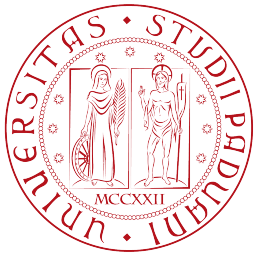

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DIPARTIMENTO DI SCIENZE
CARDIO-TORACO-VASCOLARI E SANITÀ PUBBLICA

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TESI DI LAUREA

Prognostic biomarkers for progressive fibrosing interstitial lung disease

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Abstract

Background

Le malattie interstiziali polmonari fibrose progressive (PF-ILD) rappresentano un gruppo di patologie polmonari croniche e debilitanti caratterizzate dalla progressiva cicatrizzazione del tessuto polmonare. L' obbiettivo dello studio è quello di identificare quali siano i potenziali biomarcatori prognostici di progressione verso la fibrosi e i bersagli terapeutici per queste malattie. Nel febbraio 2022 sono state pubblicate le linee guida aggiornate
riguardanti la "Fibrosi polmonare idiopatica e la fibrosi polmonare progressiva negli adulti". Tali disposizioni si propongono di fornire raccomandazioni basate
sull'evidenza sia per la diagnosi che per la gestione dell' IPF e della PF-ILD. Le novità presentate in queste nuove linee guida hanno suscitato la nostra curiosità, e ci hanno portato ad interrogarci sul potenziale dei fattori predittivi esistenti. Attualmente, sono stati studiati diversi potenziali biomarcatori predittivi di PF-ILD, sono tuttavia necessarie ulteriori ricerche per convalidarne pienamente l'utilità come strumenti predittivi nella pratica clinica.

Scopo dello studio

Lo scopo primario di questo studio è quello di identificare la presenza di variabili istologiche che suggeriscano la presenza di PF-ILD. Il secondo obiettivo consiste nel riconoscere quali siano gli aspetti clinicamente rilevanti nei pazienti con patologia progressiva. Come ultimo obiettivo infine, lo studio si propone di quantificare la presenza di citochine proinfiammatorie nei campioni di tessuto a nostra disposizione.

Materiali e metodi

Il nostro studio, di tipo retrospettivo e monocentrico, ha portato ad una completa revisione della letteratura e ad un'analisi retrospettiva dei dati clinici, radiologici e patologici. Inizialemente, sono stati raccolti i dati da una coorte di 215 pazienti affetti da malattia polmonare interstiziale e riferiti al Dipartimento di Malattie Respiratorie di Padova con diagnosi effettuata tra marzo 2016 e marzo 2022. Di questi, solamente 48 sono stati selezionati e arruolati nello studio

poichè rispettavano tutte le caratteristiche richieste. I pazienti sono stati seguiti per almeno un anno e la progressione della malattia è stata valutata con test di funzionalità respiratoria e scansioni di tomografia computerizzata ad alta risoluzione. Lo studio ha analizzato un pannello di potenziali biomarcatori, tra cui i livelli tissutali di citochine, i parametri di funzionalità polmonare e le caratteristiche radiologiche.

Risultati

Abbiamo riscontrato che i pazienti progressivi tendono ad avere volumi di FVC più bassi alla diagnosi rispetto ai pazienti non progressivi (2.42L progressivi vs 3.37L non progressivi p=0.004). Lo stesso trend statisticamente significativo è stato riscontrato anche con i valori di TLC (p=0.03). L'analisi univariata ha mostrato che i sintomi all'ultimo follow-up peggiorati è un dato che ha raggiunto la significatività statistica (p=0.007). L'analisi multivariata ha mostrato invece che la presenza di microhoneycombing all'analisi istologica è un fattore indipendente di mortalità (p=0.046). La quantificazione delle citochine nei campioni tissutali è ancora in corso.

Conclusioni

I valori espressi in litri di FVC riscontrati alla spirometria alla diagnosi sono risultati inferiori nei pazienti progressivi rispetto ai non progressivi. L'analisi multivariata ha mostrato che la presenza di microhoneycombing è un fattore indipendente di mortalità. È stata anche valutata la sopravvivenza a dieci anni dei pazienti: l'analisi ha rilevato una differenza statisticamente significativa tra pazienti progressivi e non progressivi (p=0.02), i pazienti progressivi hanno una sopravvivenza ridotta. Si prevede di riuscire, dopo l'analisi dell'RNA estratto dai campioni di tessuto polmonare o dal fluido di lavaggio broncoalveolare, a quantificare la rilevanza di alcune citochine come biomarcatori predittivi della malattia polmonare interstiziale fibrosa progressiva.

Abstract

Background

Progressive fibrosing interstitial lung diseases (PF-ILDs) represent a group of chronic and debilitating lung disorders characterized by the progressive scarring of lung tissue. This retrospective monocentric study aimed to investigate the clinical and pathological features of PF-ILDs, and to identify potential prognostic biomarkers of progression towards fibrosis and therapeutic targets for these diseases, in a cohort of patients referred for interstitial lung diseases. An updated guideline on ''Idiopathic Pulmonary Fibrosis and Progressive Pulmonary Fibrosis in Adults" was released in February 2022. Overall, this guideline aims to provide evidence-based recommendations for the diagnosis and management of IPF and PF-ILD. The novelty of these new guidelines determined our curiosity to ask questions about the potential of existing factors that predict the evolution towards fibrosis of the interstitial lung disease.At the moment, there are several potential predictive biomarkers for fibrosing progressive interstitial lung disease (PF-ILD) that have been studied. It is important to note that while these biomarkers show promise in predicting PF-ILD progression, more research is needed to fully validate their utility as predictive tools in clinical practice.

