Original article
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Changes in the bronchial epithelium between the second and the fifth day after the previous biopsy

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BACKGROUND: *Regenerative changes may be found in bronchia, in the area of previous biopsy. Superficial epithelium in the injured area proliferates, migrates and differentiates, primarily towards the squamous cells, and then towards cup-shaped, i.e. column cells with cilia. Regenerative changes can be misinterpreted for epidermoid carcinoma. The highest level of similarity of regenerative changes and epidermoid carcinoma is between the second and the fifth day after the previous biopsy.*

MATERIALS AND METHODS: *In this research, we used 66 biopsy samples. They were classified into two groups (33 re-biopsied between the second and the fifth day and 33 with diagnostically confirmed epidermoid carcinoma). In both groups, the same morphological parameters were monitored and then tested by means of z test with 5% tolerance (α=0.05).*

RESULTS: *Morphological changes that are typical for epithelium in regeneration, are the following: fibrin (z=4.41; P=0.000), granulocytes (z=4.79; P=0.000), granulocytes in epithelium (z=6.92; P=0.000), infiltration into granulation tissue. Tumor changes are the following: presence of mononuclear cells (z=-3.63; P=0.0003), diskeratosis (z=-4.29; P=0.000), nuclear polymorphism (z=-4.22; P=0.000), hyperchromatism (z=-3.83; P=0.000), and infiltration into connective tissue (z=-5.76; P=0.000). Changes, which are useless for differentiation of regeneration and carcinoma, are the following: nucleoli (z=-1.77; P=0.0763), multinuclear cells (z=0.25; P=0.8041), mitoses (z=-1.44; P=0.151), interruption of the basal membrane (z=1.07; P=0.2866).*

CONCLUSION: *Presence of squamous epithelium located in fibrin and/or granulation tissue saturated with granulocytes is a morphological characteristic of regenerative epithelium. Presence of squamous epithelium, with symptoms of severe polymorphism of nuclei, with hyperchromatism, with monocellular diskeratosis, located in cellular tissue saturated with mononuclear cells , is a characteristic of malignant epithelium.*

KEY WORDS: *Bronchi; Epithelium; Regeneration; Biopsy; Carcinoma, Squamos Cell*

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INTRODUCTION

Regenerative changes in bronchial epithelium can be found in patients treated with biopsy, patients suffering from primary or secondary pulmonary diseases, tuberculosis, sarcoidosis, mycotic infections, pneumoconiosis or other granulomatoses, primary or secondary pulmonary tumors, injuries caused by caustic materials or gases, in intubated patients, patients treated with laser for therapeutic or diagnostic purposes, lung grafts, at the site of resection of bronchi - in cases of segmentoctomia or lobectomia, after pulmonary infarctions (1-4).

Basically, regeneration of any superficial epithelium develops in the same way (5). Regeneration of bronchial epithelium is conducted from basal undifferentiated cells.

Macroscopic appearance depends on the size of a defect in mucous membrane, its depth, etiologic element that caused the defect, as well as on the development stage of the regenerative process. In re-bronchoscoped patients, the appearance of regenerative changes depends on the pervious bronchoscopy, as well as on the type of bronchoscopic material taken at that time, or the instrument used and number of days passed since the previous biopsy. Usually, the location of the preceding biopsy is in the area

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of bifurcation of bronchi, it appears changed in the form of endobronchial excrescence, reddish in color, and uneven surface. In some cases, the defect on mucous membrane - light red in color, of uneven granular surface in the central area, with the pearl-white border - is registered. The described mutations are extremely vulnerable and they bleed excessively, even when only touched slightly with the bronchoscope (4,6,7,8). All aforementioned leads to a conclusion that it is the case of primary lesion, therefore bronchologists decide for performing biopsy (Figure 1).

