From basic lesions to a pathological staging of pulmonary fibrosis

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Abstract
Idiopathic pulmonary fibrosis is a severe disease, with unpredictable evolution that frequently leads to respiratory failure and death, despite some progresses made in the field of therapy. Basically, the bad prognosis and failure of therapy are the consequence of the lack of data about the molecular events that have as result the extensive fibrosis. Although the basic lesions were defined many years ago, the pathological classification of pulmonary fibrosis is controversial. In the present work, we analyzed the prognostic impact of basic microscopic lesions on a possible new classification that could be related to the patient outcome. For this purpose, we have investigated 20 cases with idiopathic pulmonary fibrosis and samples of lung parenchyma were obtained by video assisted thoracoscopy. The specimens were processed by usual histological technique and sections were stained with Hematoxylin–Eosin, Masson’s trichrome and Gordon–Sweet silver staining. There were evaluated the lung architecture, the chronic inflammatory infiltrate, macrophages and fibrosis. The distribution and severity of each parameter was converted into points and finally graded from I to IV, with corresponding score from 1 to 12. We found four cases with degree II, 12 with degree III, and four with degree IV. Our results support the hypothesis that the evaluation of basic lesions could be the basis for a more objective classification and staging of lung fibrosis and, possibly, a better prognostic method and, eventually, a predictor for the response to targeted therapy.

Keywords: pulmonary fibrosis, classification, staging, prognosis.

Introduction
Pulmonary fibrosis is defined as a complete disorganization of the normal lung architecture because of alterations in quantity and quality of the pulmonary interstitium. Pulmonary fibrosis occurs and develops in the evolution of over 200 diffuse parenchyma lung diseases [1, 2]. Fibrosis is thought to be an irreversible process but its progression may be stopped through an effective targeted therapy applied in the early stages. When the fibrosis is already constituted, it significantly changes the therapeutic options and prognosis.

By microscopy, pulmonary fibrosis is characterized by the coexistence of some basic lesions, which could be the morphologic expression of multiple pathogenic models [3–6]. A precise diagnosis of pulmonary fibrosis is complex and dynamic, because in the evolution of this disease, the proportion to which the basic lesions are associated is different.

However, in order to a better understanding of the disease, activity, the individual response to therapy and also to control disease progression, a conventional histological description and confirmation of fibrosis seems not to be enough. Current guidelines recommend for the diagnosis and management of fibrosis the histological classification of the morphologic lesions, each histopathologic subset having a different prognosis [7, 8]. The idiopathic pulmonary fibrosis is characterized by the presence of usual interstitial pneumonia (UIP) that has the worst prognosis [9, 10]. The classical UIP pattern is found in a minority of patients and represents an advanced stage of the pulmonary fibrosis. Frequently, in clinical practice some biopsies can reveal a pattern of fibrosis that does not meet the UIP criteria and cannot be included in another histopathological subset. These cases are considered as non-classified fibrosis and they represent usually a less advanced evolutionary stage than UIP, but possible progressive [7]. The development of novel tools for the evaluation of fibrosis stage is crucial for an accurate and proper diagnosis. Along the years, there were proposed many grading systems of pulmonary fibrosis, based on cell and tissue changes. Many of these are controversial, as the final result does not predict the clinical outcome. Nowadays the most accepted score is a semi quantitative pathologic scoring system that comprises four factors: fibrosis, cell types and distribution, granulation/nectoic tissue and desquamation [11–13]. Notably, almost all scoring systems proposed in the literature are based on a subjective evaluation of biopsies and there are differences between the significance of each basic lesion on the final evaluation. Moreover, the relationships between the extension of fibrosis, density of the chronic inflammatory infiltrate and the degree of lung parenchyma damage were not clarified until now. Therefore, we believed that it is an urgent need for a new pathological classification of
pulmonary fibrosis in order to evaluate its molecular biology and the response to specific available therapy.