Aim of study

The purpose of the study is to quantifying the presence of certain histological variables that suggest the presence of progressive fibrosing pathology in the patient. The second aim of the study is to identify clinically relevant aspects in patients with progressive pathology, third aim to quantify the presence of proinflammatory cytokines in tissue samples.

Material and method

Our retrospective monocentric study involved a comprehensive literature review and a retrospective analysis of clinical, radiological and pathological data from a cohort of 215 patients of some with interstitial lung disease referred to the Respiratory Disease Department of

Padova between march 2016 and march 2022, of which 48 were selected and enrolled in the study. Among the patients excluded from our study were those with diagnosis of idiopathic pulmonary fibrosis, sarcoidosis and interstitial lung disease associated with autoimmune rheumatic disease. The patients were followed up for a minimum of one years, and their disease progression was assessed using pulmonary function tests and high-resolution computed tomography scans. The study analyzed a panel of potential biomarkers, including tissue levels of cytokines and chemokines, lung function parameters, and radiological features.

Results

We found that progressive patients tend to have lower volumes of FVC at diagnosis compared with non-progressive patients (2.42 L progressive vs. 3.37 L non progressive p=0.004). The same statistically significant trend was also found with TLC values (p=0.03). Univerate analysis showed that symptoms at last follow up worsened was a finding that reached statistical significance(p=0.007). Multivariate analysis showed that the presence of microhoneycombing at histologic analysis was an independent factor in mortality (p=0.046). The quantification of cytokines is a process of research is still ongoing.

Conclusions

We found that progressive patients have a low FVC value found already at diagnostic spirometry.The analyses also showed that worsening of symptoms during follow-up is more present in progressive patients, the finding reached statistical significance. Multivariate analysis showed that the presence of microhoneycombing is an indepedent factor in mortality. Ten-year survival of patients was also evaluated, the analysis revealed a statistically significant difference between progressive and non-progressive patients, progressive patients have a reduced survival. It is expected, after analysis of RNA extracted from lung tissue or bronchoalveolar lavage, to quantify the relevance of certain cytokines as predictive biomarkers for progressive fibrosing interstitial lung disease.

Contents

Chapter 1

Introduction

Interstitial lung disease is a term used to describe a heterogeneous set of lung disorders classified on the basis of shared clinical, radiographic, physiological or pathological factors [1].

1.1 Interstitial Lung Disease

The interstitial lung diseases constitute a heterogeneous group of diseases that causes an involvement of the lung parenchyma, of the alveoli, of the alveolar epithelium, of the capillary endothelium and the space between these structures, the interstitium; moreover, they are pathologies that also affect the perivascular tissues and lymphatics. In healthy lungs, the interstitium provides structural support to the alveolus and is only a few micrometres thick, thereby facilitating efficient gas exchange. Interstitial lung diseases are characterised by inflammation or fibrosis within the interstitial space; as a consequence we observe impaired gas exchange, thus giving rise to breathlessness and, in many cases, respiratory failure and death [2]. Patients with interstitial lung disease, or interstitial disease, often present to the doctor for the presence of progressive dyspnea on exertion or the presence of a persistent irritable cough; the patients may also complain of hemoptysis episodes, wheezing and chest pain. Findings of interstitial opacities on chest radiography also guides the diagnostic approach towards researching interstitial lung diseases.

1.1.1 Classification

The classification of this group of pathologies is complicated by the high number of entities grouped within it and the complexity of these clinical presentations.

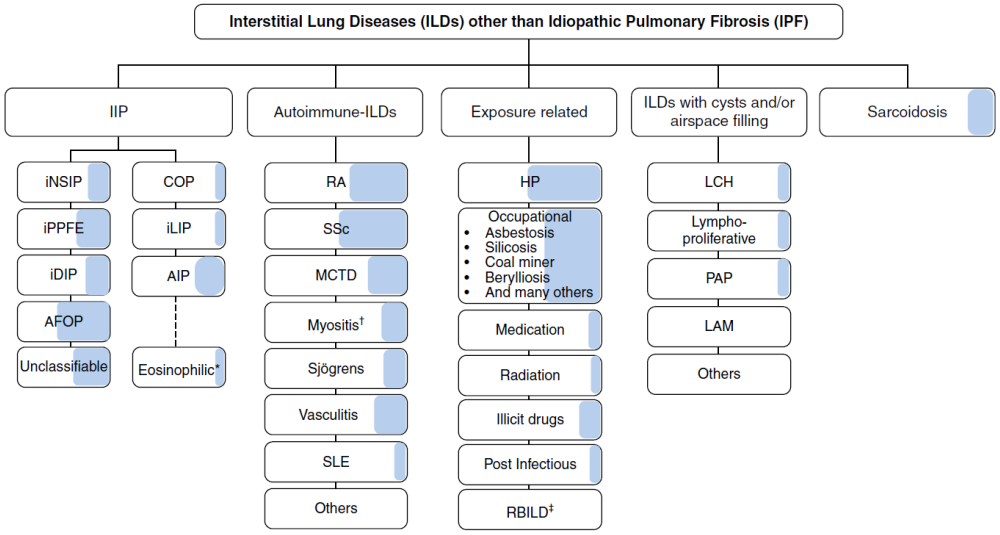

Figure 1.1: Classification [23]

The classification of the ILDs sees a first distinction in two macrogroups; ILDs of unknown cause and ILDs of known cause; there is also an intermediate group consisting of granulomatous lung diseases.

