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Original Research Article

Distribution of CD4+ and CD8+ T cells in tumor islets and stroma from patients with non-small cell lung cancer in association with COPD and smoking

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ABSTRACT

Background and objective: The immune system plays an important role in non-small cell lung cancer (NSCLC) and chronic obstructive pulmonary disease (COPD). The aim of this study was to evaluate the infiltration patterns of CD4+ and CD8+ T cells in NSCLC and to analyze their relation to COPD, smoking status and other clinicopathologic variables.
Materials and methods: Lung tissue specimens from 50 patients who underwent surgery for NSCLC (stages I–III) and 10 control group subjects were analyzed immunohistochemically.
Results: NSCLC patients had a greater number of CD4+ and CD8+ T cells infiltrating the lung tissue than the control group ($P = 0.001$) with predominant infiltration in the tumor stroma. We found a significant association between the number of total and tumor stroma-infiltrating CD4+ and CD8+ T cells, and smoking status ($P < 0.05$).

There were more CD8+ T cells in the tumor stroma and fewer in the tumor islets in NSCLC patients with COPD as compared to NSCLC patients without COPD ($P < 0.05$). However, there was no such association between CD4+ T cells and COPD status. A high level of CD8+ T cell infiltration in the tumor stroma was independently associated with the coexistence of COPD in multivariate analysis ($P < 0.05$).
Conclusions: According to our data, COPD but not smoking seems to be associated with higher infiltration of CD8+ T cells in the tumor stroma of patients with NSCLC. It allows us to

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hypothesize that NSCLC patients with coexisting COPD may have a more favorable outcome due to anticancer properties of stromal $CD8^+$ T cells.

1. Introduction

Lung cancer and chronic obstructive pulmonary disease (COPD) are the leading causes of morbidity and mortality worldwide [1]. The association between COPD and lung cancer has been reported in numerous studies [2]. COPD and lung cancer are disorders characterized by an abnormal local and systemic inflammatory response with smoking being as a major environmental risk factor [3]. Tobacco smoke stimulates both local and systemic inflammation, which may play a crucial role in both lung cancer and COPD. On the other hand, COPD has been reported to be a risk factor for lung cancer despite the smoking status [4,5]. The annual incidence of lung cancer arising from COPD has been reported to be 0.8%–1.7% [6,7]. Coexistence of COPD and lung cancer has an impact on the disease course and the outcome of cancer patients with conflicting results being reported [8].

The mechanisms by which COPD increases the risk of development of lung cancer and the influence of COPD on the prognosis of patients with lung cancer are not clear although it is accepted that chronic immune inflammation probably plays a role in the pathogenesis of lung cancer in these patients. Chronic inflammation is associated with malignant transformation and an increased incidence of local cancer in such a way how reflux esophagitis is associated with esophageal carcinoma; *Helicobacter pylori* gastric inflammation, with stomach cancer; viral hepatitis, with liver cancer; and inflammatory bowel diseases (chronic ulcerative colitis and Crohn's disease), with colon carcinoma [9–11]. The inflammatory responses that characterize COPD drive a repetitive cycle of injury and repair throughout the lungs with ongoing recruitment of host inflammatory cells and increase the risk of transformation of normal bronchial epithelium to a malignant phenotype [12,13]. Macrophages, neutrophils, and lymphocytes are the main players in chronic immune inflammation in COPD as well as lung cancer. In both airway and alveolar compartments, the $CD8^+$ cytotoxic T cell is the predominant T cell in patients with COPD. The number of pulmonary $CD8^+$ T cells in COPD increases substantially with higher stages of airflow limitation and emphysema. Smokers with COPD have increased numbers of $CD4^+$ T cells in the airways and lungs [14,15].

In lung cancer, the essential role in the immune response to cancer cells is played by tumor-infiltrating $CD4^+$ and $CD8^+$ T cells [16,17]. $CD8^+$ T cells represent a major arm of the antitumor response because they have cytotoxic cell-mediated activity toward tumor cells expressing tumor-associated antigens [18]. It is thought that $CD4^+$ T cells also play a significant role in antitumor response by allowing $CD8^+$ T cells to entry into the tumor site [19] and infected mucosa [20], and they also are required for angiogenesis inhibition at the tumor site [21]. Contradictory data have been published about the influence of the different infiltration patterns of immune cells in lung cancer on the prognosis of these patients [11].

Nevertheless, the data about the importance of accumulation and distribution patterns of $CD4^+$ and $CD8^+$ T cells in the lungs of patients with lung cancer in the background of chronic inflammation due to COPD are scarce. Coexistence of lung cancer and COPD could influence the functions of these cells considerably by promoting or suppressing tumor growth depending on the status of the lung immune [22].