Figure 1. Macroscopic appearance of the site of the previous biopsy

Histological examination of such material registers regenerative changes. The regenerative process within epithelium can be divided into four stages:
- Coagulation and inflammation,
- Regeneration of epithelia of damaged surface,
- Multiplication of new cells,
- Differentiation of the new epithelium (7,9-11).

Regeneration in bronchial epithelium is made with squamous epithelium. Epithelium shows signs of cell and nuclei polymorphism, hyperchromatism of nuclei, and often, multinuclear cells. Nucleoli are registered, too. Individual cells are diskeratotic.

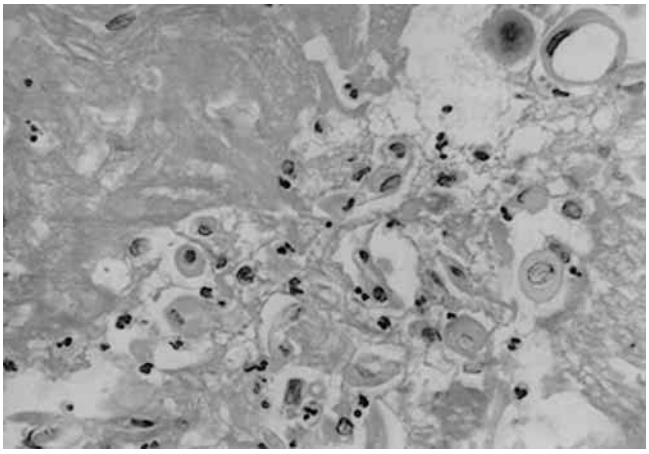

Figure 2. Fibrin with scarce neutrophil granulocytes and individual squamous cells, HE X 400

Mitotic activity of squamous cells is high. Theses morphologic characteristics of regenerating epithelium (regenerative atypia) can be misinterpreted as for epidermoid carcinoma (10-13) (Figures 2, 3).

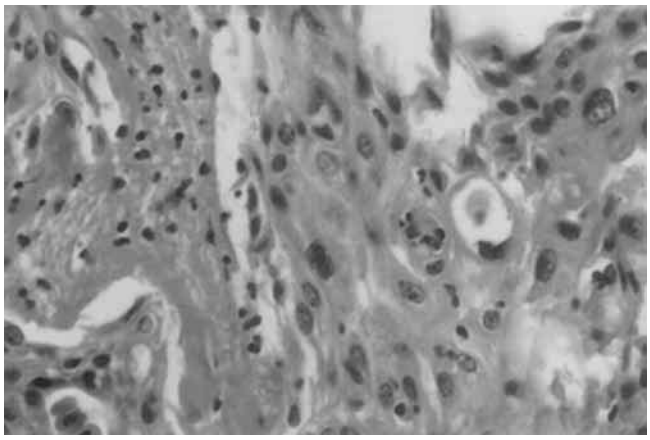

Figure 3. Squamous cells with fibrin, showing slight level of nuclei polymorphism, nucleoli, eosinophil cytoplasm; intercellular bridges can be seen; individual cells are diskeratotic, HE X 400

Aforementioned characteristics of regenerative epithelium are more emphasized in the days just after the damage (after the previous biopsy), and the chance for misinterpretation is the highest in the same period (10,11).

Epidermoid (planocellular) carcinoma is a malignant epithelial tumor with symptoms of keratinization and/or intercellular bridges (14). It is most frequently located in the area of main bronchi or bronchial branches. In early stages, it occurs as rough whitish or red swelling of bronchial mucous membrane. It very often grows endophytically in the bronchial walls as well as in surrounding pulmonary parenchyma, but most often, the growth is exophytic with cauliflower-like tumor formations that very often close the lumen of bronchi. Tumor consists of solid aggregates of polygonal cells, epithelial pearls, individual keratinizated oval cells and intercellular bridges. Presence of stated morphological characteristics is rather variable and it is connected to the level of differentiation. Tumor cells are atypical, with large nuclei, irregular nuclear membrane, rarely multinuclear, mitotically highly active, with atypical forms of mitosis present, as well. Surrounding bronchial epithelium contains very often basal hyperplasia flat metaplasia, dysplasia, or in situ carcinoma. Tumor stroma is fibrotic and contains varying quantity of lymphocytes, plasma cells and macrophages (14-17) (Figure 4).