The macrogroup of ILDs of known cause includes: ILDs caused by exposure: the exposure can be occupational or environmentaland this group includes asbestosis and silicosis; there are also other important entities studied within this group of etiology called hypersensitivity pneumonitis linked with exposure to an extended array of inhalants, respiratory bronchiolitis interstitial lung diseases and desquamative interstitial pneumonia usually linked with exposure to smoking.

ILDs related to the agents that are administered to the patients can be related to chemotherapy, radiotherapy, and two drugs in particular: an antiarrhythmic called amiodarone and a chemotherapy drug called methotrexate. Always in the category of exposure linked ILDs, we find the lung defective process linked with inflammation after infectious various diseases.

The second macrogroup into which the ILDs are divided are the ILDs of unknown cause; the various entities that make up this group are grouped into two subgroups, the idiopathic interstitial pneumonia and others. The other entities includes different pathologies of relevance to the study carried out such as pleuroparenchymal fibroelastosis and Langherans cell histiocytosis.

In idiopathic interstitial pneumonias, the pathology with the most important epidemiological relevance is found within this group, and is idiopathic pulmonary fibrosis, which has been widely studied, but also a second group of pathologies defined as non-specific interstitial pneumonias. In non-specific interstitial pneumonia some pathologies whose importance should be emphasized are desquamative interstitial pneumonia, lymphocytic interstitial pneumonia and organizing pneumonia.
To complete the classification between diseases of unknown cause and diseases of known etiology there is a distinct group including sarcoidosis and this constitute the group of granulomatous lung disease.
A new group of interstitial diseases with aiutoimmune features is emerging. Following the discovery of interstitial disease, guidelines recommend that an evaluation be conducted to detect connective tissue disease [7]. This evaluation has produced a new subgroup of ILD patients with symptoms and autoantibodies indicative of an underlying autoimmune condition but who nevertheless do not meet the criteria defined by the American College of Rheumatology (ACR) for connective tissue disease.
In the past, these patients were thought to have idiopathic interstitial pneumonia secondary to undifferentiated connective tissue disease; in fact, it would be better to classify them as a distinct subgroup since the characteristics of this group differ from the pulmonary pathology usually seen in patients with connective tissue disease.
Therefore, this subgroup includes patients with ild who have a serologic positivity that reflects an autoimmune process.

1.1.2 Epidemiology

Many fibrosing lung diseases over time tend to become chronic and progressive.
However, while idiopathic pulmonary fibrosis has been well characterized through time and therapeutic options for it are now available, the epidemiology of other chronic fibrosing ILDs with a progressive phenotype has not been extensively studied. [15].
Incidence rates of interstitial lung disease is difficult to estimate.
Probably the data available to us today see an underestimation of cases linked with underdiagnosing.
Underestimated prevalence exists due to the lack of recognition of the disease because ILDs are pathologies with an extremely complex diagnosis, which require

highly specific study and most times invasive diagnosis.
In the United States the estimated incidence is 30 per 100'000 patients, every year. The overall prevalence is 80.9 per 100'000 patients per year in males and 67.2 per 100'000 per year in females [1].

1.1.3 History and physical

As already mentioned, the worsening dyspnoea and the pesky cough are the primary symptoms that bring patients to medical check-ups.
The symptoms then differ according to the specific underlying pathology, for example an insistent cough is often found in cases of bronchiolitis obliterans, while chest pain is more frequent in some types of sarcoidosis. Hemoptysis is a rare event that usually occurs in cases of acute alveolar damage. Many patients present with flu-like illness, including fever, chills, muscle or joint pain and important tiredness like in patients with hypersensitivity pneumonitis.
However, the patients could also present completely asymptomatic with impaired imaging [1].
The value of the anamnesis in the diagnosis of these pathologies is extremely high, occupational risk factors, exposure to smoke, radiotherapy, the list of current and past medications must be investigated, finally evaluate if there is a history of inhalations of pneumotoxic substances and dust.
It is necessary to evaluate whether the patient presents characteristic symptoms of rheumatic disease since many rheumatic pathologies cause pulmonary involvement.
Even the family history must always be investigated because for some pathologies genetics is important.
Physical examination in the early stages may be completely silent. In patients with advanced disease it is possible to find bibasal crackles on auscultation, or a marked digital clubbing may be observed, finally, signs correlated to the development of pulmonary hypertension may also occur.

1.1.4 Approach to the diagnosis of ILD

Diagnosing progressive fibrosing interstitial lung disease (PF-ILD) involves a comprehensive approach that combines clinical evaluation, radiological imaging, pulmonary function tests, and often, histopathological examination. The key components involved in diagnosing PF-ILD are:

1. Clinical Evaluation:
    - Medical History: The healthcare provider will gather detailed information about the patient's symptoms, duration, progression, exposure history, and any associated comorbidities.
    - Physical Examination: A thorough physical examination may help identify signs of respiratory distress, clubbing of the fingers, crackles on lung auscultation, or other relevant findings.
2. Radiological Imaging:
    - High-Resolution Computed Tomography (HRCT): HRCT plays a central role in evaluating PF-ILD. It helps visualize lung abnormalities, assess their distribution and patterns (e.g., UIP, NSIP), identify the extent of fibrosis, presence of honeycombing, and other characteristic features.
3. Pulmonary Function Tests (PFTs):
    - Spirometry: Measures lung function parameters, including forced vital capacity (FVC), forced expiratory volume in one second (FEV1), and the ratio of FEV1/FVC. It helps assess lung volumes and airway obstruction.
    - Diffusing Capacity: Measures the ability of the lungs to transfer gases, providing information about gas exchange and fibrotic involvement.
4. Laboratory Investigations:
    - Blood Tests: Routine blood tests are conducted to evaluate for underlying causes or associated conditions, such as autoimmune markers (e.g., antinuclear antibodies, rheumatoid factor), specific antibodies (e.g., anti-CCP), serum protein electrophoresis, and other relevant tests based on clinical suspicion.
5. Multidisciplinary Discussion:
    - Due to the complex nature of PF-ILD, a multidisciplinary team, including pulmonologists, radiologists, and pathologists, often reviews the patient's clinical, radiological, and histopathological information to reach a consensus on the diagnosis.

