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CORRELATION BETWEEN THE STAGE OF CYSTIC FIBROSIS AND THE LEVEL OF MORPHOLOGICAL CHANGES IN ADULT PATIENTS

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The aim of this study was to evaluate the correlation between the stage of cystic fibrosis and the intensity of accompanying morphological changes – including transmission electron microscopy – within bronchial mucosa. The stage of the disease was assessed on the basis of clinical status and radiological and endoscopic examination. We focused on morphological changes in epithelial cells, the presence of metaplasia and/or dysplasia, the type of inflammatory infiltrate, and the presence of epithelial ulcerations, thickening of epithelial basement membrane and collagenization of lamina propria. We found two clinically different patients groups. The first one was in a poor clinical condition, advanced inflammatory fiberoptic bronchoscopy and radiological changes, multiple exacerbations, and with chronic inflammation and only focal appearance of ciliated epithelium. Moreover, squamous cell metaplasia and dysplasia was diagnosed in 3 and 4 cases, respectively. The other group, in a better clinical condition, had normal BMI and small changes on chest X-ray. In this group the diagnosis of cystic fibrosis was made at later age. Two patients from this group displayed features of acute phase; ciliated epithelium was covering the whole sample. After statistical analysis, we found a correlation between the clinical course and the morphological changes in bronchial mucosa. Bronchial ulcerations, squamous cell metaplasia and dysplasia were found in the group with the more severe clinical course.

Key words: cystic fibrosis, morphological changes, stage of disease

INTRODUCTION

Cystic fibrosis (CF) is the most common single-gene related disease in Caucasians with autosomal recessive trait of inheritance. The carrier status was estimated as 1:25 in general population, while the incidence of the disease as 1 per 2500 born alive (1). The basic abnormality was established as a mutation of the gene encoding cystic fibrosis transmembrane conductance regulator (CFTR) protein located on the long arm of chromosome 7. CFTR protein serves also as cAMP-dependent chloride channel which is found on the apical surface of cells in exocrine glands. The ducts of exocrine glands are plugged by accumulation of viscous and dehydrated mucus. Bacterial colonization due to chronic inflammation leads to progressive respiratory dysfunction. Although CF is a multisystem disease depending on several factors, the main clinical symptoms are related to abnormalities in the respiratory and gastrointestinal systems. The length of life and its quality depend on anatomical changes and functional changes in the respiratory system. CF-related chronic infections and prolonged inflammation lead to functional changes in the respiratory system which are related to several morphological changes (2). There have already been described the possible stages of changes in bronchial epithelium. They might include the sequence of chronic mucosal inflammation, followed by squamous cell metaplasia and dysplasia; the latter treated as a possible risk factor for carcinoma (3).

In the present study we attempted to evaluate the association between CF progression and its stage with morphological changes in bronchial epithelial cells in adult patients.

MATERIAL AND METHODS

The study was approved by an institutional Ethics Committee and was performed in accordance with guidelines of the Declaration of Helsinki of 1975 for Human Research.

We investigated 14 (8 female, and 6 male) adult patients with CF, aged 18-38 years (mean 23.6 yr) in good and in poor clinical conditions, who were selected from 26 CF cases being supervised and treated at the Department of Pulmonary Diseases during the last two years. CF diagnosis was based on the diagnostic and treatment criteria established by the Polish Working Group on Mucoviscidosis in the year 2002.

Stages of the disease were assessed on the basis of clinical status and radiological and endoscopic examination. The degree of clinical advancement was assessed by applying grade scale based on clinical criteria published by Shwachman and Kulczycki (4). Changes in the chest cavity, evaluated radiologically, were assessed by a grading scale, according to Chrispin and Norman (5) in later modifications by Brasfield *et al.* (6, 7). Spirometric analysis with a Flowscreen (Jaeger, Germany) was also done for a better accuracy of estimation of disease stage. We assessed forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC) and FEV1/FVC. The criteria for spirometric abnormalities were consistent with the ERS/ATS guidelines of 2005. Patients' nourishment was estimated by body mass index (BMI).

