HISTOLOGIC AND IMMUNOHYSTOCHEMICAL STUDY OF THE VASCULAR AND PERIVASCULAR STRUCTURES IN PATIENTS WITH CHRONIC OBSTRUCTIVE BRONCHOPNEUMOPATHY (COBP)

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Summary. Histologic and immunohistochemical changes of the lung vascular and perivascular structures in 37 clinical and paraclinical COBP diagnosed were studied. We noted the deterioration of an important part of the pulmonary capillary network because of the destruction of interalveolar septae, with an intense vascular congestion within restant capillaries. The venules and arterioles have had a fibrosis wall by the replacing of the elastic and muscular fibers with inextensible fibers of collagen. The perivascular stroma was infiltrated by fibroblasts, macrophages, lymphocytes and plasma cells.

Key words: chronic obstructive bronchopneumopathy, immunohistochemistry vascular lesions.

INTRODUCTION

COBP is an irreversible, progressive disease characterized by the limiting of the air flux through the respiratory pathways as a consequence of an abnormal inflammatory response of the lung to the foreign particles, antigens and gases circulating through the inspired air.

The disease appears after the age of 40 and it is characterized by gradual dyspneea determined by an obstructive syndrome of the intrapulmonary air pathways associated to chronic bronchitis lesions and centrolobular emphysema.

COBP incidence was 60% increased during the last 20 years and 23% mortality the last 10 years (Matei, 1995). Nowadays, COBP is the fourth cause of morbidity and mortality all over the world including USA where about 14 millions people suffer with COBP (12.5 millions suffer with chronic bronchitis and 1.5 millions with emphysema); these figures are 40% higher than in 1982 (Bethesda, 1998).

In Romania, the studies performed in the last decade revealed an incidence of 4.6% COBP in males and 2.3% in females aged over 40 years old. Most of the patients in Romania were diagnosed with COBP after they have reached the

chronic hypoxia and chronic pulmonary cord stages (Stoicescu *et al.*, 1999). Pathologic lesions were not limited only to the respiratory pathways. They are also present at the pulmonary parenchyma and blood vessels levels; 2/3 of the patients with COBP presented clinical signs of chronic pulmonary cord and right cardiac failure. That is why we suggested histologic investigations of the vascular and perivascular changes in the patients diagnosed with COBP.

MATERIAL AND METHODS

In order to conduct the present study we removed lung fragments from 37 patients with COBP who had passed away in the Emergency Hospital and Nr. 3 Clinical Hospital of Craiova, Pneumophtysiology, between 1997–2002.

The biologic materials were fixed in 10% neuter formaldehyde for 72 hours at the lab temperature. Then the fixed fragments were processed by the classic histologic technique of wax embedding. Sections of 3–5 μm were performed using the wax microtome and then they were coloured with:

– haematoxylin–eosin (H.E.);

– light green Goldner-Szeckeli (G.S.) technique, for an elective revealing of collagen fibres;

– Unna technique for orceina to reveal the elastic fibres.

We used biotine–streptavidine system enzymatic conjugated (LSAB) for the immunohystochemical study. It was performed to reveal the smooth muscle cells and the myofibroblasts by using antiactine antibody for the smooth muscle fiber and the vimentin antibody for vimentin revealing.

RESULTS

Vascular and perivascular changes in patients with COBP were extremely various from a case to another and from a lung area to another even if clinically and paraclinically the patients belonged to the same evolutive stage of the disease. This microscopic aspect is due to the fact that the morphologic lesions have not the same intensity all over the lung lobes.

Though COBP is defined as a chronic disease affecting especially the small air pathways, we noted that histologic changes comprised the pulmonary parenchyma too, so that intrapulmonary air pathway lesions correlated to the lung degree of affecting on the whole and the histologic changes of the blood vessels. We generally noted that the main morphologic lesions were centrolobular emphysema and chronic bronchitis ones. The former were more intense in the upper lung side while the latter were more intense in the lower side of the lung.