6. Histopathological Examination (when necessary):

    - In certain cases, a surgical lung biopsy or less invasive methods like transbronchial lung biopsy (TBLB) or cryobiopsy may be performed to obtain lung tissue samples for histopathological evaluation.
    - Histological examination helps differentiate PF-ILD from other lung diseases, identify specific patterns (e.g., UIP, NSIP) and assess the severity of fibrosis, presence of inflammation, or any concurrent pathological findings.

It's worth noting that the diagnosis of PF-ILD requires careful exclusion of other potential causes, consideration of the clinical context, and correlation with the radiological and pathological findings. It is essential to consult with a qualified healthcare professional who specializes in interstitial lung diseases for accurate diagnosis and appropriate management.

1.2 Interstitial lung disease

We choose to go on to describe the most frequent pathologies within the group we considered.
We will not discuss idiopathic pulmonary fibrosis, which while remaining the progenitor of the fibrosing disorders of interstitiopathies is not the subject of our interest.

1.2.1 Desquamative interstitial pneumonia

Desquamative interstitial pneumonia (DIP) is a rare disease included in the group of interstitial lung diseases.
The term "desquamative" dates back to the first observations made under the microscope of lung tissue from patients affected by the disease [26].
Infiltrates were present in the lung parenchyma of these patients, they were stemmed from alveolar epithelial cell desquamation.
Desquamative interstitial pneumonia is a pathology characterized by extensive alveolar infiltration of macrophages followed by some degree of inflammation and fibrosis.
90% of DIP cases are associated with cigarette smoking, but there are also cases where smoking is not the culprit and occupational risk factors or taking certain

medications come into play. Is most prevalent in the male sex between the ages of 40 and 60.
Histology is dominated by the accumulation of macrophages within the bronchial as a response to cigarette smoking subsequently an inflammatory reaction and development of fibrosis comes to develop [27].
Pigmented macrophages that are the hallmark of the disease have a bronchiolocentric distribution. The accumulation of macrophages in the DIP is uniform and this gives the histologic preparation a strongly eosinophilic appearance.
The macrophages within them contain a brown pigment, the alveolar septa are infiltrated by lymphocytes, sometimes eosinophils, and tertiary lymphoid structures may also form.
The architecture of the organ is fairly well preserved, but focal thickening of the interstitium is quite common, in advanced stages of the disease the proportion of fibrosis becomes substantial. Multinucleated giant cells are also reported.
The symptoms complained of most often by patients are cough with or without sputum, and dyspnea on exertion. There is clubbing in about half of the patients, and on auscultation, coarse ralesales can be appreciated.
On laboratory examination there is an elevation of neutrophils and eosinophils.
Respiratory function tests show a restrictive picture with impaired diffusing capacity as well.
Chest x-ray show a reticulonodular infiltrate, although it is not strongly disease specific. High resolution TC is gold standard imaging method, shows bilateral ground-glass opacities predominant in the lower lobes . In more advanced stages of disease, traction cysts and bronchiectasis may be observed,fibrosis is not so much present unlike other diseases.
It is a non-reversible pathology the greatest effects have been found with the use of corticosteroids and immunosuppressants, the use of which allows in most cases to keep the pathology stable for years. If the pathology is particularly advanced, one may consider nominating the patient for transplantation.

1.2.2 Organizing Pneumonia

Organizing pneumonia (OP) was formerly known as bronchiolitis obliterans. It is a widespread form of idiopathic interstitial lung pneumonia which is the result of the pulmonary reaction to various unidentified injurious agents [8].
Organizational pneumonia is believed to result from alveolar damage, which is followed by the formation of granulation tissue buds that obstruct the alveolar

lumen and bronchioles resulting in respiratory failure.

Typically, the diagnosis of organizing pneumonia is a diagnosis of exclusion. The exact etiology of pneumonia in organization is not known; it is believed to develop secondary to damage to the alveolar epithelium contributed by an unknown agent.

Several etiological agents have been suggested to be responsible for the development of organizing pneumonia including viral infections, toxicants, drugs, gastroesophageal reflux disease, radiotherapy, and connective tissue disease, smoking is not considered a risk factor.

Precise data on the incidence of organizing pneumonia is lacking, however, it is believed that there are 1-3 cases per 100'000 hospitalizations, both sexes are equally affected, and the disease is most common around the fifth-sixth decade of life.

The pathogenesis has not been fully elucidated; it is believed that following alveolar damage there is release of plasma proteins into the alveolar space and this causes recall of inflammatory cells [9]. The subsequent process of organization consists of three stages: there is a first stage called intra-alveolar which sees the formation of fibrin bands and an infiltrate of mononuclear inflammatory cells. The second stage is characterized by the proliferation of fibroblasts, and the third and final stage sees the organization of fibroblasts and connective tissue matrix. Endothelial growth factor VEGF plays a central role in pathogenesis, metal proteinases also appear to be upregulated.

