

THE RETICULAR NETWORK CONTRIBUTES TO THE STAGING OF IDIOPATHIC LUNG FIBROSIS

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Abstract - The aspect of reticular fibers is not considered in the current classifications of lung fibrosis. The aim of our study was to evaluate the distribution and the architecture of the reticular fibers for potential use as a tissue marker of fibrosis severity. We included in our study 25 pulmonary samples obtained by video-assisted thoracoscopy surgery (VATS) from a number of 20 cases. The cases were subdivided according to four criteria into: degree II, III and IV. We noticed no significant changes in the reticular network from interalveolar septa to the cases scored with 0, an accumulation of reticular fibers in the interalveolar septa (stage II), the condensation and thick bundles with network disorganization in all areas affected by fibrosis (stage III), partial to full depletion of reticular fibers (stage IV). Depletion of reticular fibers was constantly associated with advanced fibrosis stages.

Key words: Reticular network, idiopathic lung fibrosis, staging

INTRODUCTION

Since 1944 when Hamman et al. (1944) described the pathological features of patients with unexplained interstitial pneumonia, idiopathic interstitial pneumonias have been extensively studied. Liebow (1969) presented a histopathological classification of the idiopathic interstitial pneumonias (IIPs) consisting of five patterns. The classification of the American Thoracic Society/European Respiratory Society (2002) comprises seven clinicopathological entities of IIPs. Idiopathic pulmonary fibrosis is one of the more frequent chronic interstitial lung diseases. Incidence was estimated at 10.7 and 7.4 per 100 000 per year for males and females, respectively. Despite numerous

studies, many questions regarding the epidemiology, radiological features, clinical presentation, diagnosis, classification and therapy have remained unclear and should be elucidated.

Pulmonary fibrosis represents one of the particular responses to various lung parenchyma injuries. Idiopathic pulmonary fibrosis (IPF) is a disease characterized by lung parenchyma distortion by fibroblastic proliferation with extracellular matrix deposition and an inflammatory cell infiltration (Jones et al., 2011). Once established, lung fibrosis is a chronic, progressive, irreversible process with major impact on prognosis and increased mortality of diffuse interstitial pneumopathies.

The role of reticular fibers in the induction and progression of pulmonary fibrosis is relatively understudied and publications on this issue in the literature are rare. The changes in reticular fiber features may be hypothesized in lung fibrosis, based on their biochemical structure. Therefore, major changes of the reticular network are expected to occur in advanced stages of lung fibrosis. On the other hand, the aspect of reticular fibers is not included in the current classifications of lung fibrosis.

Quantification methods for fibrosis are not well standardized at the present time. Kumar (2005) described simple histological staining methods; morphometric, immunohistochemistry, *in situ* hybridization techniques used to quantify fibrosis in specific tissue compartments. Shahzeidi et al. (1993) showed that the activation of interstitial fibroblasts with enhanced type III collagen gene expression represent a part of the mechanism leading to increased collagen deposition in bleomycin-induced fibrosis. This phenomenon occurs before detection of fibrosis by conventional histological methods. Raghu et al. (1985) demonstrated that idiopathic pulmonary fibrosis and adult respiratory distress syndrome showed types I and III collagen accumulated in the interstitium. Type III collagen was initially predominant in the thickened alveolar septa and interstitium. None of these methods, taken alone or together can predict the behavior and prognosis of lung fibrosis.

The aim of our study was to evaluate the distribution and the architecture of the reticular fibers involved in the pathological diagnosis of pulmonary fibrosis for potential use of reticular fibers as tissue markers of fibrosis severity and the predictive value of the evolutionary potential of fibrotic process.