In the present study, we have quantified the morphologic basic lesions that characterized pulmonary fibrosis, a quantification that emphasizes the degree of lesions and consequently of the pulmonary function. This makes possible a good evaluation of the prognosis and in the future could be a good predictor for the effective targeted therapy. Moreover, by assessing fundamental lesions of pulmonary fibrosis, we intend to elaborate a new pathological staging, correlated with patient evolution and potentially, with therapeutic response.

Materials and Methods

We have investigated 25 pulmonary samples obtained by video assisted thoracoscopy surgery (VATS) from a number of 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% buffer formalin for 48 hours and then paraffin embedded, using the conventional histological procedure by using Shandon embedding center. From each paraffin block, there were performed 3 μm thick serial sections. Sections from each case were stained with Hematoxylin–Eosin (HE) method, Masson’s trichrome stain and Gordon–Sweet silver stain for the pathological evaluation. All reagents were from Sigma, USA.

It was established the morphological diagnosis of pulmonary fibrosis based on the accepted criteria and than a quantitative assessment was undertaken using a scale from “0” to “5” for four histological features: pulmonary parenchyma architecture, the inflammatory infiltrate (in terms of presence, density and distribution), the macrophages (density and type) and the presence and extension of fibrosis (the scoring system is shown in Table 1).

The total pathologic fibrotic score was generated by adding the score of each lesion and the final result ranges between 0–12 points. According to the value of final score, cases have been divided in degrees of severity (from I to IV), each degree having as corresponding score. The correspondence between the range and the degree of severity is highlighted in Table 2.

Results

For normal parenchyma we obtained score 0. From the 20 cases with pulmonary fibrosis included in the present study, none meets the criteria for the degree I (1 to 3 point final score). The cases were subdivided according to the four criteria into: degree II (four cases, 20%), degree III (12 cases, 60%), and degree IV (four cases, 20%). Three cases with degree II had a score of 6 points and one with score 4. Three cases with degree score IV received 10 points and one received maximum.

In the subset with degree III, the majority of cases had score 9. The distribution of cases based on this classification signals out the advanced stage of pulmonary fibrosis, as detected in the present series.

In the mild (II) and moderate (III) degree of fibrosis, the architectural changes have interested particularly the alveoli that displayed different sizes and shapes. In these cases, the inflammatory infiltrate is often diffusing with heterogeneous distribution, but with a moderate density. Despite on HE stained slides we have not notice fibrotic changes of the interalveolar septa, these could be identified in the trichromic slides that identifies them in blue in the form of some thin collagen fibers, distributed in variable areas surrounding alveoli. The aspect is more obvious around the small vessels of the pulmonary parenchyma, to which the adventitia fibrosis is heterogeneous and extends towards the septa. The aspect is confirmed by means of silver staining. We did not find any major change of the reticular network in the interalveolar septa (Figure 1, a–d).

Cases with fibrosis degree IV had major changes of the parenchyma. The remaining alveoli displayed irregular shapes and sizes and in some of the cases, they contained a variable number of red blood cells and macrophages in the lumen. Focally, the remaining alveoli contain a large number of macrophages, rich in hemosiderin pigment (Figure 2b). Lambert ducts
hyperplasia was constantly observed like a bronchial type epithelium proliferation. This feature partially or completely replaces the respiratory surfaces (Figure 2a).

![Image 1](image1.png)

Figure 1 – (a) Minimum fibrosis of interalveolar septa (Masson’s trichrome stain, ×100); (b) Perivascular fibrosis with extension to interalveolar septa (Masson’s trichrome stain, ×200); (c) Extensive fibrosis (Masson’s trichrome stain, ×200); (d) The change of the reticulin fibers network (Gordon–Sweet silver stain, ×400).

![Image 2](image2.png)

Figure 2 – (a) Proliferation of Lambert channels (HE stain, ×200); (b) Macrophages with hemosiderin in the remaining parenchyma (HE stain, ×200).