Based on previous data on the link between COPD and non-small cell lung cancer (NSCLC), we aimed to evaluate the infiltration patterns of $CD4^+$ and $CD8^+$ T cells in tumor islets and stroma from patients with NSCLC and to analyze their relations to COPD, smoking status and other clinicopathologic variables.

2. Materials and methods

2.1. Subjects

Lung tissue specimens from 50 newly diagnosed and untreated NSCLC patients who underwent surgical resection for NSCLC (pathological stage I-III) and 10 control group subjects who underwent surgery due to recurrent spontaneous pneumothorax at the Hospital of Lithuanian University of Health Sciences, Kaunas Clinics from September 2012 to April 2014 were studied. For eligible patients, demographic data including smoking habit, data on COPD and other comorbidities were collected. Subjects were excluded if they had a history of another malignancy, connective tissue diseases or any unstable systemic disease (including active infections, significant cardiovascular disease). None of NSCLC patients received preoperative radiotherapy or chemotherapy. Lung specimens were evaluated by two qualified pathologists. Histological classification of tumors was based on the World Health Organization criteria [23]. Tumors were staged according to the TNM Classification of Malignant Tumors, the seventh edition [24]. The clinical stage and tumor type were recorded at the time of diagnosis. NSCLC patients were divided into two groups according to their COPD status: NSCLC patients with COPD and NSCLC patients without COPD. COPD was defined according to the criteria of the Global Initiative for Chronic Obstructive Lung Disease (GOLD) [25]. None of included subjects had an exacerbation of COPD or clinical signs of an acute upper respiratory tract infection or had received systemic glucocorticoid therapy 1 month before the surgery. At the same, patients were divided into following two groups based on a smoking history: nonsmokers and smokers. Patients who reported smoking more than 100 cigarettes during their lifetime were defined as smokers. Smoking history

was calculated in pack-years as the product of tobacco use (in years) and the average number of cigarettes smoked per day divided by 20 (years × cigarettes per day/20). Pulmonary function was tested by using a pneumotachometric spirometer ''CustovitM'' (Custo Med, Germany). The highest value of forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC) and FEV_1/FVC ratio from three reproducible measurements were recorded. The results were compared with the predicted value matched for age, body height and sex according to the standard methodology [26].

The study protocol was approved by Kaunas Regional Ethics Committee for Biomedical Research (No. BE-2-20). The study was registered in the U.S. National Institutes of Health trial registry *ClinicalTrials.gov* with identifier NCT02214303.

Written informed consent was obtained from all study subjects.

2.2. *Immunohistochemical analysis*

Lung tissue samples were fixed in formalin and, after dehydration, embedded in paraffin. Tissue sections 3- to 5-μm thick were cut, subsequently de-waxed and rehydrated through graded alcohols. Slides immunohistochemically analyzed for the expression of $CD4^+$ and $CD8^+$ T cells. A Roche Ventana Benchmark XT automated slide stainer (Ventana Medical Systems, Roche, France) was used for immunohistochemistry. Immunohistochemical staining was performed according to the manufacturer's instructions. Monoclonal rabbit anti human antibodies were used for identification of $CD4^+$ T cells (anti-CD4, SP35, Ventana) and $CD8^+$ T cells (anti-CD8, SP57, Ventana). Quantitative evaluation of $CD4^+$ and $CD8^+$ T cells was done in 5 most representative high-power fields (HPFs 400× magnification) per tissue section using an Olympus BX50 microscope (Olympus Co, Japan). The number of cells with positive staining was counted manually in two locations: tumor stroma and tumor islets (Fig. 1). Slides were coded, and microscopic analysis was carried out blindly to the clinical data.

2.3. *Statistical analysis*

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 20.0. The number of $CD4^+$ and $CD8^+$ T cells is presented as median with range. The associations between tumor-infiltrating $CD4^+$ and $CD8^+$ T cells and clinicopathologic characteristics were analyzed using the chi-square (χ^2) test or the Fisher exact test. Differences among all study groups were evaluated by using the Kruskal–Wallis test or the Mann–Whitney *U* test for independent samples and the Wilcoxon test for related samples. Correlation was assessed by the Spearman rank test for continuous variables. Multivariate logistic regression analysis was used to identify factors independently associated with the biomarkers of interest. *P* values of <0.05 were considered to indicate statistical significance.

3. Results

Demographic, clinical, and histological characteristics of studied subjects are shown in Table 1. The groups of NSCLC patients by COPD status and smoking history were homogenous, except for FEV_1/FVC ratio, forced expiratory volume in 1 s (FEV_1), and smoking history. Our study included 24 patients with adenocarcinoma, 22 patients with squamous cell carcinoma, 3 patients with large cell carcinoma, and 1 patient with adenosquamous carcinoma (the latter two were grouped in other histological group).