MATERIALS AND METHODS

We included in our study 25 pulmonary samples that were obtained by video-assisted thoracoscopy surgery (VATS) from 20 cases – 10 men and 10 women, with high-resolution computer tomography (HRCT) showing an interstitial pattern and for whom a specific etiology was not identified. The specimens were

fixed in 10% buffered formalin for 48 h and paraffin embedded using the routine histological procedure. Five-micrometer-thick serial sections were performed from each paraffin block and sections were mounted on silanized slides. Sections from each case were stained with hematoxylin and eosin, Masson's trichrome staining method and Gordon and Sweet's silver staining (Sigma Reagents). Microscopic observation was performed by three independent observers using a Nikon Eclipse E600 (Nikon Corporation, Tokyo, Japan). Images were captured and processed with Lucia G software system.

As a first step, we established the morphological diagnosis and stage of lung fibrosis by using basic lesions according to current standard. The following parameters were evaluated: lung parenchyma: (score given for normal architecture – score 0; minor alterations – emphysema, collapse – 1; severe alterations – 2; major changes – 3; major changes, replacement – 4); chronic inflammatory infiltrate (absent – 0; isolated, rare, small groups – 1; focal, high density – 2; diffuse – 3; focal, even absent – 4); macrophages (rare – 0; small groups, focal distribution, intraalveolar – 1; diffuse, high density, heterogeneous – 2; diffuse, high density, homogeneous – 3; few macrophages, dust cells – 4); fibrosis (absent – 0; thin collagen fibers, not organized in bundles – 1; collagen bundles, heterogeneous pattern – 2; collagen bundles homogeneous pattern – 3; nodular, extensive fibrosis – 4). By summing up the values, the following stages of severity were given by the final score, as follows: I (1-3), II (4-6), III (7-9) and IV (10-12). In the second step, we analyzed the organization and density of reticular fibers in each section and compared with the different stages of fibrosis. The general score "0" corresponds to normal lung parenchyma, as was found in five cases included in the study as control. Biopsies from these cases were taken from patients operated on for other lesions of the lung, with the approval of the Committee of Ethics of the University.

RESULTS

All five cases diagnosed with normal parenchyma were scored with 0. From the 20 cases with pulmo-

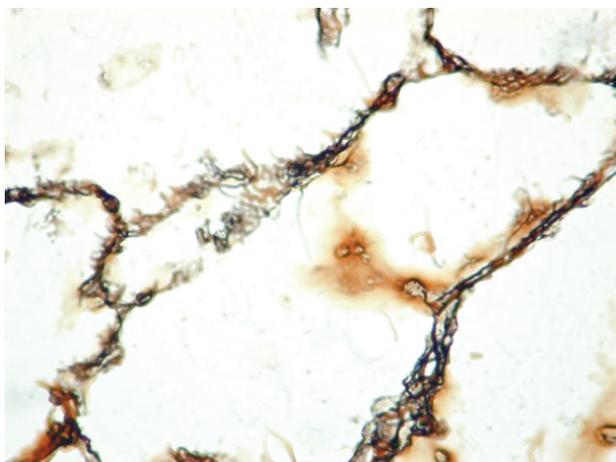


Fig. 1a. Normal distribution of reticular fibers, Gordon & Sweet's silver staining, X 100.

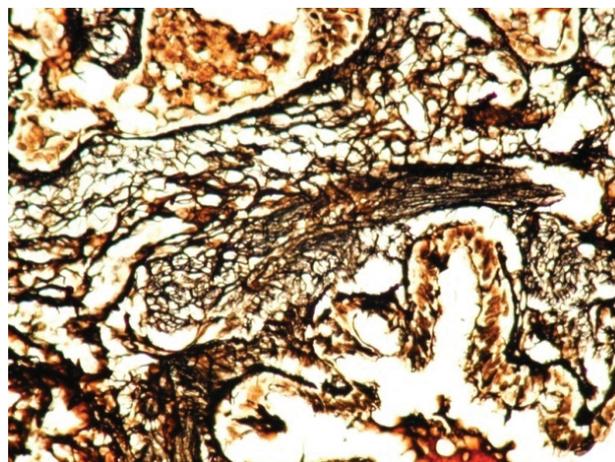


Fig. 1c. Massive accumulation of the reticular fibers, Gordon & Sweet's silver staining, X 100.