From the point of view of the distribution and arrangement of the collagen fibers in the pulmonary parenchyma, we identified several models – including extensive nodular fibrosis. Nodular fibrosis is characterized by thick fibers of collagen disposed into disorderly arranged fascicles that converge in the likeness of nodules with reduced cellularity, without the inflammatory infiltrate and without macrophage accumulation (Figure 3). We suppose that the formation of the fibrotic nodules goes through various stages and, at particular times, we can observe spindle cells concentrically-disposed around a nodule composed of collagen almost exclusively, which displays hyalinization tendency. The pulmonary parenchyma surrounding the
nodules consists of polygonal cells associated to numerous macrophages. Judging by their architecture, these areas remind the hepatization phase in the evolution of pneumonia. On slides stained with the trichromic method, all the fibrotic nodule components are intensely stained with Aniline Blue.

Figure 3 – Nodular extensive fibrosis (Masson’s trichrome stain, ×200).

The blood vessels displayed intense stasis, especially in the peripheral area of the lesions, both in the venous and arterial components. The fibrosis was usually extensive and is indicated by the presence of some thick bundles of collagen fibers (one case) and nodular fibrosis (three cases) that partially or completely replace the lung parenchyma. In the areas where the modified alveoli still persist important quantities of collagen were accumulated at the level of the interalveolar septa, which are significantly thicker than those of a normal lung or with minor changes.

We found the severe septal changes surrounding the blood vessels as well, where they are associated with partial fibrosis of the vascular wall (Figure 4a). The first changes of this type we have perceived around the smaller-sized vessels (Figure 4b), but also in the adventitia of vessels of larger size and have been constantly related to advanced stages of illness.

The inflammatory infiltrate was present in the majority of the cases with a variable organization and extension and it predominantly composed of lymphocytes (Figure 5a), occasionally plasma cells and, more rarely, was heterogeneous and it contained granulocytes. For the cases with advanced stages, we have observed a partially converse relationship between the infiltrate density and the extension of the fibrosis.

We have identified several types of macrophages. In the histological evaluation of the stages of fibrosis, we have taken into account the macrophages that contained a yellow-orange colored pigment, from the group of siderophages (Figure 5c), located mainly in the alveolar space and, occasionally, in the respiratory bronchioles and terminal bronchioles. The dusty macrophages (Figure 5b), that contained important quantities of black-colored pigment, were noticed as isolated or disposed in small groups, in the subpleural space, as well as the macrophages with foamy cytoplasm without any observed inclusions which were found only for a number of cases, were not taken into consideration in the histological assessment of the fibrosis staging.

We want to point out that the fibrotic changes do not appear around the bronchial tree branches larger in size unless these are at a certain distance beyond the muscular stratum. These aspects are further confirmed by the silver staining, which show a disorganized or absent network of reticular fibers.

In some cases with severe and diffuse inflammatory changes, we noticed a distinct distension of the bronchioles, Lambert ducts hyperplasia with an obvious distension and mucus hypersecretion. The distended areas have been bound by the respiratory-type epithelium, focal of Clara cells and more rarely, by alveolar cells type II. Numerous macrophages without inclusion bodies to the usual methods and, occasionally, lymphocytes are present in the luminal mucus. Focally, giant multinucleated cells that are not part of the granulomatous-type structures are present (Figure 5d). Intermediate aspects can be remarked and suggest the fusion of the macrophages in order to form the giant cells.

Figure 4 – (a) Fibrosis of the adventitia of a medium-sized vessel (Masson’s trichrome stain, ×200); (b) Fibrosis around a post-capillary venule wall (Masson’s trichrome stain, ×200).
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Discussion

According to the pathological classification of idiopathic interstitial pneumonia, fibrosis is described as a common morphological feature consisting of seven histological patterns that differ in prognosis and response to therapy [14, 15]. The concept of severity and the pathological degree of fibrosis have lost their value when the UIP pattern with worst prognosis was recognized as the histological hallmark of idiopathic pulmonary fibrosis (IPF) [14, 16]. The histological patterns represent the pathological basis for the classifications of idiopathic interstitial pneumonia in clinical entities. A histological pattern does not represent a degree of fibrosis, although in each of these the distribution, intensity and nature of fibrosis are different. Currently, the most important goal of the histological examination is to distinguish the UIP pattern from other histological patterns that have a better prognosis [17, 18]. Pulmonary fibrosis, an irreversible pathological change is associated with poor survival because the therapeutic options are still limited. The only way to improve prognosis is to control disease progression through an effective targeted therapy [19, 20].