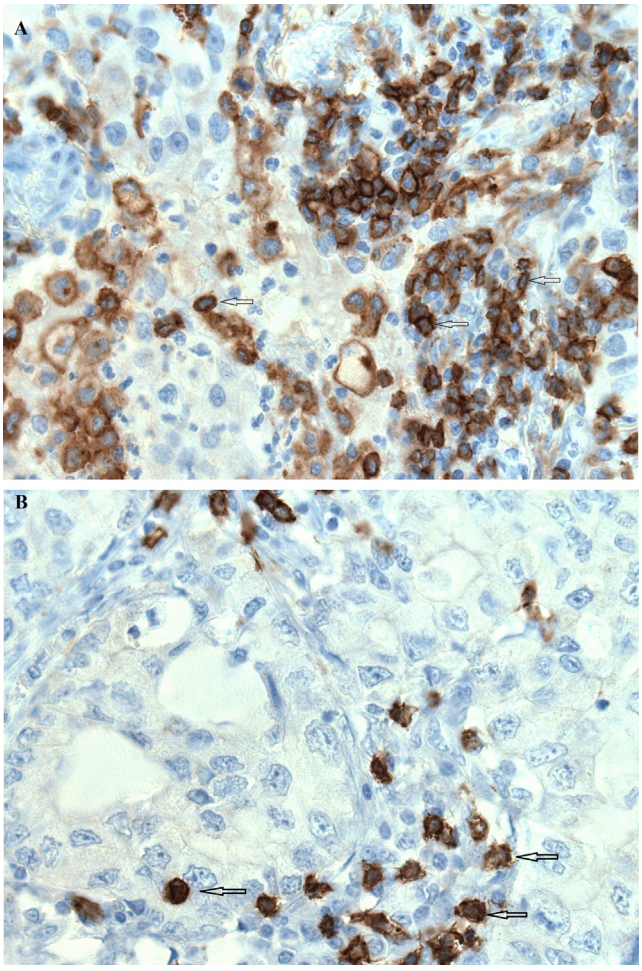

Fig. 1 – $CD4^+$ and $CD8^+$ T cells in non-small cell lung cancer tissue. Arrows indicate CD4 (A) and CD8 (B) positive stained cells (original magnification, 400×).

While analyzing the NSCLC and control group patients, we compared total numbers of $CD4^+$ and $CD8^+$ T cells due to different morphological structure of tumor tissue. NSCLC patients had a greater number of lung tissue-infiltrating $CD4^+$ and $CD8^+$ T cells compared with the control group (Fig. 2).

Predominant infiltration of $CD4^+$ T cells as well as $CD8^+$ T cells was observed in tumor stroma compared to tumor islets (139.0 [40–326] vs. 11.0 [2–55], $P = 0.0001$ and 137.5 [47–230] vs. 23.5 [6–129], respectively; $P = 0.0001$). $CD8^+$ T cells predominated over $CD4^+$ T cells in the tumor islets ($P = 0.0001$), but no significant difference between the amount of these cells was found in the tumor stroma.

In addition, a significant correlation was found between the total number of $CD4^+$ T cells and $CD8^+$ T cells ($r = 0.35$; $P = 0.012$); $CD4^+$ T cells in the tumor stroma and total number of

Table 1 – Characteristics of study population.

Variable	NSCLC without COPD $n = 33$	NSCLC with COPD $n = 17$	Healthy subjects $n = 10$
Gender, *n* (%)			
Female	8 (24.2)	2 (11.8)	1 (10)
Male	25 (75.8)	15 (88.2)	9 (90)
Age, years, median (range)	64.0 (46–77)	65.0 (45–75)	36.5 (20–77)[#]
Smoking history, *n* (%)[**]			
Nonsmokers	8 (24.2)	0 (0.0)	7 (70)
Smokers	25 (75.8)	17 (100.0)[*]	3 (30)
Smoking pack-years, median (range)	20 (0–52)	30 (10–60)	0 (0–50)
FEV_1% of pred., median (range)[***]	89 (64–144)	61 (33–102)	93 (77–114)
FEV_1/FVC ratio, % of pred., median (range)[***]	94 (79–122)	73 (49–95)	107 (86–114)
Histological NSCLC type, *n* (%)			
Adenocarcinoma	17 (51.5)	7 (41.2)	–
Squamous cell carcinoma	12 (36.4)	10 (58.8)	
Other	4 (12.1)	0 (0.0)	
NSCLC stage, *n* (%)			
IA	2 (6.1)	0 (0.0)	
IB	6 (18.2)	4 (23.5)	–
IIA	9 (27.3)	5 (29.4)	
IIB	3 (9.1)	0 (0.0)	
IIIA	12 (36.4)	7 (41.2)	
IIIB	1 (3.0)	1 (5.9)	
T status, *n* (%)			
T1a	1 (3.0)	1 (5.9)	
T1b	4 (12.1)	0 (0.0)	–
T2a	15 (45.5)	9 (52.9)	
T2b	5 (15.2)	2 (11.8)	
T3	6 (18.2)	5 (29.4)	
T4	2 (6.1)	0 (0.0)	
N status, *n* (%)			
N0	16 (48.5)	5 (29.4)	
N1	9 (27.3)	6 (35.3)	–
N2	7 (21.2)	5 (29.4)	
N3	1 (3.0)	1 (5.9)	
Differentiation, *n* (%)			
Poor	18 (54.5)	10 (58.8)	–
Other	15 (45.5)	7 (41.2)	
COPD, *n* (%)			
Mild	–	4 (23.5)	–
Moderate		9 (53.0)	
Severe		4 (23.5)	

