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Expression of Epidermal Growth Factor (EGF) and its receptors (EGFR1 and EGFR2) in chronic bronchitis

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Abstract

Chronic Obstructive Pulmonary Disease (COPD) is one of the leading causes of morbidity and mortality in the industrialized and in the developing countries. It is believe, at least in part, that some of the structural changes that occur in COPD would be a result of epidermal growth factors (EGFs) and their receptors. Therefore, our study aims was to examine the expression patterns of EGF and their receptors (EGFR1 and c-erbB2) in the bronchial mucosa from the biopsy specimens harvested from smoking and non-smoking CB patients, compared with their expression in normal controls. The statistical analysis proved that for both EGF and EGFR1 reactivity were significant correlation with smoking status and FEV1% scores. Thus, we found that the highest levels of its expression were recorded in smoker CB patients with higher FEV1% scores. Regarding cellular localization and staining pattern, we noticed a cytoplasmic and nuclear immunostaining for EGF in bronchial epithelium both for control and CB subjects at the level of basal and ciliated cells. For the receptors, reactions were at the membrane level especially at the lower lateral junctions between ciliated cells and their junctions with basal cells. This reactivity proved the pathogenic implication of the EGF and their receptors in patients with CB and suggests that blockade of the EGFR pathway can be an alternative successful therapy.

Keywords: chronic bronchitis, EGF, EGFR1, c-erbB2.

Introduction

Chronic Obstructive Pulmonary Disease (COPD) is one of the leading causes of morbidity and mortality in the industrialized and the developing countries. Studies from the last two decades indicate that 4–6% of the adult European population suffered from clinically relevant COPD [1], while in 2006 the *National Center for Health Statistics* estimated that 4% of the USA population was diagnosed with chronic bronchitis [2]. According to the *European Respiratory Society*, during 1988–1991 the mortality rates in men ranged from 41.4 per 100 000 in Hungary to 2.3 per 100 000 in Greece [3], while in the United States was estimated that COPD represents fourth most common cause of death, with 15 933 deaths due to chronic bronchitis (CB) [4]. For Romania was reported one of the highest rates of mortality with more than 60 per 100 000 populations [5].

As we proved in a previous study on biopsy specimens harvested from patients with CB, we found: squamous metaplastic change and goblet cell hyperplasia in bronchial epithelium, hypertrophia of submucosa glands, and an increased number of inflammatory cells in different bronchial compartments, predominantly of CD3+ lymphocytes, macrophages and mast cells types [6]. It is believe, at least in part, that some of these structural changes would be a result of epidermal growth factors (EGFs) and their receptors [7–16]. So, it has been demonstrated that normal bronchial epithelium expresses EGF-like factors and their receptors [8, 10, 11] and that EGF and ErbB receptors 1 and 3 reactivity was higher in smokers with CB than in non-smokers [8–10; 16]. Also, had been demonstrated that EGF and ErbB receptors regulates mucins production in airways and goblet cell metaplasia, features that are present in CB [7, 9, 13–15].

In the light of these results, the purpose of our study was to examine the expression patterns of EGF and their receptors (EGFR1 and c-erbB2) in the bronchial mucosa from the biopsy specimens harvested from smoking and non-smoking CB patients, compared with their expression in normal controls.

Materials and Methods

Subjects

As we specified in the previous study we enrolled 17 smoker patients and eight non-smoking patients, both with respiratory symptoms of CB, clinically stable, and that had not used inhaled or oral corticosteroids within three months before the study [6]. As control group, we

enrolled five non-smoking, healthy subjects (<2 pack years). We reviewed the medical records, noting for each patient parameters such as: gender, age, smoking status and FEV1% (FEV1/FVC) ratio (Table 1). The institutional medical ethics committee approved the study and all patients gave their written informed consent.

Table 1 – ***Clinical characteristics and comparison of bronchial inflammatory cells in patients with and without chronic bronchitis***

	No.	Age [years]	Gender	Smoking status	FEV1%	EGF+	EGFR1+	c-erbB2+
Smoker CB patients	1.	53	M	+	79	1	1	1
	2.	54	M	+	77	1	1	1
	3.	57	M	+	75	2	2	1
	4.	61	M	+	73	2	2	1
	5.	63	M	+	72	2	1	2
	6.	65	M	+	69	2	2	1
	7.	59	M	+	71	1	1	1
	8.	67	M	+	72	2	2	2
	9.	72	F	+	66	2	1	1
	10.	75	F	+	64	2	2	2
	11.	69	M	+	69	2	1	1
	12.	76	F	+	67	2	2	1
	13.	64	M	+	73	2	2	1
	14.	58	M	+	76	1	1	1
	15.	73	M	+	69	2	2	2
	16.	71	M	+	64	2	2	1
	17.	70	F	+	68	2	1	1
Non-smoker CB	18.	68	F	–	71	1	1	1
	19.	58	M	–	81	1	1	1
	20.	56	M	–	79	2	2	1
	21.	71	M	–	73	2	1	1
	22.	63	M	–	79	2	1	1
	23.	66	M	–	78	2	2	1
	24.	73	M	–	72	1	1	2
	25.	65	M	–	83	1	1	1
Control	1.	65	M	–	83	1	1	0
	2.	68	F	–	79	1	1	1
	3.	72	M	–	76	1	1	1
	4.	56	M	–	89	1	1	1
	5.	63	M	–	85	1	1	1

FEV1% – Proportion of the forced vital capacity exhaled in the first second; Epit. – Bronchial epithelium.