Bronchoscopy with bronchial mucosa biopsies, for histopathological examinations, and bronchoalveolar lavage (BAL) were performed using a Pentax fiberscope. All fiberoptic procedures

were done after previous intravenous premedication. During the procedure we focused on anatomical abnormalities and the intensity of inflammatory changes in bronchial mucosa. From the areas of most intensive changes, BAL was obtained. Samples were sent for bacteriologic, mycological, and cytological diagnostic examinations. Finally, light and transmission electron microscopic examinations were made after routine specimen preparations (EM 900, Zeiss, Germany). We focused on morphological changes in epithelial cells, the presence of metaplasia and dysplasia, the type of inflammatory infiltrate, and the presence of epithelial ulcerations, thickening of epithelial basement membrane, and collagenization of the lamina propria.

An analysis of similarities according to selected clinical and morphological data was performed. As a discriminating factor we used morphological changes in the bronchial mucosa and submucosa. We used the Ward agglomeration method (joining of objects). Statistical analyses were done using Statistica 6.0.

RESULTS

In present study we distinguished two clinically and histopathologically different groups of patients (*Table 1*). The first one consisted of 8 patients with poor clinical condition, less than 40 points on the Shwachman-Kulczycki scale, and with advanced inflammatory fiberoptic bronchoscopy changes along the whole bronchial tree. There was mucosal edema, with diminished picture of cartilages and with concentric narrowing of bronchi. Multiple petechiae and contact hemorrhages were observed. Bronchi were plugged by purulent content. The advanced changes in CF patients were accompanied by more diffuse and more advanced changes on the chest X-ray studies. These abnormalities were more commonly observed in the lower parts of lungs. They included nodular, cystic, and macular and annular changes, which suggested the presence of

Table 1. Essential description of the cystic fibrosis patients.

Severe disease patients							
Gender	Shwachman-Kulczycki score	Brasfield score	Exacerbations	BMI	FEV1%	Metaplasia/Dysplasia	<i>Pseudomonas aeruginosa</i>
F	25	15	> 40	16.4	49.8	metaplasia	+
M	38	9	> 40	17.8	38.1	dysplasia	+
F	18	10	10	21.4	29.3	metaplasia	+
M	30	9	13	20.3	36.2	metaplasia	+
F	29	13	23	15.0	21.5	dysplasia	+
F	30	13	>30	24.1	27.3	-	+
M	33	10	19	16.8	34.8	dysplasia	+
F	40	12	15	21.2	43.4	dysplasia	+
F	100	4	3	21.8	94.6	-	-
F	92	6	10	21.3	113.0	-	-
M	100	4	4	24.2	93.5	-	-
F	90	6	3	22.8	80.4	-	-
M	96	3	1	23.2	84.2	-	-
M	89	4	3	21.6	81.6	-	-

bronchiectases (Fig. 1). All these patients had several or multiple clinical exacerbations, calculated as the number of hospitalizations. In BAL samples, *Pseudomonas aeruginosa* was found in all studied patients. Light and transmission electron microscopic studies revealed chronic inflammation of bronchial mucosa and only a focal presence of ciliated epithelium (Fig. 2). The basement membrane was thickened and the lamina propria was collagenized. In 2 patients, lack of inner dynein arms was found. We also found cilia with abnormal number of microtubular doublets and with displacement of peripheral microtubules toward the central microtubular pair (Fig. 3). Additionally, there were numerous cilia (from 2 to 20) covered by a common cell membrane. There were also single microtubules replacing normal doublets. In a few cases, we observed three single cilia occupying the central area. In several cases, the axonemal membrane was detached from the groups of cytoplasmic filaments. The rete rigis was interrupted and irregularly deeply situated in the cytoplasm, sometimes even encircling the nuclear area. In another few cases, there were squamous-like cells in the bronchial mucosa areas deprived of ciliated cells. Desmosome-like or zonula adherens connections were seen between cells. Moreover, squamous cell metaplasia and dysplasia was diagnosed in 3 and 4 cases, respectively (Fig. 4). The spirometric examination revealed severe obstruction. We found body weight deficiency in half of the patients. Two patients died during the follow-up period.