FEV_1, forced expiratory volume in one second; FVC, forced vital capacity.
[#] $P < 0.05$, healthy subject compared to NSCLC groups (Mann–Whitney *U* test).
[*] $P < 0.05$, NSCLC patients with COPD compared to NSCLC patients without COPD (Fisher exact test).
[**] $P < 0.05$, chi-square (χ^2) test.
[***] $P < 0.05$, Kruskal–Wallis test.

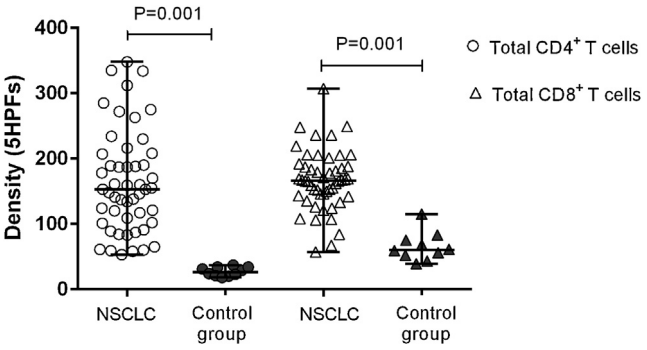

Fig. 2 – Distribution of total $CD4^+$ and $CD8\ T^+$ cells in lung tissue of NSCLC and control group subjects.

$CD8^+$ T cells ($r = 0.35$; $P = 0.012$) as well as $CD8^+$ T cells in the tumor islets ($r = 0.28$; $P = 0.045$); total number of $CD4^+$ T cells and $CD8^+$ T cells in the tumor islets ($r = 0.3$; $P = 0.036$).

There was no association between the numbers of total $CD4^+$ or $CD8^+$ T cells in tumor stroma or islets and NSCLC patients' gender, age, pathological T status, lymph node status, or tumor differentiation (Tables 2 and 3). While analyzing the total number of $CD4^+$ T cells, they were more frequently found in squamous cell carcinoma than adenocarcinoma ($P = 0.04$) (Table 2). $CD4^+$ and $CD8^+$ T cells were found more abundantly in the stroma of squamous cell carcinoma compared with adenocarcinoma (175.5 [52–326] vs. 121.0 [40–258], $P = 0.03$ and 149.5 [49–223] vs. 129.0 [78–230], respectively; $P = 0.06$).

While analyzing the patients according to their smoking status, smoking patients with NSCLC had a significantly greater number of tumor stroma-infiltrating $CD4^+$ and $CD8^+$ T cells than nonsmokers with NSCLC ($P < 0.05$); however, in the tumor islets, there were no significant differences in the number of $CD4^+$ and $CD8^+$ T cells between the groups (Figs. 3 and 4). Significantly higher amount of total tumor infiltrating $CD4^+$ and $CD8^+$ T cells was found in smoking NSCLC patients compared to non-smokers NSCLC patients ($P = 0.03$) (Table 2).

Our data showed that there were significantly more smokers with NSCLC having a high level of $CD4^+$ and $CD8^+$ T cell infiltration as compared to nonsmokers with NSCLC ($P = 0.02$) (Table 2). There were no significant differences in the level of $CD4^+$ or $CD8^+$ T cell infiltration either in the tumor islets or stroma comparing both patients' groups (Table 3).

