examination.

**Flow cytometry analysis.** The analysis of Fas expression was performed on lymphocytes from the venous blood. The following mixtures of antibodies were used for the cell phenotyping: anti-CD45-FITC and anti-CD14-PE, negative isotype controls (Ig2a-FITC and Ig2b-PE), anti-CD4-FITC and anti-CD95-PE, anti-CD8-FITC and anti-CD95-PE (Becton-Dickinson Immunocytometry Systems, San Jose, California). Two-color analysis of cells was performed as described previously [4]. Anti-CD45-FITC and anti-CD14-PE was used for the lymphocyte gate setting at FSC/SSC graph. The samples were analyzed using a FACS Calibur flow cytometer (Becton-Dickinson, San Jose, California). The cells were collected by CELLQuest software.

**Statistical analysis.** Nonparametric Mann-Whitney U-test was applied with  $p<0.05$  being regarded as significant. The relationship between the proportion of cells and smoking history expressed as pack-years was assessed by the Spearman correlation test.

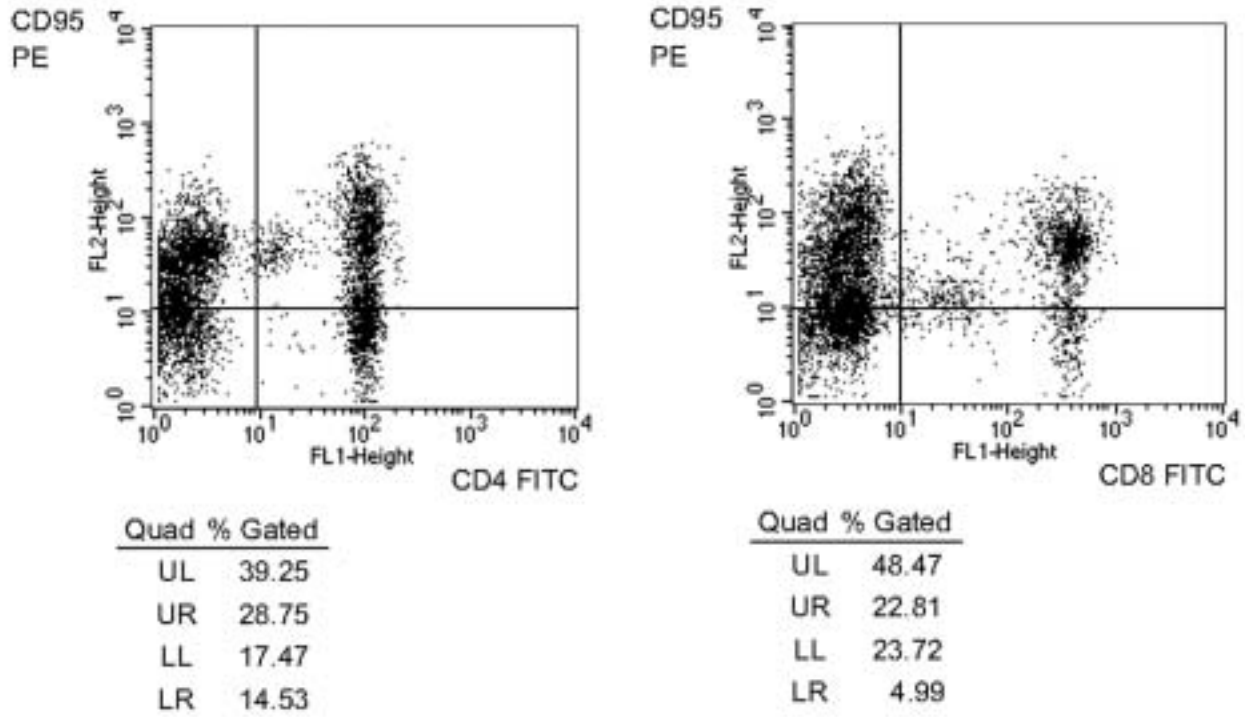
## Results and discussion

We found elevated proportion of lymphocytes with expression of Fas (CD95) in the peripheral blood of pa-