In the second group consisting of 6 patients, who were in a better clinical condition, had normal BMI and small changes on chest X-ray, cystic fibrosis was diagnosed at a later age. Fiberoptic bronchoscopy revealed in these patients rounded

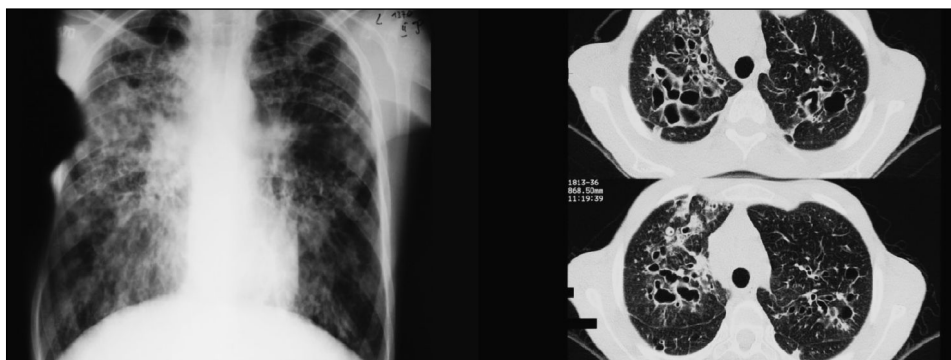


Fig. 1. A picture of advanced pathological lung changes. In both images, there are increased bronchial tree shadows visible. There are numerous annular shadows and single macular lesions of variable size. These changes are more prominent in the middle portion of the right lung. The annular shadows correspond to bronchiectases and emphysematous lesions. Widened lung hili, especially the right one, are clearly present. There also is a wide and non-sharply demarcated right peritracheal region. One can see there increased lung aeration with a large sagittal chest diameter and low diaphragm.

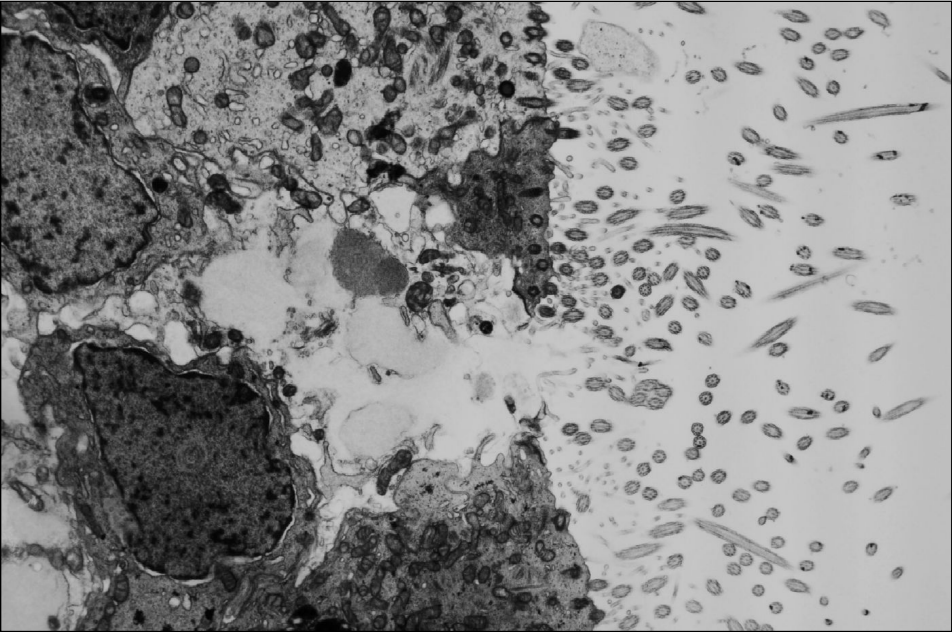


Fig. 2. The free surface of ciliated epithelium. The cilia are scanty and there are microvilli present between them. Several microtubular complexes are visible under a common cell membrane. There is a mucous-secreting goblet cell.