There were more $CD8^+$ T cells in the tumor stroma in NSCLC patients with COPD compared with NSCLC patients without COPD ($P = 0.01$). However, the number of $CD8^+$ T cells in the tumor islets was significantly greater in the group of NSCLC patients without COPD than their counterparts with COPD ($P = 0.04$) (Fig. 5). The total number of tumor-infiltrating $CD4^+$ and $CD8^+$ T cells did not differ significantly between these groups; however, a trend toward higher numbers of tumor-infiltrating $CD8^+$ T cells in NSCLC patients with COPD was observed ($P = 0.06$) (Table 2).

The high level of $CD8^+$ T cell infiltration in the tumor islets was found more frequently in NSCLC patients without COPD compared to NSCLC patients with COPD, and contrary, the high level of $CD8^+$ T cell infiltration in the tumor stroma was found more frequently in NSCLC patients with COPD compared to NSCLC patients without COPD ($P = 0.01$ and $P = 0.02$, respectively) (Table 3). There were no significant differences in the level of $CD4^+$ T cell infiltration either in the tumor islets or

Table 2 – Association between total CD4+ and CD8 T+ cells infiltration in non-small cell lung cancer tissue and clinicopathological features.

Characteristic	Cells, median (range)				No. of patients with particular infiltration level					
	$CD4^+$ T cells	P	$CD8^+$ T cells	P	$CD4^+$ T cells			$CD8^+$ T cells		
					Low	High	P	Low	High	P
Gender										
Female	140.0 (60–312)	NS	159.5 (57–205)	NS	7	3	NS	6	4	NS
Male	159.5 (53–348)		167.0 (67–307)		17	23		19	21	
Smoking status										
Nonsmokers	110.5 (60–155)	**0.03**	142.5 (57–205)	**0.03**	7	1	**0.02**	7	1	**0.02**
Smokers	160.5 (53–348)		168.5 (67–307)		17	25		18	24	
Histological NSCLC type										
Adenocarcinoma	142.0 (58–285)		160.5 (84–249)		14	10		14	10	
Squamous cell carcinoma	188.0 (59–348)	**0.02**	178.5 (67–307)	**0.07**	6	16	**0.01**	8	14	NS
Other	93.0 (53–134)	**0.02**[*]	146.0 (57–205)	NS[*]	4	0	**0.04**[**]	3	1	NS[**]
pT status										
pT1a-2b	155.0 (53–348)	NS	165.0 (57–249)	NS	17	20	NS	21	16	NS
pT3-4	134.0 (40–322)		179.0 (84–307)		7	6		4	9	
Lymph node status										
Negative (pN0)	161.0 (58–348)	NS	162.0 (67–248)	NS	10	11	NS	11	10	NS
Positive (pN1-N3)	153.0 (53–335)		167.0 (57–307)		14	15		14	15	
Differentiation										
Poor	159.5 (58–348)	NS	173.5 (67–307)	NS	12	16	NS	11	17	NS
Other	143.5 (53–335)		158.5 (57–205)		12	10		14	8	
COPD										
Absent	148.0 (53–335)	NS	153.0 (57–307)	**0.06**	17	16	NS	20	13	**0.07**
Present	187.0 (65–348)		171.0 (84–249)		7	10		5	12	

[*] Mann–Whitney test for difference between adenocarcinoma and squamous cell carcinoma.
[**] Fisher exact test for association between adenocarcinoma and squamous cell carcinoma with T cells.

Table 3 – Associations between the level of $CD4^+$ and $CD8^+$ T cell infiltration in tumor islets and stroma and clinicopathological features in NSCLC.

Characteristic	No. of patients with NSCLC						No. of patients with NSCLC					
	$CD4^+$ T cells in islets		P	$CD4^+$ T cells in stroma		P	$CD8^+$ T cells in islets		P	$CD8^+$ T cells in stroma		P
	Low	High		Low	High		Low	High		Low	High	
Gender												
Female	4	6	NS	6	4	NS	4	6	NS	7	3	NS
Male	20	20		19	21		21	19		18	22	
Smoking status												
Nonsmokers	3	5	NS	6	2	NS	3	5	NS	6	2	NS
Smokers	21	21		19	23		22	20		19	23	
Histological NSCLC type												
Adenocarcinoma	14	10	NS	13	11	**0.06**	10	14	NS	15	9	**0.07**
Squamous cell carcinoma	9	13	NS[*]	8	14	NS[*]	13	9	NS[*]	7	15	**0.045**[*]
Other	1	3		4	0		2	2		3	1	
pT status												
pT1a-2b	20	17	NS	17	20	NS	18	19	NS	20	17	NS
pT3-4	4	9		8	5		7	6		5	8	
Lymph node status												
Negative (pN0)	9	12	NS	9	12	NS	8	13	NS	14	7	**0.09**
Positive (pN1-N3)	15	14		16	13		17	12		11	18	
Differentiation												
Poor	12	10	NS	12	15	NS	9	13	NS	13	9	NS
Other	12	16		13	10		16	12		12	16	
COPD												
Absent	14	19	NS	18	15	NS	13	20	**0.01**	21	12	**0.02**
Present	10	7		7	10		12	5		4	13	

The level of infiltration of $CD4^+$ and $CD8^+$ T cells was defined as either "high" or "low" according the median of $CD4^+$ and $CD8^+$ T cells.
[*] Fisher exact test for association between adenocarcinoma and squamous cell carcinoma with T cells.