The bronchial biopsies processing and staining was done as previously described [6].

Immunohistochemical processing consisted in xylene deparaffinization, ethanol dehydration, and immersion of the slides in distillated water containing 3% hydrogen peroxide for 30 minutes to block endogenous peroxidase activity. Next, an antigen retrieval step was performed by 20 minutes boiling in Citrate buffer, pH 6 or Tris buffer-EDTA, pH 9. Subsequently, the unspecific binding sites were blocked with 5% BSA/PBS for one hour. For amplification, we used a standard Streptavidin–Biotin peroxidase system. The primary antibodies were used in dilutions specified in Table 2, incubating the slides overnight at 4^0C.

The reactions were amplified with LSAB2 (Dako, Redox, Romania – code K0675) and visualized with 3,3'-diaminobenzidine (DAB) (Dako, Redox, Romania – code K3468). For counterstaining, we used Mayer's Hematoxylin. For negative controls, the first antibody was omitted from this procedure and for positive controls, we used samples of tissue specimens specified in Table 2.

To estimate the reactivity only of the bronchial epithelium to EGF and their receptors (EGFR1 and c-erbB2) we use a double immunohistochemistry reaction. The first antibody (CK 5/6) was developed in the same manner as above and the second (EGF and their receptors) was visualized with LSAB+, AP. Rabbit/Mouse/Goat (Dako, Redox, Romania – code K0689) and as chromogen was used the Vulcan Fast Red (Biocare Medical, CYBER, Romania – code FR805).

Table 2 – ***The antibodies utilized in this study***

Antibodies	Clone, Manufacturer	Dilution	Antigen retrieval	Tissue used as positive external control
EGF	Rabbit polyclonal, SDIX	1:200	Citrate buffer, pH 6	Stomach
EGFR1	Rabbit polyclonal, Sigma	1:100	Citrate buffer, pH 6	Colorectal carcinoma
c-erbB2	Rabbit polyclonal, Dako	1:400	Citrate buffer, pH 6	Breast carcinoma
CK5/6	Mouse, D5/16 B4, Dako	1:50	Tris buffer-EDTA, pH 9	Skin

Analysis of bronchial biopsies and assessment of immunohistochemical staining

The stained sections were screened at a magnification of ×200 under a Nikon Eclipse 55i microscope (Nikon, Apidrag, Bucharest) equipped with a 5-megapixel cooled CCD camera and the Image ProPlus AMS7 software (Media Cybernetics Inc., Buckinghamshire, UK) to identify the regions with intact bronchial epithelium.

The immunoreactivity assessment was done independently by two-experienced pathologist and, in case of disagreement, the cases was reviewed until a consensus was reached. All three investigated markers were scored semi-quantitatively based on their distribution pattern in the bronchial epithelium thickness, on ×200 magnification according to the methodology describe by Merrick DT *et al.* [17]. In the absence of immunoreactivity was given score of 0. Presence of the reactions in the lower half of bronchial epithelium was assed as score 1, while the reactivity confined to the upper half and/or involving the entire thickness of the epithelium was scored 2. Because the intensity of the reactions varied very wide on specimens collected from all investigated patients, in this study we did not assessed it quantitatively but only qualitatively.

Statistical analysis

The statistical analysis was done with SPSS version 16.0 for Windows, using the *chi*-square test for dependence assessment, and ANOVA testing for multiple group comparison, the results being considered statistically significant if the *p*-value was <0.05. To assess the association between EGF, EGFR1 and c-erbB2 expression and other clinicopathologic variables, we have taken into account only quantitatively score of immunoreactivity distribution, the results of this investigations being grouped in two classes of measurements: one considered negative (that includes scores 0 and 1) and the other positive (scores which includes score 2).

Results

Patients and histopathological features

As we already described [6] in our casuistry CB developed especially in men of 65-year-old or older, especially in smokers with a median FEV1% at around 71 years. We could not manage to show significant differences between average FEV1% and age values depending on the study and control groups. Histopathologically, patients with symptoms of CB, regardless of smoking status, presented on bronchial biopsies with focal squamous metaplastic change, goblet cell hyperplasia and enlargement of the bronchial gland mass because of the inflammatory process, consisting predominantly of mononuclear cells in the bronchial wall.

Characterization of EGF expression patterns

In control groups at the bronchial mucosa specimens, we observed that EGF was expressed at the level of basal and ciliated cells from the bronchial epithelium (Figure 1A). The staining pattern was granular, both in cytoplasm and nuclei (Figure 1B). The mucous cells do not stain with this marker, and the ciliated cells presented reactivity even at the level of cilia. Also, some reactivity was present at the level of submucosal glands especially in serous acinar cells (Figure 1C) and in the rare inflammatory cells scattered through the bronchial specimen's thickness.