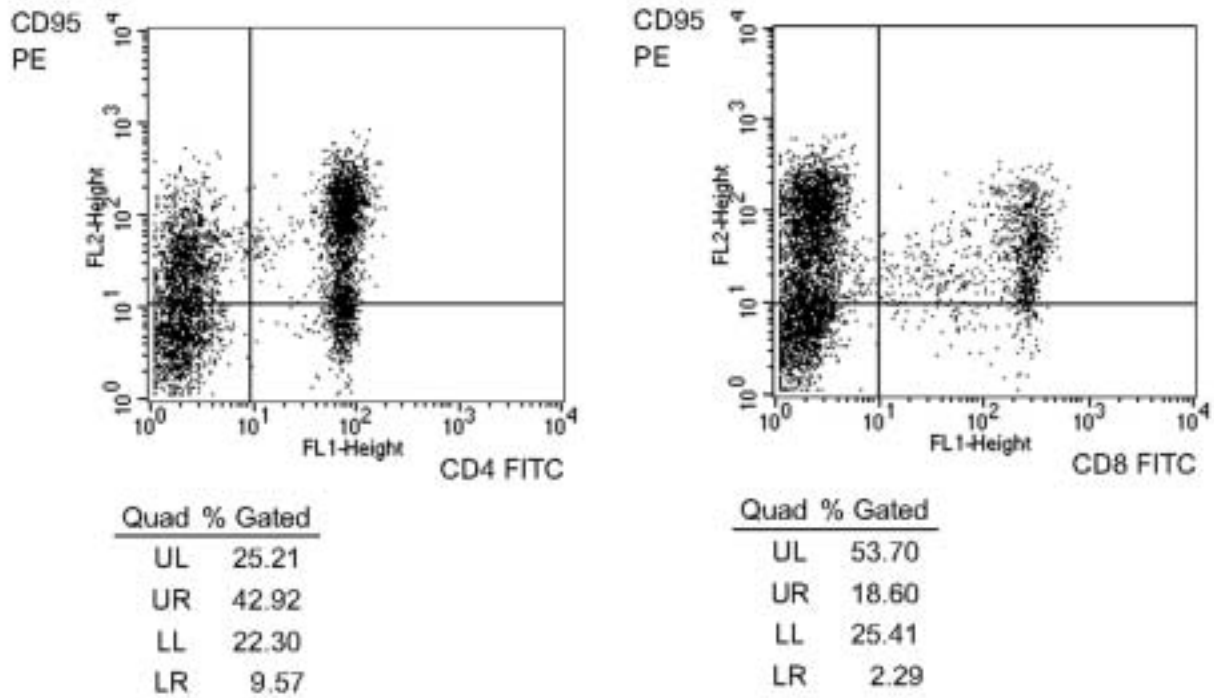
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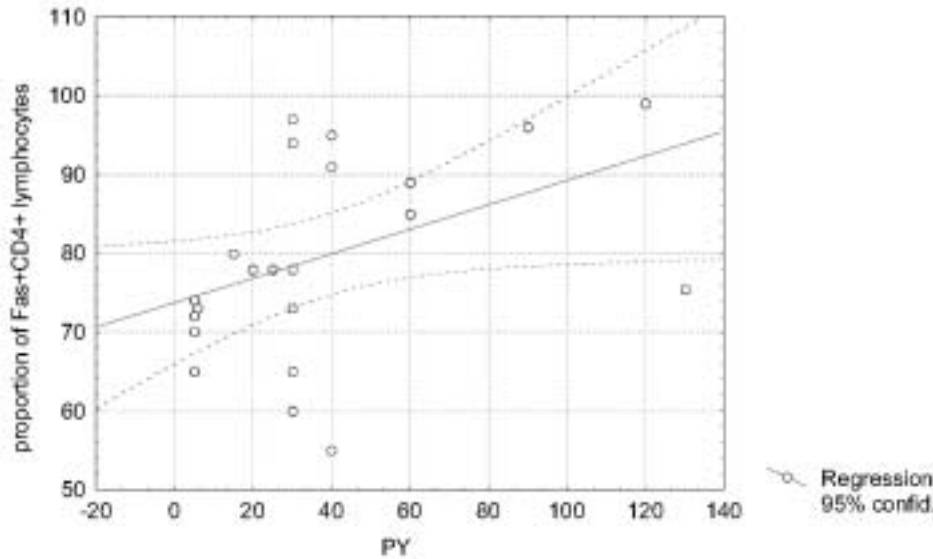
A. Peripheral blood lymphocytes from healthy person



B. Peripheral blood lymphocytes from lung cancer patient



**Fig. 1.** Flow cytometry profile of peripheral blood (PB) lymphocytes from: A - healthy person, B - patient with lung cancer. Left dot plots: lymphocytes CD95+/CD4+ in the upper right quadrant; right dot plots: lymphocytes CD95+/CD8+ in the upper right quadrant.



**Fig. 2.** Correlation of proportion of Fas-positive CD4+ lymphocytes in the blood of smokers with smoking history expressed as pack-years (PY) (significant in Spearman test, R = 0.6, p<0.05).