The first and more constant histologic change noted at the vessels level was the lung capillary bed reducing as a consequence of the changes appeared at the

interalveolar septae level. In the patients wits COBP III stage, the lung parenchyma was totally disorganized thus revealing big air cavities replacing the lung alveolae.

We consider that interalveolar septae destruction is firstly due to the small air pathways chronic obstruction, which led to the residual volume increasement at the alveolae and lung acinary levels, followed by their relaxing and finally interalveolar septae destruction.

This air cavities formed by interalveolar septae destruction determined a deterioration of the lung vascular network especially of the sanguine capillaries, the place where changes of respiratory gases between the internal and external environment occurs. The remaining lung capillaries presented enlarged lumen and some reactive parietal changes in the most part of them.

Vascular congestion often noted in some lung areas may be explained also by the presence of an inflammatory process existing in the peribronchovascular stroma which, by chemical mediators released by the immune system cells, influences the lung circulation but also for the remaining capillary network has to assume the same blood volume and manage the same flow.

Arterioles and venules presented an unhomogenous thickness of the vascular walls by some increased quantity of collagen fibers replacing the muscle and elastic fibers of the their tunica media. In the structure of these vessels, collagen fibers appeared much more developed both in the external and in tunica media where they either partly replaced the smooth muscle fibers or they disorganized the muscle disposition of the vessels. We consider that collagen fibrosis of the vascular wells is one of the factors disturbing the lung flow and leads to the chronic lung cord installing.

Elective staining with orceina revealing the elastic fibers of the lung blood vessels allowed us to note remarkable changes of that component. In some arterioles and venules, we noted a thickness of the parietal elastic fibers, a microscopic aspect pointing an adaptation process of the vessel wall at a higher blood pressure, presented from the very beginning of the disease.

In the more advanced stages of the disease we noted a turbulence, a fragmentation and even a destruction of the elastic lamellae into the blood vessel walls, which is a histologic aspect pointing that the vascular adaptive mechanisms had been exceeded.

By using the immunohistochemical technique with smooth muscle antifiber antibody to reveal the miocytes of the tunica media structure of these vessels we noted that for the first stage of the disease a hypertrophy and a hyperplasia of the smooth muscle fibers appeared. At the same time to the evolution of the disease the smooth muscle fibers appeared more and more disorganized and replaced by the collagen fibers.

Immunomarking with anti-vimentine antibodies for the vascular endothelium studying allowed us to noted discontinuities of the vascular endothelium in almost all the blood vessels.

This microscopic aspect raises the endothelial harming mechanisms problem in COBP. Perivascular stroma was extremely rich with connective (fibroblasts) and inflammatory cells (neutrophiles, macrophages, lymphocytes and plasma cells).

The main cells playing a part both in the collagen fibers genesis and lung fibrosis appearance of both the peribroncho-vascular conjunctive stroma and the blood vessels walls are considered to be the fibrocytes–fibroblasts.

At the lung level, normally, the fibrocytes (fibroblasts) are found in a reduced number into the peribronchovascular stroma and into the interalveolar septae. Under the influence of some local factors, fibroblasts rapidly proliferate to repair lesions produced by the pathogenic agents.

The increasement of the proliferation index associates to the fibroblast metabolism intensification, with collagen and glicosaminoglicans synthesis increasement and other components of the extracell matrix causing the appearance of a scar tissue (Figures 1, 2, 3 and 4).

The inflammatory cell number increasement is due to the pulmonary affection recrudescence, expositions to favoring factors the decreasement of the defense capacity of the body (Figures 1, 2, 3, and 4).

As concerning the inflammatory process intensity, on the pieces studied by us, we noted that the cell aspects vary from a stage of the lung disease to another, from a patient to another and from a lung area to another. These different histologic aspects can be explained by the presence of some various etiopathogenic factors.