The histopathologic hallmark is an excessive proliferation of granulation tissue within the alveolar sacs, which also comes to engage the bronchioles.

Plugs of granulation tissue are known as Masson's bodies. Granulation tissue has a uniform appearance, the structure of the lung is preserved. Mild infiltrates consisting of mononuclear cells and foamy macrophages can be observed at the level of the tissue surrounding the lesion.

Generally the condition involves subacute symptom development, the first flu-like symptoms preceding the definitive diagnosis by 2-3 months.

Patients typically present with fever cough and shortness of breath, cough may be more or less productive. Rarely there is weight loss, night sweats and chest pain.

A careful history is necessary for diagnosis, one must go to rule out the presence of connective tissue diseases that can often associate with polmonitis in organization,a list of medications taken by the patient should also be obtained.

Laboratory tests reveal marked neutrophilia and inflammation indices are also

typically high. Testing for the presence of an autoimmune disease should always be done when COP is suspected. Rx shows irregular opacities. HRCT reveals bilateral patchy consolidations present in the periphery, and may also show ground-glass opacities. The sign of "the atoll" can also sometimes be observed. It consists of a thick outer edge given by a consolidation surrounding an area of ground glass opacity. Spirometry shows a restrictive pattern.
As far as lung biopsies are concerned, the transbronchial biopsy can be attempted but generally it does not guarantee an adequate quantity of tissue for the diagnosis and therefore it is generally necessary to perform a surgical biopsy.
Most patients with symptoms and signs of progressive disease are treated with oral steroids with symptom relief. Glucocorticoid therapy is long, it can last from six months to a year. The initial dose is maintained for about two months and then slowly reduced. Relapses unless the dose is scaled can be frequent. Four different types of OP are known, fibrosing OP characterized by a variable amount of fibrosis, it is a rare entity, the disease does not respond to steroid therapy, and the prognosis is poor, secondary OP, it is pneumonia in organization that is associated with rheumatological disorders such as rheumatoid arthritis, acute fibrinous and organizing pneumonia it is a rare form of idiopathic origin that manifests itself acutely with respiratory insufficiency, it can also be secondary to the intake of certain drugs, pathologies of the connective tissue. Finally there is the acute fulminant form organizing pneumonia, where there is a rapid worsening of respiratory function with the development of hypoxemia, it is a form characterized by high mortality however it responds well to treatment with glucocorticoids.

1.2.3 Hypersensitivity Pneumonitis

Hypersensitivity pneumonitis (HP) is an immune-mediated disease that manifests as an ILD in the lungs. An unambiguous definition of hypersensitivity pneumonitis is lacking, and the treatment and clinical management of patients differs greatly among different countries [19].
Hypersensitivity pneumonitis is classified as one interstitial lung disease characterized by the development of a complex immune reaction that occurs in response to repeated inhalation of an allergen to which the patient is sensitized.
The severity in the manifestation of the pathology depends on the type of inhaled antigen and on the quantity. Hypersensitivity pneumonitis is divided into acute, or inflammatory HP, and chronic, or fibrotic HP [10].
There are numerous antigens at the basis of this immune response which occurs in

predisposed subjects and favors the development of the pathology. The responsible antigens can be roughly divided into categories such as animal proteins, plant proteins,fungi, low molecular weight chemicals, and metals. Exposure to these antigens occurs either for occupational reasons or based on the person's hobbies, however exposure can also occur within the patient's own home.
Only a small percentage of people exposed to these antigens subsequently develop the pathology, which suggests that the pathology is determined by environmental but also genetic factors. Genetic predisposition to the development of HP is due to certain polymorphisms of the major histocompatibility complex class 2. Smokers appear to have a reduced risk of developing HP, however in cases where the smoker develops hp it manifests with increased severity. The agents that most commonly give hp have a specific name in general, a few are reported:

- Farmer's lung: Seen in agricultural workers involved mostly in livestock farming.
- Bird or Pigeon fancier's lung: Caused by exposure to organic antigens in bird (particularly pigeon) excreta. Indirect exposure from feather bedding or down comforters have also been reported to cause disease.
- Hot tub lung: Lung parenchymal inflammation in response to Mycobacterium avium complex (MAC) in immunocompetent individuals.
- Cheese workers lung

Since there is a lack of defined diagnostic criteria the incidence and prevalence are poorly assessed. European registries report that hp covers 1.5 to 12% of ILD cases. Among the most affected workers are bird breeders and farmers. It is a disease that is more common in middle-aged men.
The inflammation of the lung parenchyma is due to a mixture of type 3 hypersensitivity, characterized by immune complexes which subsequently give way to type 4 hypersensitivity; how the fibrosing process evolves is not yet fully understood. Once sensitization occurs, the injurious agent triggers an immune complex-mediated hypersensitivity reaction, so in the acute form of HP, in the blood elevated levels of immunoglobulins can be detected. If exposure to the agent is prolonged over time, a delayed type IV hypersensitivity response occurs, during this response toxic CD8 T cells are activated that release chemokines. The chemokines attract and activate macrophages resulting in the formation of granulomas. In the transition from acute to subacute and finally chronic HP, lifocyte T action increasingly prevails.

The acute form of HP manifests with fever, cough that arise 1-2 days after exposure to the particular agent. Symptoms usually resolve 1-2 in one to two days. Patients also report shortness of breath, general malaise and weight loss.