**Table 1.** Expression of Fas (CD95) on peripheral blood (PB) lymphocytes in lung cancer patients and healthy individuals

	Patients n = 18	Healthy n = 18
CD95+ CD4+ <sup>a</sup>	86.5 ± 8.7*	63.9 ± 11.9*
CD95+ CD8+ <sup>b</sup>	85.9 ± 9.1*	64.8 ± 14.9*

<sup>a</sup>cells with expression of CD95 as percentage of all CD4+ cells (mean ± SD)

<sup>b</sup>cells with expression of CD95 as percentage of all CD8+ cells (mean ± SD)

\*p<0.05 in Mann Whitney U-test

**Table 2.** Expression of Fas (CD95) on blood lymphocytes in smokers and nonsmokers

	Patients	Healthy	
	smokers n = 18	smokers n = 10	nonsmokers n = 8
CD95+ CD4+ <sup>a</sup>	86.5 ± 8.7*	69.7 ± 8.1*	56.6 ± 12.2*
CD95+ CD8+ <sup>b</sup>	85.9 ± 9.1*	72.1 ± 6.1*	55.7 ± 17.9*

<sup>a</sup>cells with expression of CD95 as percentage of all CD4+ cells (mean ± SD)

<sup>b</sup>cells with expression of CD95 as percentage of all CD8+ cells (mean ± SD)

\*p<0.05 in Mann Whitney U-test

tients with non-small cell lung cancer when compared with healthy persons. Table 1 shows the mean proportion of Fas+ CD4+ lymphocytes as percentage of all CD4+ lymphocytes and the proportion of Fas+ CD8+ lymphocytes as percentage of all CD8+ lymphocytes. The proportion of Fas-positive CD4+ lymphocytes was significantly higher in patients when compared to healthy persons. The proportion of Fas-positive CD8+ lymphocytes was also significantly higher in patients

when compared to healthy persons. These differences between patients and healthy persons were significant. Figure 1 presents the representative examples of flow cytometry assay of Fas-positive lymphocytes. In each group, the proportion of Fas-positive CD4+ and Fas-positive CD8+ lymphocytes were similar (Tab. 1).

A significantly higher percentage of Fas-positive lymphocytes was found in healthy smokers when compared to healthy nonsmokers, however, the proportion of Fas-positive lymphocytes in smokers with lung cancer when compared to healthy smokers was still higher (Tab. 2). A significant positive correlation was found between proportion of Fas-positive lymphocytes and smoking history expressed as pack-years (Fig. 2).

In this study we examined lymphocytes expressing "death receptor" Fas derived from the peripheral blood of patients with lung cancer. We have not found data on this subject in the literature. The impairment of host defense against cancer may result from depletion of lymphocytes. One way of this depletion is apoptosis of lymphocytes after contact with tumor cells. It was documented that many tumor cells express Fas ligand (FasL) and may induce apoptosis of Fas-positive lymphocytes [3, 9, 11, 13]. Little is known about the mechanisms of apoptosis of lymphocytes in the lung cancer environment, the results presented by Niehans *et al.* [7] suggested that lung cancer cell lines express functional FasL and that lung cancer cells were capable of killing T cells. We found a significantly higher proportion of Fas-positive lymphocytes in the blood of patients with lung cancer when compared with healthy individuals. These findings are in agreement with the observations in other types of neoplasms [3, 8, 9, 13]. We demonstrated previously a significant depletion of peripheral blood CD8+ in patients with lung cancer [2]. Saito *et al.* [8] reported that mainly CD8+ cells undergo apoptosis

in melanoma, Hoffmann *et al.* [3] observed greater proportion of apoptotic CD8+ cells in patients with head and neck cancer. In this study, we did not find any differences between the proportions of Fas+ CD8+ lymphocytes and Fas+ CD4+ cells in cancer patients. It seems possible that other or more complex mechanisms than Fas-FasL interaction are involved in blood CD8+ lymphocyte depletion in lung cancer.

The main exogenous cause of lung cancer is tobacco smoke. In this study we found elevated proportion of Fas+ lymphocytes in smokers (both, patients and healthy). The data on the influence of tobacco smoke on lymphocyte apoptosis is very scarce. Bijl *et al.* [1] found an increased expression of Fas on blood lymphocytes in smokers, while Suzuki *et al.* [10] did not find any differences in the expression of Fas on lymphocytes between smokers and nonsmokers [10]. We observed before an elevated proportion of activated lymphocytes in the blood of smokers [5]. Fas is upregulated on lymphocytes after their activation [12] what may explain our present observation in smokers.

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