In the smoker CB group, the semiquantitative assessment showed that the majority of cases (13 of 17 patients) were scored as 2, EGF reactivity being present in the full bronchial epithelium thickness. For the non-smoker CB group, we noticed an equal distribution of scores 1 and 2, while in the control group all subjects had score 1. In the CB patients, the EGF immunoreactivity was mainly localized on ciliated and basal bronchial epithelial cells (Figure 1D) and in the metaplastic epithelium. The reaction pattern was similar to that from the control specimens. The reactivity appeared to be reduced in smoker CB specimens, which presented much more areas of bronchial goblet cell hyperplasia (Figure 1E). Also, some reactivity was observed in inflammatory cells that infiltrating the bronchial specimens (especially at the level of macrophages, eosinophils, and lymphocytes), in endothelial blood vessels cells, on smooth muscle and mucous glands (Figure 1F).

For EGF immunoreactivity, the statistical analysis showed no significant correlations with age {[$F(1.28)$= 3.915, p=0.058]}, sex {$\chi^2(1, N=30)=0.305$, p=0.580} and c-erbB2 reactivity {$\chi^2(2, N=30)=2.477$, p=0.290}. Regarding smoking status the *chi*-square test proved significant differences for EGF reactivity {$\chi^2(1, N=30)=6.266$, p=0.012}, the highest scores being associated to smoker CB patients (Figure 2A). Also, the ANOVA test indicated an significant inverse association of EGF reactivity with FEV1% {[$F(1.28)=15.5$, p=0.000]}, the highest EGF scores being recorded in CB patients with lowest FEV1% values (Figure 2B). Comparing the EGF reactivity with the EGFR status, the *chi*-square test indicated a positive correlation between these two variables {$\chi^2(1, N=30)=13.282$, p=0.000}.

Characterization of EGFR1 expression patterns

Immunohistochemical analysis of normal bronchial specimens revealed positive staining for EGFR1, in bronchial epithelium, submucosal glands and vascular endothelium. In the bronchial epithelium, the reactivity was restricted to the basal and ciliated cells, and the staining pattern was predominantly membranar with occasional cytoplasmic positivity (Figure 3A). The reaction was more obvious at the lower lateral junctions between ciliated cells and their junctions with basal cells, and a weak reactivity was also seen on the brush border of the bronchial epithelium (Figure 3B).

In the pathological specimens, the EGFR1 seems to be higher in the smoker CB specimens then in that from the non-smoker CB subjects, and for both it was superior to that from control specimens. Quantitatively, in the smoker CB group prevailed the score 2 (in 53% of the cases), while in the non-smoker subject the most encountered score was 1 (75% of cases).