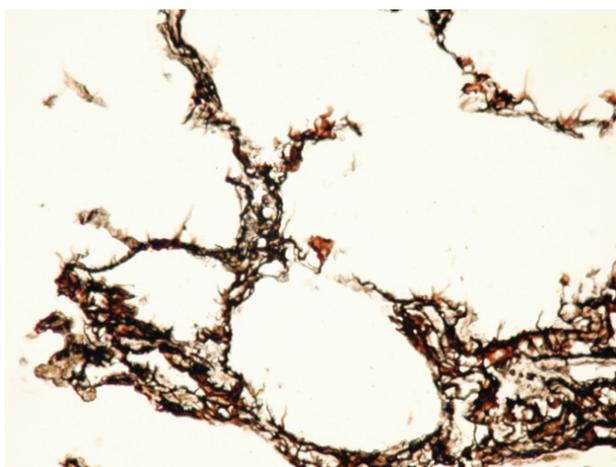


Fig. 1b. Accumulation of the reticular fibers in the interalveolar septa, Gordon & Sweet's silver staining, X100.

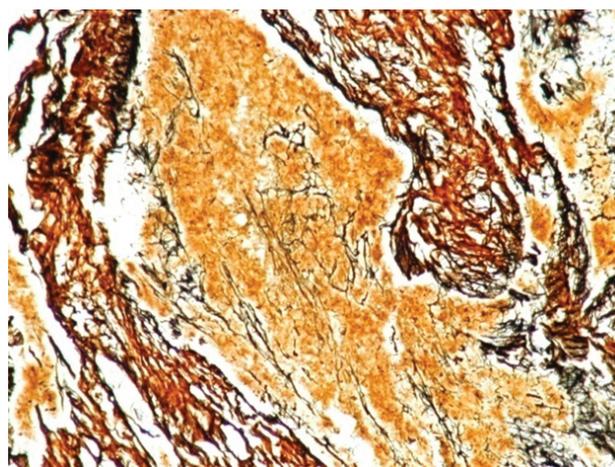


Fig. 1d. Disorganization of the reticular network, Gordon & Sweet's silver staining, X 100.

nary fibrosis included in the present study, none met the criteria for degree I (1 to 3 point final score). The cases were subdivided according to the four criteria into: degree II (4 cases, 20%), degree III (12 cases, 60%), and degree IV (4 cases, 20%). We noticed that the number and organization of reticular fibers were different depending on the morphologic stage of pulmonary fibrosis. We noticed no significant changes in the reticular network from interalveolar septa to the cases scored with 0. Reticular fibers appeared fine, filigreed, located in the network; this is considered to be the normal reticular network (Fig. 1a).

Along with progression of fibrosis and architectural changes of lung parenchyma, we found changes in the quantity, quality and organization of reticular network. At stage II of fibrosis, we noticed a high number of reticular fibers that accumulated in the interalveolar septa (Fig. 1b) causing significant thickening of these areas. Reticular fibers are still laid out in a partially organized network. Later, at stage III, the massive accumulation of reticular fibers leads to their condensation, and thick bundles with network disorganization in all areas affected by fibrosis were found (Figs. 1c, 1d).