DISCUSSIONS

COBP specific histopathologic changes prevalently appear at the small air pathways levels. Through the lung owns numerous defence mechanisms and a great self-defence capacity their function in COBP can be affected by genetic factors (alpha-1-antitripsine deficiency) or the repeated long exposing to environment risk factors (atmosphere pollution, infections, etc.) (Pride and Burrows, 1995).

The most frequent form of parenchyma affecting in COBP patients in the study was the centrolobular emphysema, which involves the enlargement and destruction of the respiratory bronchioles.

In the average gravity cases this phenomenon was revealed just in the apical area of the lung but in the advanced stages it appeared diffuse in the whole parenchyma also involving the areolar capillary bed destruction.

We consider that the various morphologic lesions present at the lung level are the result of some repeated inflammatory phases originated by the pathogen agents inhalation, noxae toxic gases including the cigarette smoke. Among all these factors the most incriminate is the cigarette smoke in COBP aetiology, as smoking seems to interfere the repairing processes thus contributing to the lung structure

altering. The inflammation caused by the risk factors produces at turn a series of harming-repairing cycles of the distal air pathway walls, stroma, and parenchyma and lung vessels. In the patients with COBP, the tissue repairing processes can also determine a lung tissue remoulding with parenchyma and lung stroma function and structure altering.

In any case, this cyclic harming-repairing process can produce the structural remoulding of the respiratory pathway wall, with collagen content increasement and scar tissue forming which narrows the lumen and produces fixed obstruction of the air pathways (Matsuba *et al.*, 1972), induce collagen fibrosis of the interlobullar stroma and deteriorate the lung vascularization.

As concerning vascular changes in COBP patients, some authors (Sekon, 1994) considered that they appear early in the natural history of the disease when the lung function is still well fulfilled and the lung arterial pressure is normal. These vascular changes first expresses by the thickness of the blood vessel walls. The same authors considered that the first histologic changes appear at the vascular endothelium level.

Endothelial dysfunction of the lung arteries appears early in the course of the disease due to the products in the cigarette smoke or indirectly produced by the mediators of the inflammation. It is known that the vascular endothelium plays the main part in maintaining the vascular tonus and the cell proliferation. That is why endothelial dysfunction perhaps initiates physiopathologic sequences that would lead to structural changes of the vessels.

In some other authors opinion, the thickness of intima is the first event which appears, then the smooth muscle proliferation in the vessel walls follows and also the appearance of an inflammatory infiltrate including macrophages and CD8+ lymphocytes. These structural changes correlate to the lung arterial pressure increasement, which initially appears under effort and subsenquently, during the rest. As the disease advances more and more proteoglycans stares, muscle and collagen fibers would thicken the vascular walls therefore the muscle arterioles changes associates to the increasement of the pressure in the small circulation and the appearance of the chronic pulmonary lung.

In the cases studies, into the pulmonary blood vessels structure, collagen fibers appeared more developed than normally, especially in the adventice and tunica media where they either replaced the smooth muscle fibers here and there, or they disorganized the muscle disposing of the vessel.

The increasement of the collagen fibers is the result of the game between the destructive and repairing processes, which take place into the pulmonary stroma. Finally, the process of pulmonary process would extend and reduce the pulmonary elastic recoil. The understanding of the pulmonary fibrogenesis pathogeny moves around the interaction between the ethiopathogenic factors, inflammatory process and the lung structural cells. The inflammation originated by the risk factors can determine the appearance of numerous harming and repairing cycles of the

pulmonary structures. Subsequently the inflammation mediators maintain it. Many studies prove the existence of a direct correlation between the different inflammatory cell numbers and the severity of the COBP evolution (Finkelstein *et al.*, 1998).