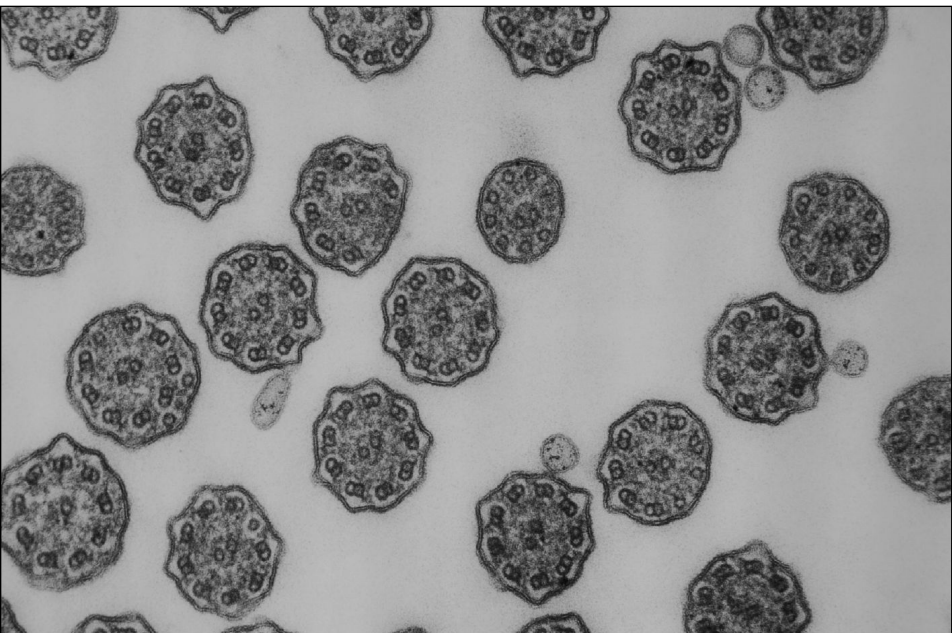


Fig. 3. Transverse sections of cilia. Lack of dynein inner arms is visible. There are single peripheral microtubules and additional microtubules under a common cell membrane.