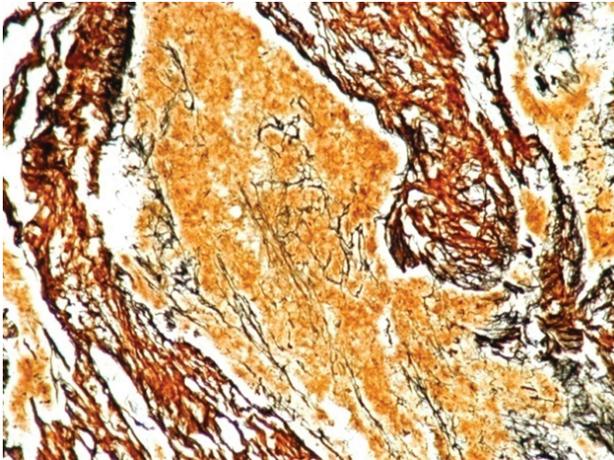


Fig. 1e. Partial depletion of the reticular network, Gordon & Sweet's silver staining, X 100.

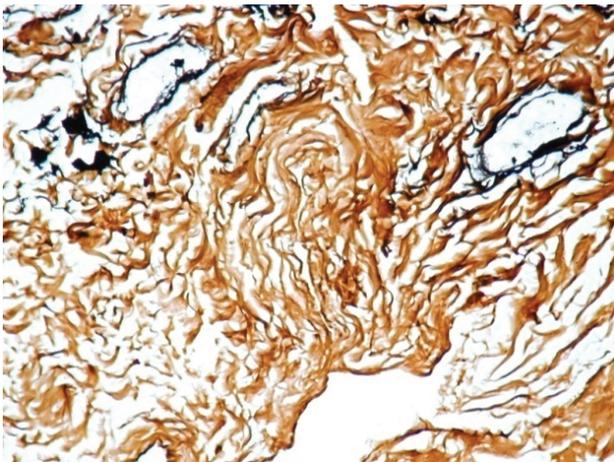


Fig. 1f. Complete depletion of the reticular network, Gordon & Sweet's silver staining, X 100.

In the severe, advanced stages of extensive fibrosis (stage IV), thick bundles of collagen fibers partially or completely replaced the lung parenchyma with partial (Fig. 1e) to full depletion (Fig. 1f) of reticular fibers. The reticular network of the septa was noticed only focally in the immediate vicinity of the alveolar epithelium and occasionally in the septal blood vessel wall.

No significant changes were found in the blood vessels' walls in terms of the reticular component, even in cases with severe injuries (Fig 1g).

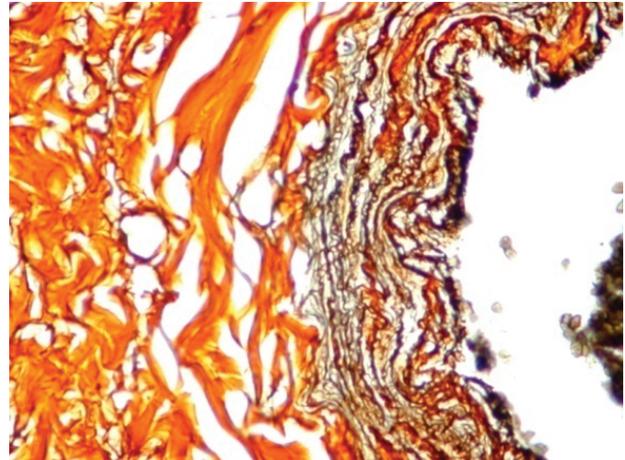


Fig. 1g. Persistence of the vessel wall reticular network, Gordon & Sweet's silver staining, X 200.

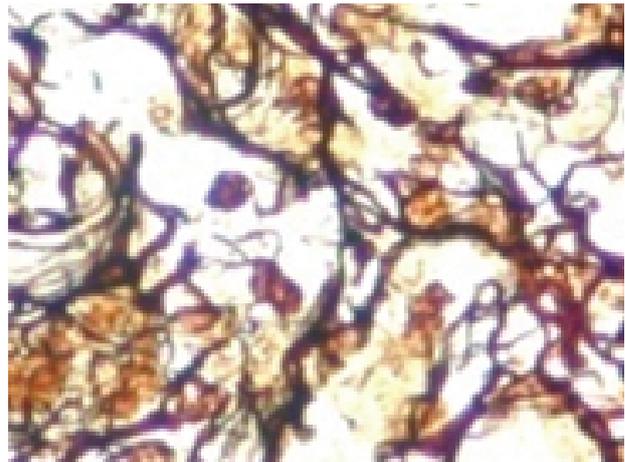


Fig. 1h. Disordered network of reticular fibers in the area of inflammatory infiltrate, Gordon & Sweet's silver staining, X200.