The objective was to define morphological criteria for differentiation of regenerative changes in bronchial epithelium of epidermoid carcinoma.

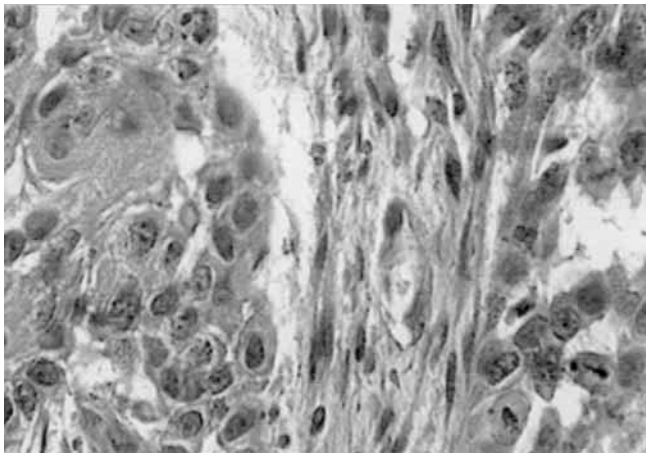

Figure 4. Well-differentiated epidermoid carcinoma, HE X 350

MATERIALS AND METHODS

The research included bioptic materials taken from 66 patients in the Institute for Pulmonary Diseases in Sremska Kamenica (Serbia) during a 4-year period. The examined material was divided into two groups. One group consisted of 33 samples of re-biopsied patients taken 2 to 5 days after the previous biopsy, with morphologic changes indicating the location of the previous biopsy. The other group included 33 samples of patients with diagnostically confirmed epidermoid carcinoma of bronchi.

The material obtained by means of biopsy was fixated in 10% formalin. After a routine preparation, paraffin blocks of tissue were made and then cut by microtome with 10-30 semi-serial sections and dyed with routine hematoxillin-eozin (HE) dying method. When necessary, additional dying methods were conducted: Mallory, Gˆmori reticule, alcian blue, periodic acid-Schiff (PAS). The materials were processed "blind", without any general information, or information regarding the date of previous biopsy or basic disease. During analysis, a binocular microscope "Olympus" was used (lens 10x, 25x, 35x, 40x; eyepiece 16x) with 1.4 mm field of vision.

With each sample, the following morphologic changes were semi-quantitatively assessed:

1. Interruption of basal membrane (0 - absent; 1 - present);
2. Fibrin (0 - absent; 1 - in small quantity; 2 - in medium quantity; 3 - abundant);
3. Granulocytes in fibrin (0 - absent; 1 - scarce; 2 - focally thick; 3 - diffusely thick; 4 -micro-abscess formed),
4. Granulocytes in squamous-stratified epithelium (0 - absent; 1 - scarce; 2 - focally thick; 3 - diffusely thick; 4 - intraepithelial microabscesses)
5. Mononuclear cells/lymphocytes/cell plasma/macrophages in fibrin (0 - absent; 1 - scarce; 2 - focally thick; 3 - diffusely thick),
6. Diskeratosis in squamous cells (0 - absent; 1 - present, 1-10 cells in one field of vision with large magnification - high-power fields (HPF); 2 - more than 10 cells in 1 HPF; 3 - keratin bulbs),
7. Nuclear polymorphism of squamous cells (0 - absent; 1 - low; 2 - medium; 3 - high; 4 - bizarre multinuclear cells),
8. Nuclear hyperchromatism of squamous cells (0 - absent; 1 - present),
9. Multinuclear cells (0 - absent; 1 - present),
10. Nucleoli in squamous cells nuclei (0 - absent; 1 - present),
11. Mitotic activity of squamous cells (0 - absent; 1 - 1 mitosis/1HPF; 2 - 2 to 5 mitoses/1HPF; 3 - more than 5 mitoses/1HPF; 4 - pathological mitoses),
12. Infiltration of squamous cells into connective tissue (x - connective tissue absent; 0 - absent; 1 - present in granulation tissue; 2 - present in cellular tissue).