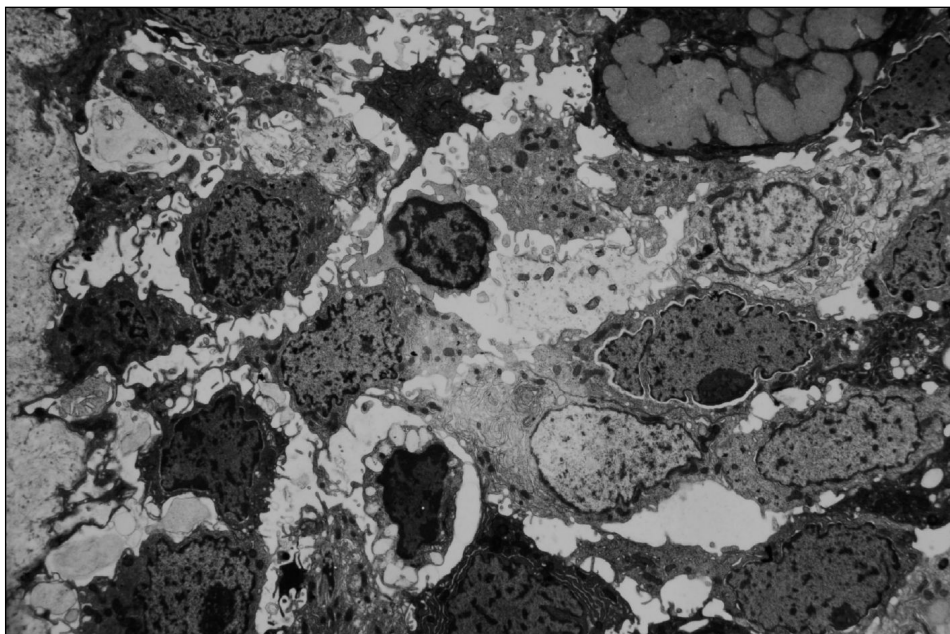


Fig. 4. Electronogram showing squamous cells on the surface of bronchial epithelium.

carinas and the development of small petechiae after contact with the instrument. The bronchial lumen was free from mucous-purulent exudates or contained small amounts of it. In the BAL samples we found the predominance of macrophages. Moreover, there was *Staphylococcus aureus* present in the specimens, which was *MRSA* in one case, and *MSSA* in all other cases. The presence of *Pseudomonas aeruginosa* was not supported. Spirometric results were within normal range. In bronchial biopsies ciliated epithelium was covering the whole specimen. Histologically, there were features of chronic inflammation, which in 2 cases appeared in acute phase. In a single case the inner dynein arms were absent.

DISCUSSION

The multisystem abnormalities observed in patients with CF and progressive anatomical and functional changes in the respiratory system lead to their death. Progression of broncho-pulmonary lesions related to inflammatory changes is observed in pulmonary structures. It should be considered that such changes might include the transformation of inflammatory changes into squamous metaplasia and even dysplasia, which may be a presage of cancer (8).

In the present study, we found that more advanced macroscopic changes in bronchial mucosa, progress of radiological changes, and more common clinical

exacerbations were correlated with the poorer patients' status. Such patients also had obstructive functional pattern, and half of them had body mass deficiency. The correlation between radiological and functional abnormalities as well as the stage of disease advancement, as assessed by the Shwachman and Kulczycki score (4), have also been described by Helbich *et al.* (9) and Asie *et al.* (10). On the other hand, Nir *et al.* (11) have described lower BMI in 90% of adult male and 83% female CF patients, which also correlated with lower functional lung parameters.

Polosukhin (3) has proved that chronic inflammation in the respiratory system is a cause of rebuilding of bronchial wall and of injury to the alveolar epithelium. Inflammation is in effect responsible for destruction of epithelial cells or abnormalities in their structure with hyperplasia of goblet cells and their maldevelopment, or even degeneration. Hyperplasia of basal cells has also been observed. Such changes are related to abnormal cell differentiation and proliferation of epithelial cells. In the present study, the presence of squamous metaplasia was observed with disappearance of ciliated epithelium, likely caused by inappropriate regeneration. Same authors have found that squamous metaplasia is correlated to more advanced chronic inflammatory changes and increased fibrosis in the submucosa (3). Kim (12) has revealed that squamous metaplasia and bronchial epithelium atrophy are the final stage in transition from inflammatory changes into fibrosis. On the other hand, Breuer *et al.* (13) have described that severe dysplasia found in bronchial mucosa samples could pass into carcinoma in situ or even invasive carcinoma. In the present study, we found lesions similar to those described previously by Polosukhin (3) and Kim (12). These lesions were typical for patients in a poor clinical condition, characterized by rebuilding of bronchial epithelium, structural changes in submucosa, and the presence of squamous metaplasia/dysplasia, all of which was accompanied by advanced inflammatory changes.

We conclude that there is a correlation between the clinical course and morphological changes in bronchial mucosa in adult patients suffering from cystic fibrosis. Bronchial ulcerations, squamous cell metaplasia and dysplasia are found in patients with more severe clinical course.

Conflicts of interest: No conflicts of interest were declared in relation to this article.

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