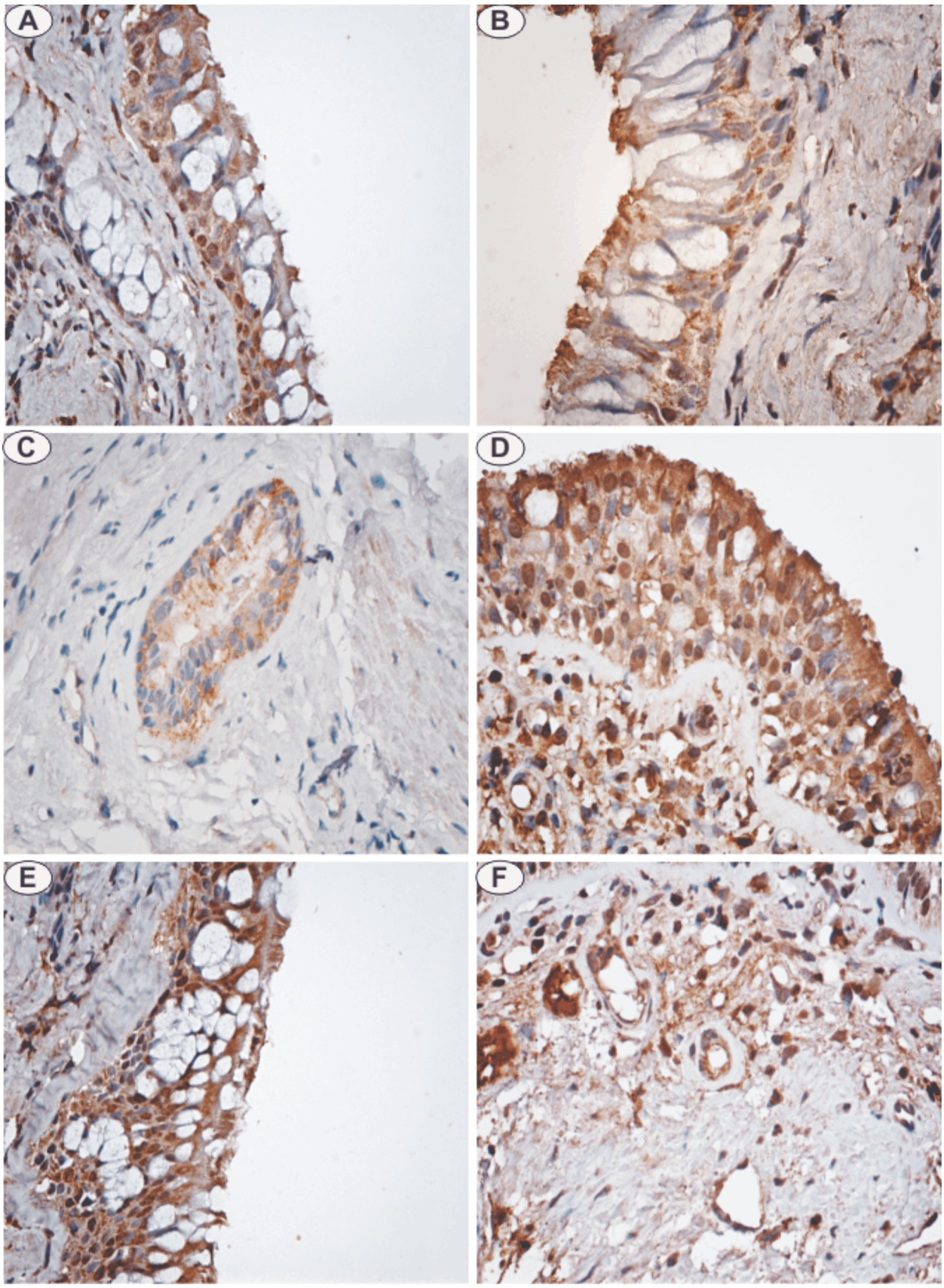

Figure 1 – ***EGF reactivity: (A) Positive reaction in the basal and ciliated cells from the control bronchial epithelium specimens. DAB, ×400; (B) Granular staining pattern in cytoplasm and nuclei and cilia. DAB, ×600; (C) Positive reaction in the serous acinar cells of submucosal glands. DAB, ×400; (D) Positive reaction in the basal and ciliated cells from the bronchial epithelium of CB specimens. DAB, ×400; (E) No reactivity at the level of mucous cells from the bronchial goblet cell hyperplasia areas of CB specimens. DAB, ×400; (F) Positive reaction in inflammatory cells that infiltrating the bronchial specimens and in endothelial blood vessels cells. DAB, ×400.***