In the cases studied by us, we found an increase number of neutrophils in the peribronchovascular stroma. The neutrophils (microphages) are cells, which came to protect the body; their main function is phagocytosis. We should mention that the neutrophils from the inflammatory hotbed secrete a series of proteinais to the extracell area; among them we name: neutrophilic elastase (EN), neutrophilic catepsin G and neutrophilic proteinase 3 (Liu, Lazarus and Caughey, 1998) which contribute to the pulmonary parenchyma, stroma and vessels destruction. Other cells identified on our preparations were the macrophages; they were present both in the small and big air pathway and also in the pulmonary parenchyma of the COBP patients. Some authors identified a great number of macrophages not only on the histopathologic sections but also into the liquid of bronchic lavage, into the bronchic secretions and the spittle exam (Peleman *et al.*, 1999).

In the patients with emphysema macrophages were found into the destructions situs of the alveolar walls. It seems that macrophages play an important part into the COBP inflammation by releasing some mediators such as the alpha-tumoral necrosis factor (TNF-alpha), interleukine-8 and leucotriene-B4, which promote the neutrophylic inflammation. Also, the environment the most aggressive of which are collagenase and elastase had a lytic action of the collagen fibers and elastine from the connecting tissue and the blood vessel walls. Macrophages secretion is made from big quantities of oxidant agents (H_2O_2, superoxides) with destructive effects on the lung structural elements.

On the histologic preparations performed by us, we identified numerous lymphocytes and plasmocytes. Some investigators found a very big number of T-lymphocytes on the pieces of bronchic biopsy and they showed that there were increasements of the cytotoxic T-lymphocytes number (CD8+) in all the lung structures (Peinado *et al.*, 1999).

Their part is incompletely known, but one of the ways they could act is the chemical mediator releasing such as: perphorine, TNF-alpha etc., which can produce cytolysis and accelerated apoptosis of the epithelial cells (Liu *et al.*, 1999), events that contribute to the inflammation maintenance.

On the whole, inflammatory cells activated in COBP release a series of mediators including a large range of strong proteinasae oxidants and toxic peptides. Among all the mediators the following are considered to be the most important: LTB4, IL-8, TNF-alpha, TGF-β end EGF. It seems that they part in scaring process, therefore in fibrosis and air pathway narrowing. Another extremely important factor in COBP appearance and development is the oxidative stress. Now it is obvious that in COBP, a lock of poise appears between the oxidants and antioxidants favoring the former ones.

Oxidants react with a larger variety of biologic molecules, including proteins, lipids nucleic acids, etc., leading to the harming or even to the death of cells and remarkable changes of the extracell matrix. Also, such oxidants are the promontories of inflammation by activating NF-KB factor, which initiated some proinflammatory gene encoding TNF and IL-8.

CONCLUSIONS

Lung vascular changes in COBP characterized by the thickness of the blood vessels walls (especially of arterioles and venules) by the increasement of the muscle component from the tunica media for the first phase, and collagene fibres number increasement from the adventitia.

In the advanced stages of the disease, a reduction of the smooth muscle fibers from the arterioles and venules walls was noted and also their replacement by collagen fibers. Lung capillaries appeared congested and reduced in number. Perivascular lung stroma presented increased quantity of collagen fibers, which appeared as a consequence of numerous harming-repairing cycles of the lung structures, consequently maintained by the chemical mediators of the inflammation.

We noted numerous type inflammatory cells (lymphocytes, granulocytes and macrophages) into the connective stroma; by chemical mediators they release and by lysosomal proteasis they are involved in disorganizing the lung structures. Immunohistochemical studies allowed us to reveal vascular endothelium discontinuities and a reduced proliferation of miofibroblastic type into the peribronchovascular stroma.