Descriptive statistic methods were used for results processing , which were then presented in tables and graphs. The groups were compared with individual morphological parameters and tested by means of z test on equality of proportions of two groups for large samples ($n>30$), with 5% error risk ($\alpha=0.05$).

RESULTS

The first group consisted of 33 samples obtained by re-biopsy, taken 2 to 5 days after the previous biopsy, with morphologic changes indicating the location of the previous biopsy. (Group I). The other group included 33 samples of patients with diagnostically confirmed epidermoid carcinoma (Group II).

Fibrin was found in every sample re-biopted between day 2 and day 5 after the previous biopsy, and in 54.54% for Group II (figure 5). Abundant quantities of fibrin were registered in 87.87% for Group I, and in 5.55% only for Group II.

Statistically, presence of fibrin is extremely different comparing Group I and Group II, $z=4.41$; $P<0.000$; it was more often found in Group I.

In Group I granulocytes were present in every sample and in Group II in 48.48% of samples. Diffuse, thick infiltrates of granulocytes were found in 69.69% of re-biopsied patients, and 18.75% in patients with epidermoid carcinoma

Statistically, presence of granulocytes in infiltrate is extremely different between the mentioned groups; it was more often found in materials of Group I, $z=4.79$; $P<0.000$. Presence of granulocytes in the two groups is presented in Figure 5. Besides granulocytes being more often found in the group of the re-biopsied patients, the infiltrate was usually diffusely thick in that group when compared to the group with epidermoid carcinoma where the granulocytes were scarce.

Intra-epithelial granulocytes were present in 96.96% of cases in Group I, and in 12.12% of Group II cases (Figure 5). Granulocytes within epithelium are much more often found in Group I, $z=6.92$; $P<0.000$. Granulocytes within epithelium of

patients with epidermoid carcinoma were rarely registered, 4 cases only.

Presence of aggregates of squamous cells in connective tissue of the re-biopsied patients group was confirmed in 24.24% cases, and in all cases they were located in the granulation tissue. In Group II, they were present in 93.93% cases, and in all cases they were located in not in granular but in cellular tissue (Figures 5,6, Table 1).

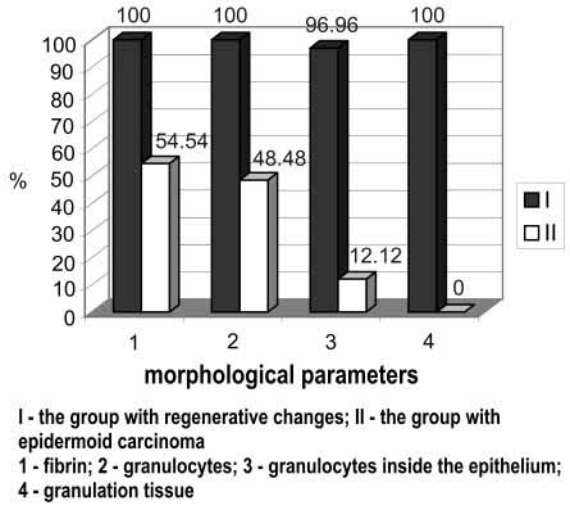

Figure 5. Presence of granulocytes and fibrin

Mononuclear cells in infiltrate are found in 36.36% of cases in Group I, and, in every single case, were present in Group II. A rare infiltrate of mononuclear cells is found in both groups, and focally and diffusely thick one is more often found in the group with epidermoid carcinoma (Figure 6).