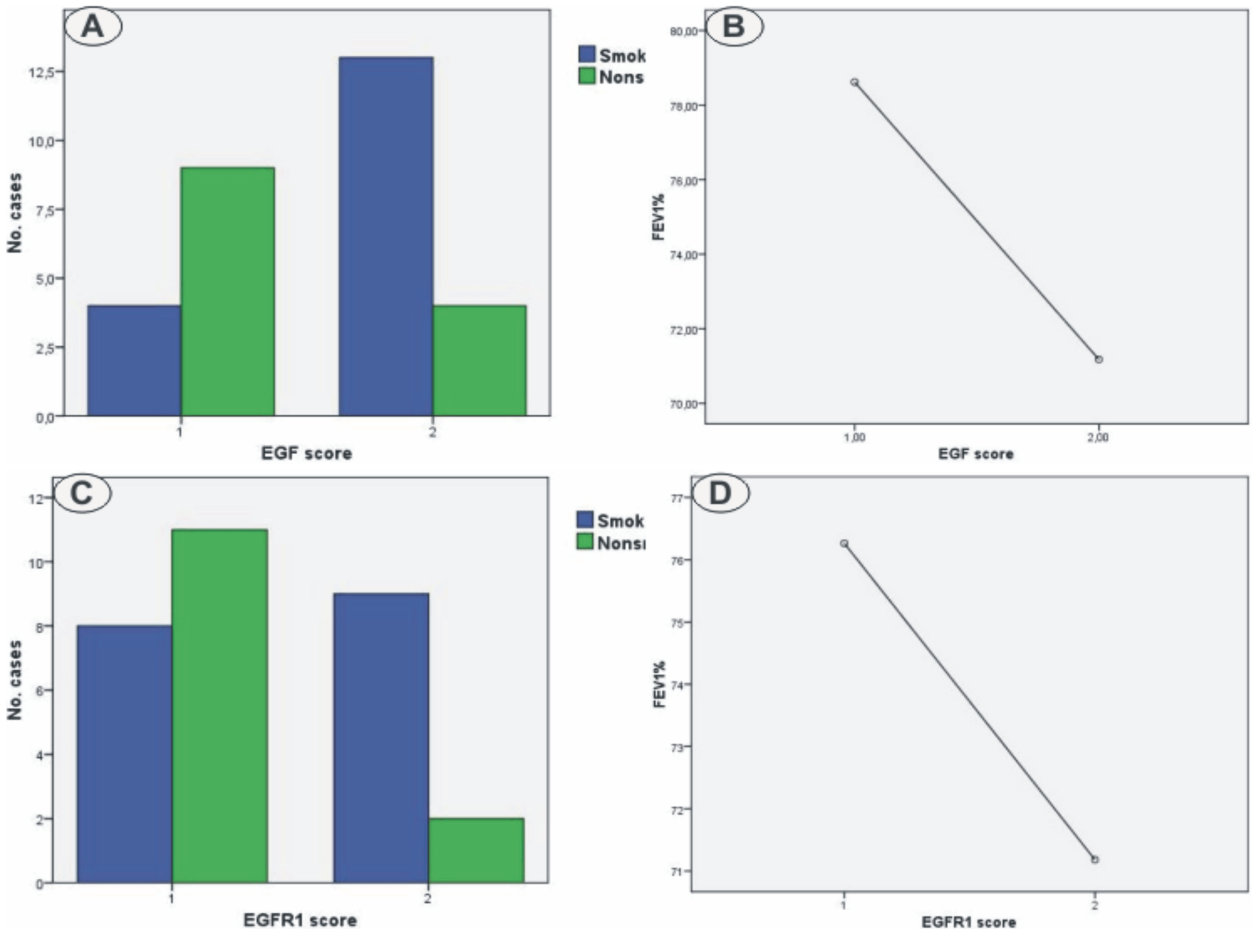

Figure 2 – ***ANOVA and chi-square testing of the EGF and EGFR1 immunoreactivity scores correlation with the smoking status and FEV1% scores of CB patients: (A) Significant differences of EGF reactivity with smoking status in CB patients; (B) A positive correlation of EGF reactivity with FEV1% scores of CB patients; (C) Significant differences of EGFR1 reactivity with smoking status in CB patients; (D) A positive correlation of EGFR1 reactivity with FEV1% scores of CB patients.***

In the bronchial epithelium, the immunostaining was weak along the basement membrane side of the basal cells and intense circumferential throughout most bronchial layers (Figure 3C). For the smokers CB specimens, the reactivity was more extensive extending to the apical surface highlighting the brush border (Figure 3, D and E). The reaction was also present in the remnants of the damaged epithelium and in squamous metaplastic areas (Figure 3F). Also, some reactivity was observed in some inflammatory cells and endothelial blood vessels cells.

Statistical analysis showed no significant correlations with age {[$F(1.28)=0.817$, $p=0.374$]}, sex {$\chi^2(1, N=30)=0.036$, $p=0.850$} and c-erbB2 reactivity {$\chi^2(2, N=30)=1.866$, $p=0.393$}. Regarding smoking status, the *chi*-square test proved significant differences for EGFR1 reactivity {$\chi^2(1, N=30)=4.474$, $p=0.034$}, the highest scores being associated to smoker CB patients (Figure 2C). Also, the ANOVA test indicated an significant inverse association of EGFR1 reactivity with FEV1% {[$F(1.28)=5.228$, $p=0.030$]}, the highest EGFR1 scores being recorded in CB patients with lowest FEV1% values (Figure 2D).

Characterization of c-erbB2 expression patterns

Generally, both for the normal and pathological specimens the c-erbB2 immunostaining was weaker than for EGFR1. In control specimens, the reactivity was present in bronchial epithelium, submucosal glands and vascular endothelium. In the bronchial epithelium, the c-erbB2 expression was more obvious between basal cells and the basal aspect of ciliated cells (Figure 4A). The reaction was also observed on lateral borders of mucous cells and even on the brush border of the bronchial epithelium. Similar to the EGFR staining, the pattern was predominantly membranar with occasional cytoplasmic positivity (Figure 4A).

In the pathological specimens, the reactivity was superior to that from normal specimens. The quantitative c-erbB2 assessment showed that in both CB groups the prevailing score was 1 (76.5% for smoker CB patients and 87.5% for the non-smoker CB subjects). Both the bronchial topography and staining pattern were similar to those from the control specimens (Figure 4B). In the bronchial epithelium, the reactivity was extended from the basal layer to the apical surface (Figure 4C). The bronchial c-erbB2 reactivity was reduced in areas with goblet cell hyperplasia (Figure 3D).

All the statistic analysis showed for c-erbB2 reactivity no significant correlations with age {[$F(2.27)=2.023$, $p=0.152$]}, sex {$\chi^2(2, N=30)=0.260$, $p=0.878$}, smoking status {$\chi^2(2, N=30)=2.477$, $p=0.290$} and FEV1% scores {[$F(2.27)=2.664$, $p=0.088$]}.