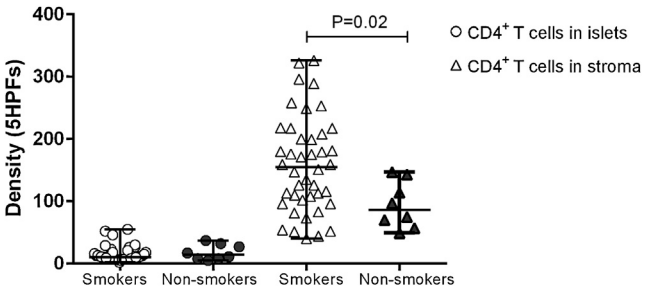

Fig. 3 – CD4⁺ T cells distribution in tumor stroma and islets among smokers and non-smokers NSCLC patients.

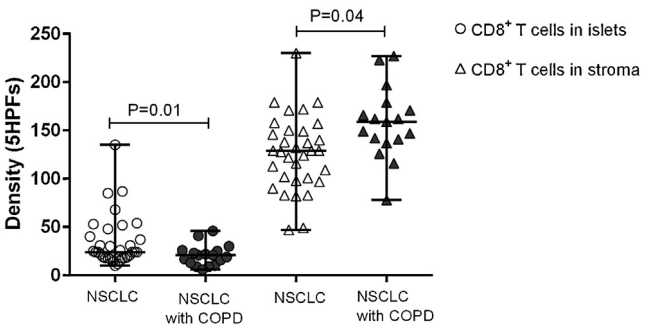

Fig. 5 – CD8⁺ T cells distribution in tumor stroma and islets among NSCLC patients with and without COPD.

stroma and total CD4⁺ and CD8⁺ T cell infiltration between these patients' groups, but there was a trend of higher CD8⁺ T cell infiltration in NSCLC patients with COPD ($P = 0.07$) (Tables 2 and 3).

To investigate the association between the level of CD8⁺ T cell infiltration in the tumor stroma and factors such as smoking status, histology, and COPD status, multivariate logistic regression analysis was performed. A high level of CD8⁺ T cells infiltration in the tumor stroma was found to be independently associated with the presence of COPD (Exp(*B*), 4.29; 95% CI of Exp(*B*), 1.02–17.99; $P = 0.046$).

4. Discussion

In this study, we aimed to determine if there is an association between the number and infiltration level of CD4⁺ and CD8⁺ T cells in tumor stroma or islets and clinicopathological features with special attention given to coexisting COPD and smoking status.

Differently from normal tissue, lung tumors have two distinct but interdependent compartments: the parenchyma (neoplastic cells) and the stroma that the neoplastic cells induce and in which they are dispersed, that is why while analyzing control and NSCLC groups we used total number (in tumor islets and stroma) of CD4⁺ and CD8⁺ T cells. As expected, the numbers of CD4⁺ and CD8⁺ T cells were greater in the NSCLC tissue than the lung tissue from control group subjects. To the best of our knowledge, there are no studies that compared T cell infiltration in the lung tissue between lung cancer patients and control subjects. In previous reports,

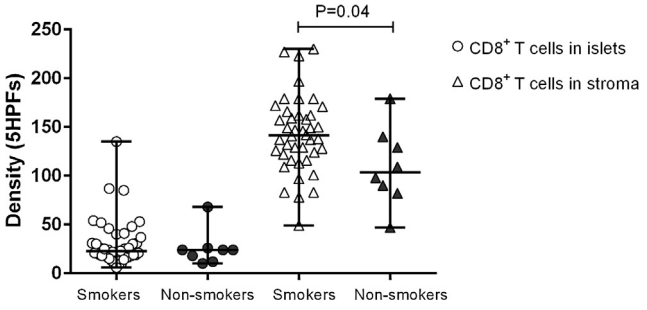

Fig. 4 – CD8⁺ T cells distribution in tumor stroma and islets among smokers and non-smokers NSCLC patients.