In our study, lung biopsies revealed fibrosis but they did not meet consistent pathological criteria for a histological pattern. The American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines have admitted the existence of non-classifiable fibrosis without establishing the morphological assessment of it [14, 21]. We have quantified the basic morphological lesions in order to see if the final pathological score for each pattern of fibrosis can be a useful tool in characterizing and determining the degree of fibrosis.

Out of the 20 assessed cases, 20% were included in the degree II, 60% in the degree III and 20% in the degree IV.

We have observed a poor survival for degree IV and a variable evolution for degree III in this preliminary study. On the other hand, degree II was more likely to remain stable, but to evaluate more precisely this aspect is necessary to investigate larger series of patients. The proportion to which the inflammatory infiltration contributed to the final degree of fibrosis was determined from the clinical improvement after the therapy. The changes of the pulmonary parenchyma and the organization of the collagen fibers were good guides to the prognosis because each of them, taken separately, contributes to the establishment of the degree of fibrosis.

The absence of cases with degree I of fibrosis from our study is explained by the manner in which we selected the cases. The series included the cases with evident HRCT changes that, during the histological evaluation, corresponded to lesions that had a score higher than three (the upper value of the interval for the degree I). The degree IV of fibrosis established through our method is showed by advanced fibrosis with pulmonary architectural changes, but is not equivalent.
to the UIP pattern, which is the honeycomb lung. The small number of degree IV is also explained by the way in which these were included in the study, the HRCT changes and the extensive bilateral reticular changes, even without the UIP pattern aspect.

Many studies have correlated the degree of cellularity with response to treatment and prognosis, but the value of these results is limited by the fact that the notion of cellularity has been used indiscriminately for the inflammatory infiltrate, the intra-alveolar accumulation of macrophages or a combination of both [15, 22, 23]. In our study, the lymphoplasmacytic inflammatory infiltrate showed density and variable distribution, but it has a strong impact on the final result. Taken individually, it did not guide significantly towards including the case in the degree. Its presence in advanced stages could be correlated with the so-called ingravescence of the process and the progressive evolutionary marker.

As the degree of pulmonary fibrosis increased, so did the density of the macrophages within the analyzed sections. In all the four cases of degree IV, the macrophages were displayed, distributed in a diffuse manner, with a high density. Only the association between the macrophages with a high density and little fibrosis was connected to a good response to treatment [15, 24]. The pulmonary parenchyma showed an inverse relation to the degree of fibrosis, and the degree of pulmonary parenchyma destruction is related to an increased fibrosis. There is strong evidence towards the association of advanced fibrosis and the honeycomb lung with a bad prognosis given that the fibrosis, in its final stage with the pulmonary restructuring, is an irreversible change [25–27]. In our cases, the nodular-type fibrosis that replaced the pulmonary parenchyma represents the final stage of fibrosis, confirmed by death of two from four cases. The degree IV is characterized by major changes of the parenchyma in all of the four cases that were analyzed. Relative to the degree of fibrosis, the absolute figure value also increases, attributed to the collagen fibers distribution and arrangement in the pulmonary parenchyma. In the degree III, homogeneous fascicles of collagen predominate, while for the degree IV the extensive nodular fibrosis.

The highest final score of each case is borne by the changes of the pulmonary parenchyma and the collagen fibers, subcomponents that taken individually can determine the degree of pulmonary fibrosis. The score by means of all of its four subcomponents accurately reflects the pulmonary fibrosis heterogeneity and its progressive evolutionary trait, the diagnosis merely highlighting one of its moments.

**Conclusions**

In the present study, we have shown the value of four tissue elements for the pathological staging of pulmonary fibrosis, namely lung architecture, macrophages, the inflammatory infiltrate and fibrosis. From 20 patients included in the study, we found the pathological degree II, III and IV in four, 12, and four cases, respectively. Our preliminary data support the clinical application of this type of staging in order to a better evaluation of the prognosis and eventually, the response to specific therapy.

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