It is important to take a 'careful history' to identify exposure to antigens that may have triggered the symptomatology, according to studies in 60% of cases the culprit exposure is identified. Objective examination may be normal in the acute form, however, crackles and squeaks may be heard. In chronic HP forms, the crackles are coarser and digital hippocratism may be present. Arriving at a correct diagnosis requires the combination of clinical and anamnestic data,the performance of HRCT, laboratory tests, and sometimes even the performance of lung biopsies.

Laboratory tests show higher than normal indices of inflammation,beyond that it is possible to go for specific precipitin panels specific for the antigen suspected to be responsible for the reaction, finding a positive however indicates that there has been exposure not that the antigen is the culprit.

At spirometry, a restrictive pattern is detected, with impairment of the parameter DLCO as well.

Chest x-ray does not always return a clear picture to help in diagnosis, sometimes opacities may be present, if the degree of fibrosis is advanced we might observe also on a reticular pattern. On CT scans, the hallmark finding is ground-glass opacities or bronchovascular nodules in the upper and middle lobes of the lung. There is also evidence of air entrapment best appreciated in expiratory scans. As the disease progresses and fibrosis increases, reticulation and traction bronchiectasis develop.

Histopathologically, in acute forms of HP, the inflammation is concentrated around the airways demonstrating the fact that the noxious agent reaches the parenchyma by inhalation. The distinctive finding consists of granulomas, not well formed, non-caseous, and an inflammatory infiltrate consisting of lymphocytes. If the pathology progresses and is not treated, fibrosis develops,in this case the characteristic malformed granulomas, the inflammatory infiltrate and a variable amount of fibrosis are observed around the bronchioles. In the chronic phase, fibrosis mainly affects the upper part of the lung where there is a loss of volume. Surgical biopsies are often needed to make a definitive diagnosis of chronic HP.

The cornerstone of the treatment of hypersensitivity pneumonitis is the identification of the responsible antigen and its eradication from the patient's environment. If the disease is diagnosed at an early stage, it is possible to have complete reso-

lution if the antigen is eliminated. Glucocorticoids exploited in the treatment of acute HP have been shown to be effective at this stage; in symptomatic patients with altered respiratory function tests, they are administered at high doses for 1-2 weeks and then scaled down the dose. In cases where there is resistance to steroid treatment in the face of progressive disease, azathioprine and mycophenolate mofetil are used. In cases of chronic fibrosing HP, the use of antifibrotic agents is suggested. Finally, in patients with advanced lung disease, lung transplantation for HP has been shown to have excellent survival.

1.2.4 Pleuroparenchymal Fibroelastosis

Pleuroparenchymal Fibroelastosis (PPFE) is an uncommon pulmonary disease with unique clinical, radiological and pathological characteristics [5], this is an extremely severe pathology detectable by high-resolution CT scan.
It is characterized by causing a loss of volume especially at the level of the upper lobes of the lung, the patient, in addition to complaining of dyspnea, undergoes a progressive loss of body mass.
The incidence of the disease is unknown certainly it appears to be underestimated. Although the etiopathogenesis of PPFE has not been clarified, studies suggest that acute or subacute damage to the lung could be at the basis of the development of the disease, this damage would lead to an exuberant inflammatory response which culminates in the development of the disease.
The term "pleuroparenchymal fibroelastosis" refers to an admixture of fibrosis departing from the visceral pleura associated with fibroelastosis predominantly involving the subpleural parenchyma.
Several possible triggers for the development of PPFE have been reported including bone marrow transplantation or lung transplantation, a history of chemotherapy or autoimmune and connective tissue diseases may also predispose to the development of PPFE.
Certain genetic mutations are detectable in individuals with PPFE, the genes are predominantly those responsible for telomere integrity and telomerase function. The presence of mutations at the level of these genes also results in clinically aggressive and progressive disease.
The most affected age is from 40 to 70 years, and are often female subjects. It is a frequent pathology in transplant recipients.
Symptoms complained of by patients at diagnosis are progressive dyspnea, cough, and a persistent pleuritic pain. There are no laboratory tests useful in defining the

diagnosis of PPFE; however, laboratory tests are useful in identifying autoimmune disease that might be associated with the development of PPFE. The hope for the future is to identify serologic markers that allow early diagnosis of the disease. A radiologically characteristic sign of PPFE is pleural thickening of the upper lobe, accompanied by subpleural fibrosis, if there is lower lobe involvement it is limited. Airway traction distortion is common, bronchiectasis formation and mosaic attenuation of the pulmonary parechyma are also often reported. For a histologic diagnosis is necessary to demonstrate the presence of intralveolar fibrosis and elastosis with associated fibrosis of the visceral pleura, features that are predominantly found in the upper lobes. At the interface between a portion of stabilized fibrosis and the lung parenchyma, foci of fibroblasts can be observed, inflammation if present is mild.
Progressive loss of lung volume causes a restrictive pattern to be observed on respiratory function tests, with decreases in forced vital capacity and total lung capacity. In patients with progressive disease, FVC may follow a trajectory of rapid decline.
Progressive forms generally have a life expectancy of around five years, slower progressing forms generally have an average survival of 10 years. Unfortunately, no treatment has been shown to be effective against PPFE. Prednisolone is empirically exploited for its immunomodulatory effects. Immunosuppressants are generally avoided because of the high infectious risk in these patients. In some cases, treatments with antifibrotic drugs have been tried with variable results.
Transplantation is an option but because of the nature of the disease, explantation of the native lung is extremely complicated and risky