peripheral blood, BAL, or lung tissue from NSCLC were used as a control. Derniame et al. showed more intense expression of CD8⁺ T cells in the tumor tissue compared with the healthy lung tissue from the same patient [27], and Jusufovic et al. found a significantly higher number of CD4⁺ and CD8⁺ T cells in BAL of patients with primary lung cancer as compared with the healthy lung tissue of the same patients [28]. Woo et al. reported that expression of all of the activation markers examined was significantly higher in CD4⁺ and CD8⁺ T cells from early stage lung cancer as compared with control from healthy donor peripheral blood [29]. Thus, our findings and previous studies support the idea that tumor-associated CD4⁺ and CD8⁺ T cells may play an essential role in immune response during oncological process as demonstrated by higher infiltration of these cells and significant state of activation in NSCLC as compared to healthy subjects.

Tumor-infiltrating CD4⁺ and CD8⁺ T lymphocytes were observed predominantly in the tumor stroma compared to islets. These findings are in agreement with the results from other studies, which found that the number of infiltrating CD4⁺ or CD8⁺ T cells in the tumor stroma was overwhelmingly higher than in the tumor islets [16,30,31]. It was shown that antitumor cytokines contributing to tumor suppression acted mainly in the stroma [32]. Thus, the abundance of tumor-infiltrating lymphocytes in the tumor stroma let suggest a significant impact of the stromal component on the modulation of cancer cells. On the other hand, in our study, CD8⁺ T cells predominated over CD4⁺ T cells in the tumor islets but not in the tumor stroma. Wakabayashi et al. also found that CD8⁺ T cells predominated over CD4⁺ T cells in the tumor islets [33]. It is well known that cytotoxic CD8⁺ T cells are the principal antitumor effector cells that recognize particular tumor-associated antigens presented on MHC class I molecules at the cancer cell surface and possess the ability to destroy cancer cells directly [34]. Previous studies showed that a higher number of epithelial CD8⁺ T cells were associated with tumor cell apoptosis [35] or IFN-γ/CD8 ratio [36] in NSCLC. Our results also suggest that CD8⁺ T cells could be directly involved in immune response against lung cancer cells in the tumor islets.

CD4⁺ T cells play a central role in orchestrating the immune response to cancer. Essentially CD4⁺ T cells recognize peptides presented on MHC class II molecules expressed primarily on antigen-presenting cells. The main role of CD4⁺ T cells in the immune response to cancer is to prime CD8⁺ T cells and

maintain their proliferation. There are data that $CD4^+$ T cells are required for $CD8^+$ T cell activation against cancer cells by secreting cytokines such as interleukin 2, which is required for the growth and proliferation of $CD8^+$ T cells [16,27] and their transformation into long-lived functional effector cells [37]. This positive interaction between immune cells was also found in our study as there was significant correlation between the number of $CD4^+$ and $CD8^+$ T cells in different compartments of cancer.

The greater number of total $CD4^+$ T cells was more frequently found in squamous cell carcinoma compared with adenocarcinoma as well as $CD4^+$ and $CD8^+$ T cells were more abundantly found in the stroma of squamous cell carcinoma compared with adenocarcinoma. Hiraoka et al. found that $CD8^+$ T cells in the tumor islets were significantly associated with the histology of squamous cell carcinoma [16]. Wakabayashi et al. reported that $CD4^+$ and $CD8^+$ T cells in the tumor islets or stroma were more frequently found in squamous cell carcinoma compared with adenocarcinoma [33]. The reason for these differences between histology and T cell infiltration could be a difference in immunogenicity of different histological subtype of NSCLC. For example, it was shown that squamous cell carcinoma of the lung had a higher frequency of mutations that adenocarcinoma [38]. These findings suggest that squamous cell carcinoma could be more immunogenic lung cancer than adenocarcinoma. On the other hand, a recent study by Alifano et al. has reported opposite results showing a lower density of infiltrating $CD8^+$ T cells in the tumor islets in squamous cell carcinoma [39].