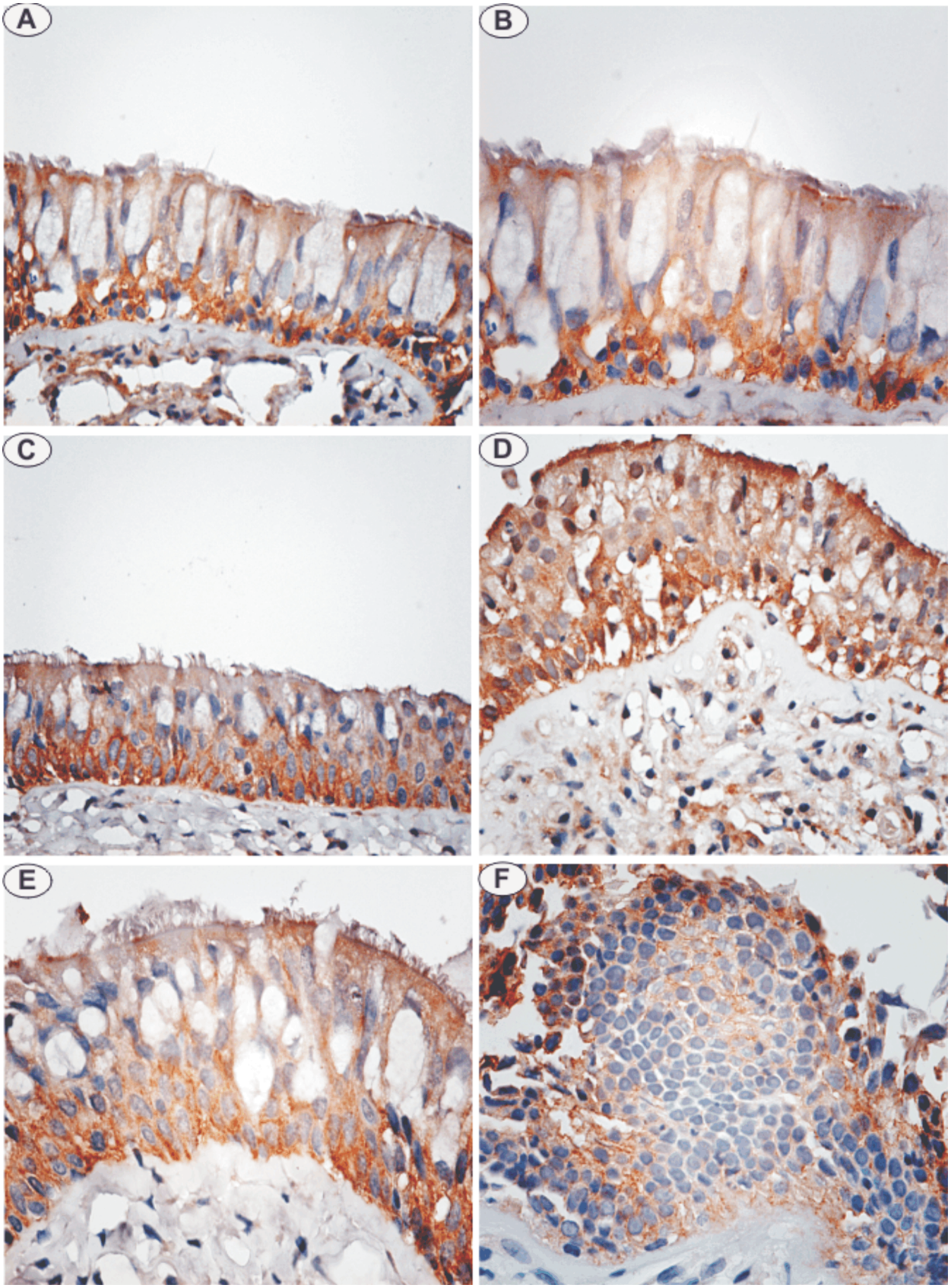

Figure 3 – ***EGFR1 reactivity: (A) Positive reaction in the basal and ciliated cells from the control bronchial epithelium specimens. DAB, ×400; (B) Positive reaction on lower lateral junctions between ciliated cells and their junctions with basal cells, and at the brush border of the bronchial epithelium. DAB, ×600; (C) Positive reaction in the bronchial epithelium of a non-smoker CB specimen. DAB, ×400; (D) Positive reaction in the bronchial epithelium of a smoker CB specimen. DAB, ×400; (E) Positive reaction extending to the apical surface highlighting the brush border in the bronchial epithelium of a smoker CB specimen. DAB, ×600; (F) Positive reaction in the squamous metaplastic area of a smoker CB specimen. DAB, ×400.***