1.2.5 Nonspecific Interstitial Pneumonia

Non-specific interstitial pneumonia (NSIP) is a distinct form of interstitial lung disease (ILD) characterized by inflammation and fibrosis within the lung tissue. It is considered a separate entity within the ILD spectrum and is recognized for its distinct clinical, radiological, and histopathological features [12].
NSIP typically presents with gradual onset and is commonly associated with symptoms such as progressive dyspnea (shortness of breath) on exertion, dry cough, and fatigue. It can affect individuals of any age, but it is most commonly diagnosed in middle-aged adults.
Radiologically, NSIP is often characterized by bilateral and symmetric lung involvement with ground-glass opacities and reticular (linear) patterns seen on

high-resolution computed tomography (HRCT) scans. Unlike some other forms of ILD, NSIP typically lacks specific radiological features such as honeycombing or traction bronchiectasis.

Histopathologically, NSIP is defined by a pattern of inflammation and fibrosis within the lung tissue. It is characterized by varying degrees of inflammation in the interstitium (the space between the air sacs) and a pattern of fibrosis that tends to be more uniform and less patchy compared to other types of ILD such as usual interstitial pneumonia (UIP).

NSIP is further classified into two subtypes: cellular NSIP and fibrotic NSIP. Cellular NSIP is characterized by increased inflammation within the lung tissue, while fibrotic NSIP demonstrates more extensive fibrosis. These subtypes have different prognostic implications, with cellular NSIP generally associated with a better prognosis compared to fibrotic NSIP [11].

The exact cause of NSIP is not fully understood, and it can occur idiopathically (with no known cause) or be associated with certain underlying conditions such as connective tissue diseases (e.g., rheumatoid arthritis, systemic sclerosis), exposure to certain environmental agents (e.g., occupational exposures), or drug-induced reactions. In some cases, NSIP may occur in a familial or genetic context, but this is relatively rare.

The management of NSIP involves a multidisciplinary approach, including pulmonologists, rheumatologists (if an underlying connective tissue disease is present), pathologists and radiologists. Treatment aims to reduce inflammation and prevent further fibrosis. Immunosuppressive medications, such as corticosteroids or other immunomodulatory drugs, are commonly used as the mainstay of therapy. The response to treatment can vary among individuals, and some cases may be refractory or have a more progressive course.

In summary, NSIP is a distinct form of interstitial lung disease characterized by inflammation and fibrosis in the lung tissue. It presents with gradual onset and commonly manifests as progressive dyspnea and dry cough. NSIP has specific radiological and histopathological features, with bilateral and symmetric lung involvement on HRCT and a pattern of inflammation and fibrosis on lung biopsy. Treatment involves a multidisciplinary approach and often includes immunosuppressive medications.

1.2.6 Unclassifiable Interstitial Lung Disease

Indeterminate interstitial lung disease refers to a category of lung diseases that present with characteristics and features that do not fit into a specific diagnosis within the existing classification schemes of ILDs.
It is a term used to describe cases where the clinical, radiological, and histopathological findings do not align with any well-defined ILD subtype.
When evaluating patients with ILD, physicians often strive to make a specific diagnosis based on the clinical presentation, radiological findings and histopathological examination if a biopsy is performed. However, in some cases, despite a thorough evaluation, the characteristics observed may not match the established criteria for a specific ILD subtype. This situation leads to the diagnosis of "indeterminate ILD." Indeterminate ILD poses a diagnostic challenge, as accurate classification is crucial for appropriate management and treatment decisions [13]. In such cases, further investigations and evaluations may be necessary to narrow down the possibilities and reach a more specific diagnosis.
These additional assessments may include clinical evaluation, laboratory tests, new bronchoscopy, lung biopsy, and mostly multidisciplinary discussions involving pulmonologists, radiologists, and pathologists. It is important to note that the term "indeterminate ILD" is not a specific diagnosis but rather a descriptive term used to acknowledge the diagnostic uncertainty and complexity of the case. It indicates the need for further evaluation and monitoring to potentially identify distinct features or patterns that may eventually lead to a more definitive diagnosis.
The management of indeterminate ILD involves a comprehensive approach that focuses on monitoring disease progression, symptom management, and supportive care. Close follow-up with periodic clinical assessments and imaging studies is crucial to track any changes or progression of the disease. Additionally, treating comorbid conditions, optimizing lung function, and providing symptomatic relief are important aspects of managing patients with indeterminate ILD.
Research efforts are ongoing to better understand the nature and underlying mechanisms of indeterminate ILD. These efforts aim to refine diagnostic criteria, identify novel biomarkers, and develop more targeted treatment strategies for this challenging subset of ILD.
In summary, indeterminate ILD refers to cases where the clinical, radiological, and histopathological features do not align with any specific ILD subtype. It represents a diagnostic challenge and often requires further evaluation and mon-

itoring. Management focuses on supportive care, symptom management, and regular follow-up. Ongoing research aims to improve diagnostic accuracy.