Cigarette smoking causes lung cancer, poses a greater risk of metastatic cancer spread and increases overall mortality in cancer patients. Smoking is a risk factor for COPD as well. A significantly higher number of total and tumor stroma-infiltrating $CD4^+$ and $CD8^+$ T cells as well as a higher level of total $CD4^+$ and $CD8^+$ T cell infiltration in smoking patients compared to nonsmokers with NSCLC were documented in our study. Some studies have reported that the number of $CD8^+$ and $CD4^+$ T cells was greater in the lungs of patients with COPD and even asymptomatic smokers than nonsmoking controls [40–43]. In one large study, Saetta et al. immunohistochemically examined alveolar walls and pulmonary arteries to identify $CD4^+$ and $CD8^+$ T cells and found that smokers with COPD had an increased number of $CD8^+$ T cells both in lung parenchyma and pulmonary arteries as compared with nonsmokers. The numbers of $CD8^+$ T cells were also increased in pulmonary arteries of smokers with COPD as compared with smokers with normal lung function [44]. Smokers with normal lung function showed increased numbers of macrophages and T lymphocytes in the lung parenchyma compared with control nonsmokers [45]. The number of $CD4^+$ cells was reported not to be changed in patients with mild/moderate COPD compared with control smokers [46]. Contradictory data have been published about the influence of smoking on $CD4^+$ and $CD8^+$ T cells in NSCLC. Some studies found that $CD4^+$ and $CD8^+$ T cells in tumor islets correlated significantly with the smoking habit, i.e., smokers had greater numbers of these cells [33], while others did not find such associations [31,39].

Our results in agreement with other previous studies let suggest that tobacco smoking interferes with the host defense system [47–49]. It is known that tobacco smoking stimulates the migration of neutrophils, macrophages, $CD4^+$, $CD8^+$, and B cell lymphocytes and smaller numbers of dendritic cells and natural killer cells into the damaged tissue [47]. On the other hand, cigarette smoking inhibits the maturation of dendritic cells. Smoke-induced defects in the function of dendritic cells may lead to impaired function of T cells and inhibit tumor immunosurveillance [48]. In mouse models, the activation of $CD8^+$ T cells has been reported to be impaired in the presence of cigarette smoke [49]. Thus, tobacco smoking through chronic inflammation could not only attract more immune cells, but also impair the function of these cells.

Smoking, COPD, and lung cancer are closely related because chronic airway inflammation is a key feature of smoking-induced lung damage and play a significant role in the pathogenesis of COPD and lung cancer. However, a direct role of chronic inflammation defined as lung tissue infiltration with T cells in the pathogenesis of lung cancer and its relation to the processes in COPD have not been completely elucidated yet. Regarding specific inflammation, $CD4^+$ helper and especially cytotoxic $CD8^+$ T lymphocytes have been increased in airways and lung parenchyma in COPD patients [40]. Even though the mechanisms by which $CD8^+$ and $CD4^+$ T cells accumulate in the lungs of patients with COPD are not yet understood, it is thought that an increased number of T cells in the lungs are due to adhesion and selective chemotaxis [50]. The excessive recruitment of T cells in the lungs could be a consequence of response to neoantigens induced by cigarette smoke or simply response to viral infections (active or latent) that are common in this disease [40].

To the best of our knowledge, we are first who presented the data about a significant association of COPD with the patterns of $CD4^+$ and $CD8^+$ T cell infiltration in NSCLC. Our results show that NSCLC patients with COPD had a greater number of $CD8^+$ T cells in the tumor stroma and a lower number in the tumor islets than those without COPD. There was a trend toward higher numbers of total tumor-infiltrating $CD8^+$ T cells in NSCLC patients with COPD as well. Multivariate analysis revealed that a high level of $CD8^+$ T cell infiltration in the tumor stroma was independently associated with the presence of COPD. We found only one study carried out by Alifano et al. that did not report associations between the density of $CD8^+$ T cells in tumor islets and COPD. However, the same investigators reported that the density of intratumoral mature dendritic cells was significantly associated with COPD status in multivariate analysis [39]. These results are in line with our results and suggest that coexisting COPD could influence the accumulation of immune cells in the tumor stroma rather than tumor islets. We suggest that lung cancer developed in COPD patients arises in the lung tissue enriched by $CD8^+$ T cells compared to lung cancer patients without COPD. In addition, smoking increases the total number of $CD4^+$ and $CD8^+$ T cells in NSCLC, and COPD independently influences the distribution of T cells between the tumor stroma and islets. The importance of this different COPD-related distribution of T cells is unknown; however, some studies suggest that it could have prognostic significance. The interaction between tumor and immune cells in the tumor microenvironment is influenced by the type of immune cells as well as their density and localization [51]. Although there are contradictory results, the majority of recent studies have shown that high intrastromal $CD4^+$ and/or $CD8^+$ T cell

infiltration is a favorable prognostic factor for overall and disease-free survival [16,30,33,52,53].

5. Conclusions

Due to chronic airway inflammation smoking, COPD and lung cancer are closely related. According to our data, COPD but not smoking seems to be associated with greater infiltration of $CD8^+$ T cells in the tumor stroma of patients with NSCLC. It allows us to hypothesize that NSCLC patients with coexisting COPD may have a more favorable outcome due to anticancer properties of stromal $CD8^+$ T cells; however, further investigations are required.

Conflict of interest

The authors declare that they have no conflict of interests.

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