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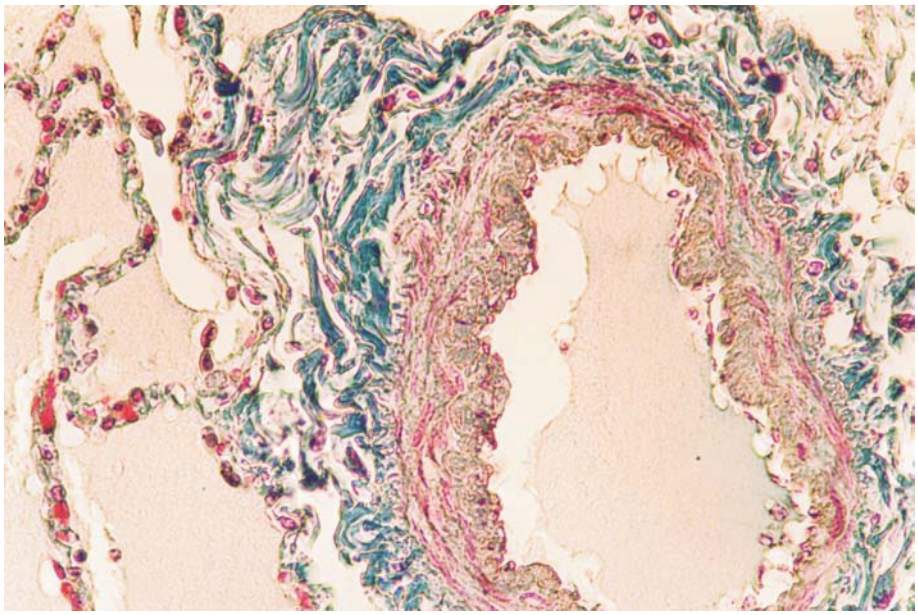

Figure 1 – Microscopic image of an arteriola with the thickening of the wall by intense collagen fibrosis within adventice; macrophages in stroma (Goldner-Szeckeli stain, ×200)

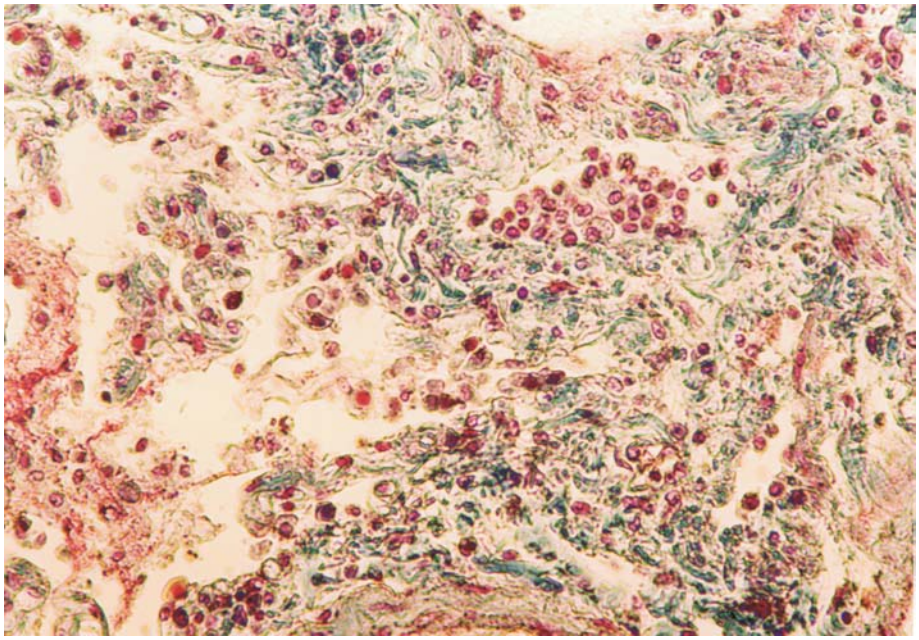

Figure 2 – Peribronchovascular stroma infiltrated with macrophages, lymphocytes and plasma cells (Goldner-Szeckeli stain, ×200)