When compared, infiltration with mononuclear cells is more often found in the group with epidermoid carcinoma, z=-3.63, P=0.0003.

Diskeratotic cells were present in 93.93% patients of Group II, and in 45.45% of Group I. Monocellular diskeratosis in the group of re-biopsied patients amounted from 1 to 10 cells in one field of vision HPF, while in the group with epidermoid carcinoma more than 10 cells per one field of vision HPF were found. Keratin bulbs could have also been found (Figure 6). Monocellular diskeratosis is, from point of statistical significance, more often found in the group with epidermoid carcinoma, z=-4.29; P<0.000.

Nuclear polymorphism in Group II was present in every single biopsy, and in Group I only in 63.63% (Figure 6). In the group of the re-biopsied patients, slight polymorphism is found, and in the group with epidermoid carcinoma, polymorphism of medium or heavy level is found, as well as bizarre multinuclear cells.

Also, nuclear polymorphism was more often found in Group III, z =-4.22; P<0.000, and the level of polymorphism was also more expressed in Group III. Nuclei were hyperchromatic in every single patient of Group II, and in 63.63% of Group I (Figure 6, Table 1). Hyperchromatism was of similar relation as nuclear polymorphism, i.e. the difference was significant, and it was more often in Group II, z=-3.83; P<0.000.

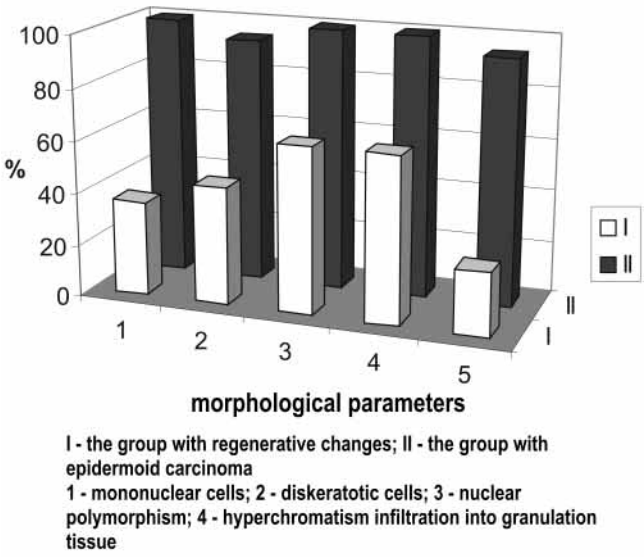

Figure 6. Other morphological parameters

Nucleoli were present in 90.9% of Group I patients, and in every single case of Group II (Figure 7). There is no statistically significant difference in presence of nucleoli in the observed groups, z=-1.77, P=0.0763.

Multinuclear cells were present in 45.45% in Group I, and in 42.42% in Group II patients (Figure 7). There is no statistically significant difference in presence of multinuclear cells in the observed groups, z=0.25, P=0.8041.

Tumor cells were mitotically active in every single case, and regenerative in 93.93% (Figure 7). There is no statistically significant difference in mitotic activity of the observed groups, z=-1.44, P=0.151.

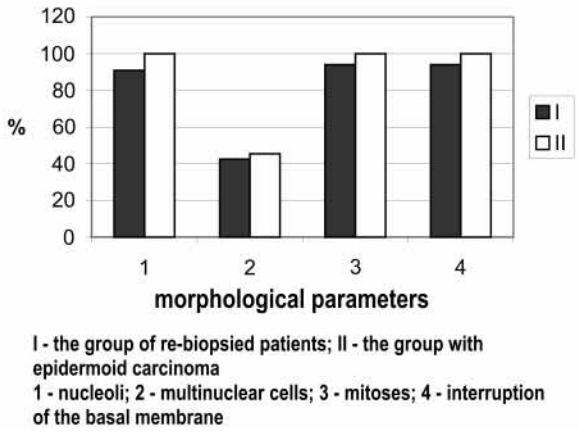

Figure 7. Interruption of the basal membrane and nuclear alteration

Interruption of basal membrane (BM) in Group I patients was found in every single case where assessment was possible.