A well developed but irregular reticular fiber network was found in the areas with inflammatory infiltrate (Fig. 1h). In these areas, the aspect mimics the lymphoid tissue architecture and not that of normal lung parenchyma.

DISCUSSION

Several studies have described the reticular fiber network in the normal lung. Toshima et al. (2004) used electron microscopy scanning and showed that collagen fibers predominate in the alveoli and alveolar

septa, smaller fibers, forming basket-like networks, in the normal lung. Mercer et al. (1990) analyzed the distribution of collagen and elastic fibers in the lungs and noticed that connective tissue fibers were present in the alveolar duct wall in both human and rat species. Suki et al. (2005) showed that connective tissue of the lung is not a static structure, even in normal situations. It is a dynamic balance between continuous breakdown and remodeling modulated by mechanical forces, influenced by external or internal changes such as a disease or environmental stimuli.

The reticular fiber network changes, which are useful in diagnosis, were noticed in different situations: to differentiate between normal hypophysis and pituitary adenoma (Ceausu et al. 2010), well-differentiated hepatocellular carcinoma and benign hepatic nodules (Hong et al. 2011), in the diagnosis of superficial cervical endometriosis (Kim, 2001), and pathological changes of the lung after prolonged inhalation of high oxygen concentrations (Matsubara et al. 1986).

Regarding lung fibrosis, Takyia et al. (1983) showed that the mode of organization of the fibrotic lung connective matrix could be correlated with the evolution, stability, remodeling ability and reversibility. Our study shows that the identification of reticular fibers in the pulmonary fibrosis areas is an important factor in the evaluating, staging and prognostic assessment of fibrosis itself. Pulmonary fibrosis, for long considered as the final stage of evolution of diffuse interstitial pneumopathies, is itself a progressive, evolving, pathologic process, diagnosed in various stages of severity.

Bateman et al. (1981), using an immunofluorescence technique, demonstrated that type III collagen increases in early active fibrosis areas in the lung. We noticed an inverse relationship between the number and organization of reticular fibers and the severity of pulmonary fibrosis. The presence of reticular fibers is correlated with early and moderate forms of fibrosis, and their depletion with severe stages.

Idiopathic pulmonary fibrosis is a disease that is currently in the stage of hypothesis regarding its etiology, pathogenesis, mechanisms, experimental model, evolution and prognosis. Leslie (2012) proposed a pathogenic sequence of events in idiopathic pulmonary fibrosis as follows: stretch injury to epithelial-mesenchymal transition, formation of the fibroblastic reticulum, local alveolar collapse, collagen deposition, vascular ingrowths, simplification of lobules, and honeycomb lung. King et al. (2011) considered that the fibrotic response is due to abnormal activation of alveolar-epithelial cells. The production of mediators, proliferation of mesenchymal cells, formation of fibroblast and myofibroblast, attraction of circulating fibrocytes and epithelial to mesenchymal transition, are the next steps.

In these conditions, we estimate that in the early stages of fibrosis reticular fibers are formed in larger quantities than normal and are then replaced by the production of precursors of collagen fibers.

CONCLUSIONS

Analysis of the cases included in the study revealed several particular aspects. These indicated reticular fiber as a potentially useful tissue marker for the histopathological classification of fibrosis stage. Depletion of reticular fibers is constantly associated with advanced fibrosis stages. This major change may explain the irreversibility of the fibrotic process and the absence of efficacy of therapy on recovery of lung function.

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