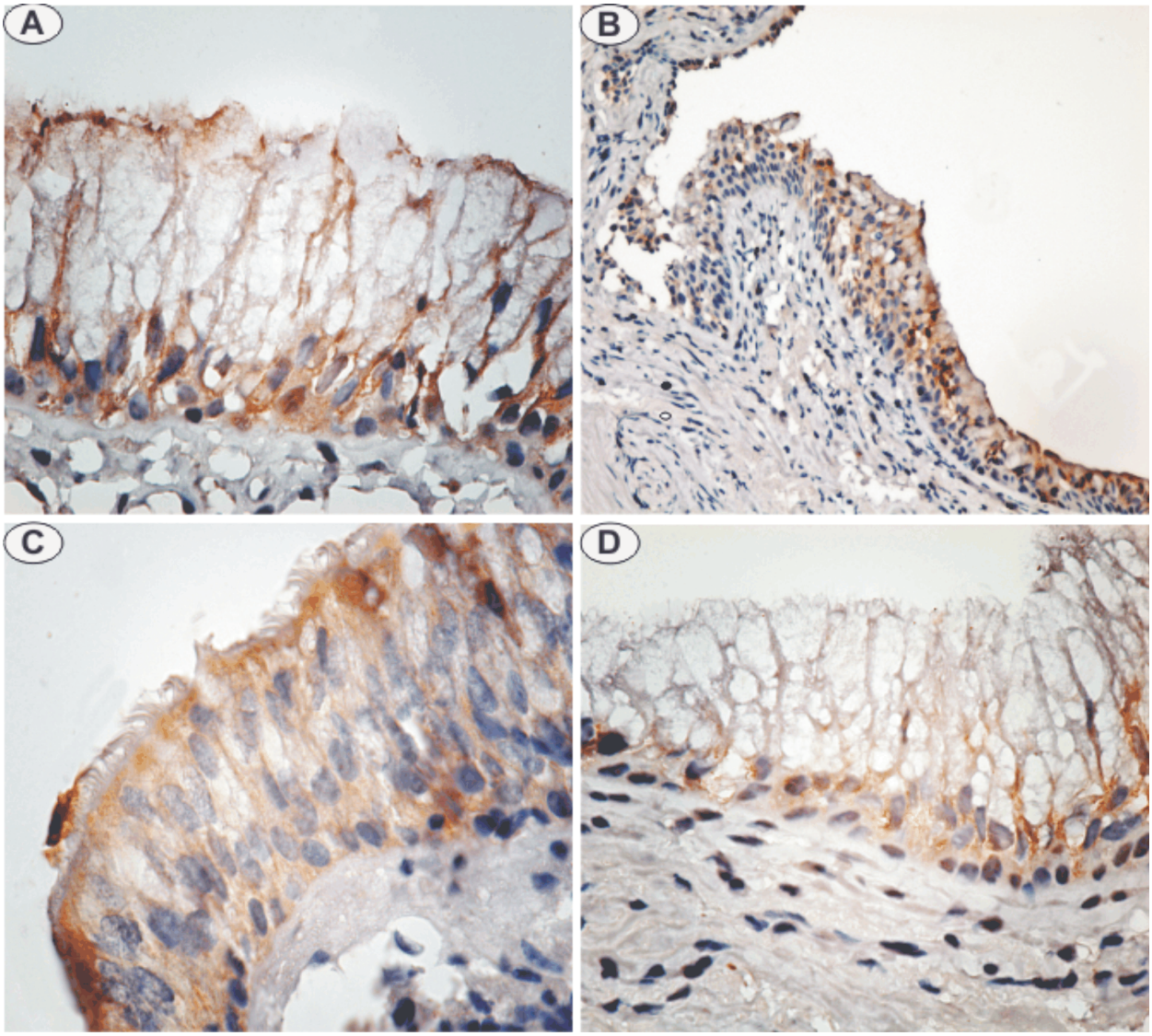

Figure 4 – ***c-erbB2 reactivity: (A) Positive reaction more obvious at the interface of basal cells and ciliated cells from the control bronchial epithelium specimens. DAB, ×400; (B) Positive reaction in the bronchial epithelium of a non-smoker CB specimen. DAB, ×400; (C) Positive reaction extended form the basal layer to the apical surface of a non-smoker CB specimen. DAB, ×600; (D) Weak positive reaction in the bronchial epithelium area with goblet cell hyperplasia of a smoker CB specimen. DAB, ×400.***

Discussion

Chronic obstructive pulmonary disease (COPD) is a slowly progressive and mostly irreversible condition characterized by airflow limitation, mainly as a consequence of an innate and adaptive inflammatory immune response triggered by smoking or other inhalation noxes in predisposed subjects [18, 19]. This abnormal inflammatory response is linked to a tissue-repair and -remodeling process that increases mucus production and causes emphysematous destruction of the gas-exchanging surface of the lung [19]. In chronic bronchitis, one of the COPD subtypes the abnormal inflammatory reactions occurs mainly in epithelium of the central airways and in the mucus-producing glands and it is associated with increased mucus production, reduced mucociliary clearance, and increased permeability of the airspace epithelial barrier [20, 21].

One of the key molecules implicated in the epithelium bronchial repair and -remodeling process is epidermal growth factor receptor (EGFR) and its ligands. The epidermal growth factor receptor (EGFR; ErbB-1; HER1 in humans) is a transmembrane glycoprotein that constitutes one of four members of the erbB family of tyrosine kinase receptors: EGFR (ErbB-1), HER2/c-neu (ErbB-2), Her3 (ErbB-3) and Her4 (ErbB-4) [22]. It can be can be activated by one of several structurally related ligands including epidermal growth factor (EGF) [23], transforming growth factor-α (TGFα) [24], heparin-binding EGF-like growth factor (HB–EGF) [25], amphiregulin (AR) [26], betacellulin (BTC) [27], and epiregulin [28]. Its activation trigger a several signal transduction cascades, principally the MAPK, Akt and JNK pathways, by which are modulated phenotypes such as cell migration, adhesion, differentiation and proliferation [29]. Both the function and expression of EGFs and their receptors have been studied extensively in keratinocytes, proving a direct role for EGF, in cutaneous wound healing [30]. More recently was proved their implication in the epithelium bronchial repair and -remodeling process *via* induction of

epithelial migration, proliferation, differentiation, and extracellular matrix synthesis [7–16].

In our study, the EGF reactivity presented significant differences depending on the smoking status and FEV1% scores. Thus, we showed that smoker CB patients with high FEV1% scores had the highest EGF reactivity, most probable due to the high degree of hyperplasia and metaplastic change in the bronchial epithelium of smoker CB patients. These results are in contradiction with those from the literature, which have revealed that there are no significant differences in the level of EGF expression depending on the smoking status [8, 10, 16]. De Boer WI *et al.* [8] showed that there was no EGF difference in its expression in intact epithelium between subjects with or without COPD irrespective of smoking status. On compared the EGF level expression in damaged epithelium in ex-smokers with COPD with those without COPD they found that the expression was higher in ex-smokers with COPD. The authors suggested that cigarette smoke can have an inhibitory role on the EGF expression in COPD. When the comparison is made with normal subjects, most authors have shown that EGF expression was higher in smokers with chronic bronchitis or mild to moderate COPD than in non-smokers control patients [8, 10, 16].