Assessment was not possible in 45.45% of cases, due to presence of regenerative epithelium only, without structural BM elements . In Group II, interruption was present in 93.93% (Figure 7, Table 1.).

Table 1. Morphological parameters that are important for differentiation of regeneration from neoplastic changes in bronchial epithelium

Regeneration	Epidermoid carcinoma	Regeneration/epidermoid carcinoma
Fibrin	Mononuclears	Nucleoli
Granulocytes in fibrin	Diskeratosis	Multinuclear cells
Granulocytes in squamous-stratified epithelium	Nuclear polymorphism	Mitotic activity
Squamous cells in granulation tissue	Nuclear hyperchromatism	Interruption of basal membrane
	Squamous cells in connective tissue	

Statistically, difference in interruption of basal membrane is not significant between the Group I and Group II ($z=1.07$; $P=0.2866$).

DISCUSSION

Morphologic differentiation between regenerative changes in bronchial epithelium and epidermoid carcinoma is a hard task. The hardest problems in differentiation are in the early days after damage, when the level of similarity is at its highest point. Regenerating cells are polymorphic, with polymorphic nuclei, prominent nucleoli, often mitotically active. Individual cells with hyperchromatic nuclei and multinuclear forms of cells, some of which diskeratotically changed, can be found, too. Cells of the above-described characteristics can be located in granulation tissue. Should only above stated morphological parameters be observed, in every case, a conclusion would be that these are tumor cells originating from epidermoid carcinoma (2, 10, 11). However, morphological changes present besides the above described cells should not be neglected either: the defect in pseudo-stratified epithelia, interruption of basal membrane, exudation of fibrin and granulocytes, formation of basal layer of cells and basal membrane, presence of goblet and column cells on flat epithelia, presence of granulation tissue; they all indicate regeneration in bronchial epithelium. If all morphological parameters are precisely analyzed, not separating them individually, changes in epithelium are marked as being atypical, of regenerative type (2,4,7,10,11).

Regenerative cells desquamate and can be found in sputum or broncholavate. Cell depends on time passed since the previous bronchial brushing or biopsy. Saito et al. claim that most of cell aggregates make rather atypical cells with large nuclei and prominent nucleoli of emphasized mitotic activity, if observed within first two or three days after bronchial brushing (18). Regenerative polymorphism is particularly expressed during the first days just after the biopsy, and it reflects from the day 11 after the previous biopsy. It is suggested to avoid taking samples for cytological analysis within above stated period after the previous biopsy, and should it be analyzed anyway, it is necessary for cytological and histopathologic findings to be correlated due to diminishing chances for misinterpretation of regenerative atypia (18,19).

CONCLUSION

- Presence of squamous epithelium located in fibrin and/or granulation tissue, interspersed with granulocites, is a morphological characteristic of regenerating epithelium.
- Presence of squamous epithelium with signs of outstanding polymorphism of nuclei, with hyperchromatism and with monocellular diskeratosis, located in cellular tissue interspersed with mononuclear cells is a characteristic of malignant epithelium.
- Multinuclear, mitotically active squamous cells with emphasized nucleoli, and interruption of continuity of basal membrane cannot be used as criteria for differentiation of regenerating from tumor changes.

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CORRIGENDUM

The author wishes to correct the authorship of the paper titled The role of rational immunotherapy for renal cell carcinoma (2003;11:17-9.) as follows:
Svetlana Salma, Borislava L. Nikolin, Jasna Trifunović.

In paper titled Effect of intratumoral application of methotrexate in vivo on frequency of micronuclei in peripheral blood lymphocytes (2003;11:1-4.) the authors Novaković T et al. would like to add the following Acknowledgement:
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