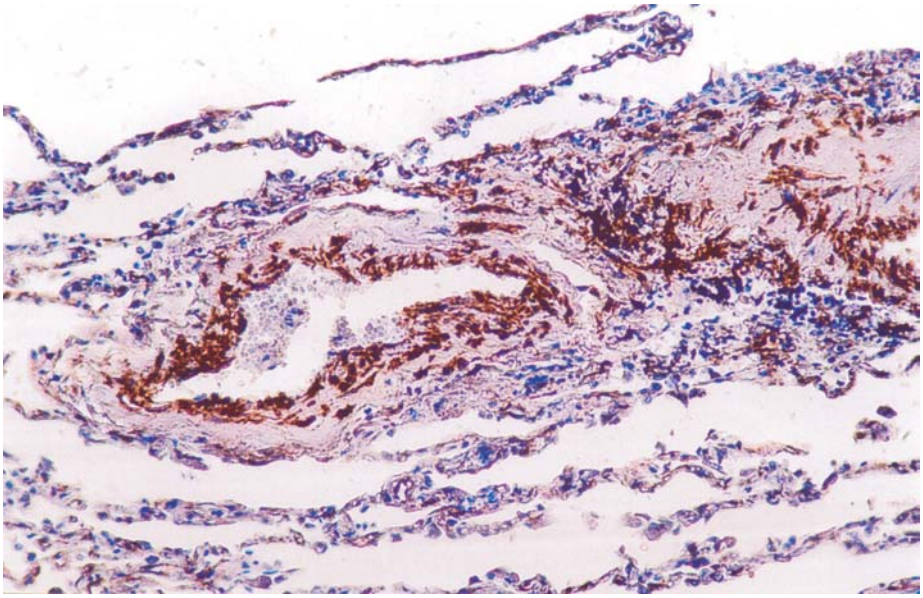

Figure 3 – Some cells in tunica media with positive immunostaining for alpha-actin antibody, probably myofibroblasts (LSAB technique, ×200)

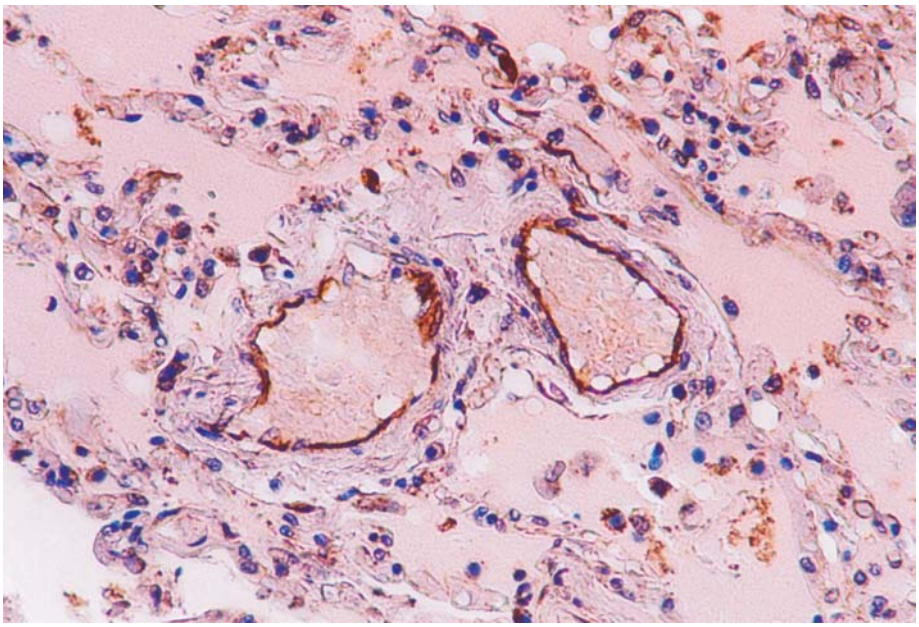

Figure 4 – Intense positive immunostaining for vimentine antibody at discontinuous vascular endothelium (LSAB technique, ×200)