As regard the cellular localization of EGF expression, most of the other authors showed that in the bronchial epithelium the reactivity was much more obvious at the lower lateral junctions between columnar cells and their junctions with basal cells, both in normal and CB subjects [8, 10, 11, 16]. In particular, we have found an EGF expression on nuclear level, especially in those specimens where the bronchial epithelium was thicker. This aspect was more obvious in the smoker CB patients with hyperplastic mucosa and in squamous metaplastic areas. In the past, nuclear positivity for EGF was documented in normal and physiopathological states [31–33], suggesting that this growth factor when is secreted can be internalized into the cytoplasm and routed to the nucleus, where they exert diverse functions such as regulation of gene transcription. Therefore, EGF can modulate the expression of genes involved in cell cycle progression, its nuclear expression being considered a bad prognostic factor for patients with cancer [32]. The EGF reactivity was also observed at the level of submucosal glands (especially in serous acinar cells), in the blood vessels endothelium, inflammatory cells (macrophages, eosinophils and lymphocytes) and smooth muscle cells.

Similar to the EGF reactivity investigation we proved that for EGFR1 expression the highest scores were assessed in the samples harvested from the smoker CB patients. On contrary the most authors did not found any significant statistical difference of ErbB receptors at bronchial level between CB patients irrespective of smoking status, but these differences were obvious when these CB patients were compared with normal subjects [8–10]. In addition, O'Donnell RA *et al.* showed that within the smoker group there was no significant difference in staining of any of the ErbB receptors between healthy smokers and smokers with COPD [10]. According to the results the authors concluded that in current smoker subjects, smoking is responsible the ErbB receptors enhanced expression, but without detectable relationship with disease severity. Moreover, the same authors observed that the expression of ErbB2 in the airway epithelium was not different from normal in the epithelium of the smoker subjects, but presented a more variability than either EGFR or ErbB3 [10]. In accordance to the strong association of ErbB2 with malignant transformation, it was speculated that higher ErbB2 expression in a subset of smokers might reflect other smoke related processes linked to the development of lung cancer. De Boer WI *et al.* did not observe differences in receptor expression between subjects with or without COPD [8], and for most of these ErbB receptors, the reactivity was higher in damaged epithelium than in intact epithelium, irrespective of the presence of airflow limitation. Therefore, the authors concluded that current smoking and the presence of airflow obstruction independently may increase the expression of growth factors and their receptors, whereas current smoking in the presence of airflow obstruction inhibits the mechanisms leading to increased expression. As an explanation for the last statement, the authors suggested that cigarette smoke inhibit the expression of mediators involved in regulation of expression of EGF ligands and their receptors or by oxidative inactivation of these mediators. Also, Lapperre TS *et al.* noticed that were no difference in EGFR expression between ex- and current smokers with COPD suggesting that in ex-smokers subjects the EGFR immunophenotype may be related to the persistence of bronchial inflammation [9]. Moreover, the same authors concluded that smoking cessation decreased epithelial mucin store, proliferation, and squamous cell metaplasia in large airways of patient with COPD, but that it does not affect EGFR expression.

Another qualitative assessment of our study was the observation that while EGF expression was restricted to basal and ciliated cells both in normal and pathological bronchial specimens the EGFR reactivity was also present in mucous cells. Moreover, Takeyama K *et al.* claimed that EGFR expression and activation causes goblet-cell metaplasia without changing the total number of epithelial cells, by mucous differentiation of Clara cells [13]. A little later, the same authors identified cigarette smoke as cause for the both mucus hyper-secretion and increased number of goblet cells through activation of the EGFR system [34].

As other authors have found our study has revealed the approximately same cellular location of EGF and its receptors suggesting that it may represent ligand bound to these receptors. The signification of this immuno-phenotype could by that autocrine as well as juxtacrine stimulatory mechanism *via* EGF receptors can be involved in tissue repair after injury at the level of bronchial epithelium [11, 35]. By immunoelectron microscopy, Aida S *et al.* were able to localize more precise the EGFR in the adult human lung, revealing that was it was restricted to basal cells of the bronchial epithelium at the intercellular lateral cell membrane position [36].

Taking into account all we discussed it is clear that

blockade of the EGFR pathway *in vivo* in patients with COPD can be an alternative successful therapy by reducing airway obstruction through limiting mucous production. Such therapies for COPD patients have been taken into account [37–39], and even more a clinical study has been started using a specific inhibitor of EGFR tyrosine kinase autophosphorylation [40]. In this clinical trial, the authors found that the doses at which therapeutic effects on mucin stores in patients with COPD may occur overlap with the doses at which reversible adverse effects occur.

Conclusions

Investigating the EGF and its receptors (EGFR1 and c-erB2) in normal and in smoker or non-smoker CB patients, we found that the reactivity was higher in pathological condition, and there was significant differences regarding smoking status and FEV1% scores, the highest level of EGF and EGFR1 expression being associated with highest FEV1% score in smoker CB patients. The cellular distribution and staining pattern of these markers are suggestive for the involvement of some autocrine and juxtacrine mechanisms *via* to these receptors that might play key roles in chronic tissue remodeling in CB patients.

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