1.3 Guidelines

Although, as can be understood from the classification just analyzed, we are faced with numerous different pathologies, the morphological similarities found, the common physiopathological mechanisms underlying the development of these diseases and the fact that they are diseases that cause a progressive deterioration of lung function in affected patients "support the concept of a progressive fibrosing PF-ILD phenotype that can be applied to a variety of ILD subtypes" [24]. ILDs are a heterogeneous group of pathologies characterized by the development of an inflammatory and fibrotic process affecting the lung parenchyma.
However, within this large group there are some diseases in which the fibrotic component is predominant over the inflammatory component (an example is idiopathic pulmonary fibrosis where fibrosis outweighs the infimmatory component). The not-IPF ILD, on the other hand, are characterized by having a particularly marked inflammatory component which exceeds the fibrosing component. Damages of various kinds of known and unknown aetiology determine the activation of inflammatory pathways at the level of the lung parenchyma of these patients. The activation of these inflammatory pathways determines the activation of fibroblasts with their consequent differentiation into myofibroblasts. Despite important differences, distinct ILD subtypes often have overlapping morphological features and common pathological mechanisms, leading to the concept of a progressive fibrosing phenotype that can be applied to a variety of fibrotic ILDs.
Recent studies support this hypothesis, also suggesting that there are shared biological mechanisms in these pathologies and this in turn implies that new treatment options are available compared to the historical approach to ILDs [24]. Based on the concept of the existence of a "common fibrosing phenotype", in 2022 the new guidelines relating to the diagnosis and treatment of idiopathic pulmonary fibrosis (IPF) were published. Furthermore, in this document, the concept of Progressive Pulmonary Fibrosis (PPF) was also introduced in patients affected by fibrosing interstitial lung diseases different from IPF. According to these new guidelines [23], 2 of the three criteria listed below are required to make a diagnosis of progressive fibrosing pathology, criteria that occurred in the last year without another alternative explanation.

The criteria are:

- Worsening respiratory symptoms
- Physiological evidence of disease progression (either of the following):
    1. Absolute decline in FVC $> 5\%$ predicted within 1 yr of follow-up
    2. Absolute decline in DLCO (corrected for Hb) $> 10\%$ predicted within 1 yr of follow-up
- Radiological evidence of disease progression (one or more of the following):
    1. Increased extent or severity of traction bronchiectasis and bronchiolectasis
    2. New ground-glass opacity with traction bronchiectasis
    3. New fine reticulation
    4. Increased extent or increased coarseness of reticular abnormality
    5. New or increased honeycombing
    6. Increased lobar volume loss

However, the use of the DLCO parameter as a criterion for defining progression is still the subject of much controversy, since it is a parameter that can be influenced by pulmonary hypertension, the presence of emphysema, tecnique and machine differences of measurement, so it is generally defined as a criterion for progression if it is associated with a decline in FVC or in association with radiological worsening.
Another novelty present in the 2022 guidelines concerns the treatment of progressive pulmonary fibrosis for which the prescription of antifibrosant therapy is recommended, even if not mandatory.The recommendation on the use of antifibrotics derives from the observation that even ILDs other than IPF can show a progressive fibrosis phenotype.
The introduction of treatment with Nintedanib has shown positive results and also from this it can be concluded that there are shared mechanisms at the basis of the pathogenesis of the various ILDs.

1.4 Risk Factors

Risk factors have been identified for some forms of ILD. To fully understand the pathogenesis of the development of occupational interstitial diseases, it is

necessary to ask what are the possible risk factors to be called into play in the development of these diseases [16]. Certainly the risk factors are both related to the genetics of the patient but also to the environment in which the patient lives. Starting with considering cigarette smoking, which is one of the major risk factors for the development of ILD. Cigarette smoking has been associated with an increased risk of developing lung cancer, development of chronic obstructive bronchitis, and idiopathic pulmonary fibrosis, in addition to which there is a whole group of ILD, defined as smoking-related interstitial lung disease (Pulmonary Langerhans cell histiocytosis (PLCH), Respiratory bronchiolitis associated interstitial lung disease (RB-ILD), Desquamative interstitial pneumonia (DIP), the pathogenesis of which is closely related to cigarette smoking [17].
Other recognized risk factors for the development of ILD are the presence of a positive history of tuberculosis, hepatitis, a positive history of recurrent pneumonia. Male sex and advanced age have also been shown to be two risk factors for the development of ILDs. Another interesting finding in the literature is the association between the development of ILDs and the comorbidities that afflict these patients. Comorbidities most frequently associated with the development of ILD are metabolic ones; in fact, many individuals have diabetes. Although reflux is an established risk factor for idiopathic interstitial fibrosis, it appears not to be too closely associated with ILD.
Going to consider interstitial lung disease a risk factor is also exposure to metal dusts that frequently promote the development of disease.

1.5 Pathogenesis of Interstitial Lung Disease

Interstitial lung diseases are characterized by having different degrees of inflammation and fibrosis of the pulmonary interstitium. The cells responsible for the fibrotic process affecting the lung parenchyma are fibroblasts and T lymphocytes. The interrelationships between stromal cells and immunity cells are mainly responsible for the pathogenesis of ILDs. Fibroblast are mesenchymal cells residing in all tissue types. The primary function of fibroblasts is the maintenance of the structural integrity of the connective tissues.

The fibroblasts contribute to the process through the secretion of extracellular matrix and thanks to the production of proinflammatory cytokines, the T-cells instead are responsible for the adaptive immunity and also for the destruction processes affecting the lung tissue.

Fibroblasts also play an essential role in the modulation of lymphocyte recruitment, in their differentiation and function, vice versa the T lymphocytes are able to balance the fibrotic sequelae [3].

Some studies have recognized fibroblasts as a not-classical branch of innate immunity. The chronic inflammation present in the lungs of ILD patients appears to be caused by the disordered behavior of fibroblastic cells; the fibroblasts lose the signal to switch off their inflammatory program and this leads to an aberrant survival of the lymphocytes within the inflamed tissue.

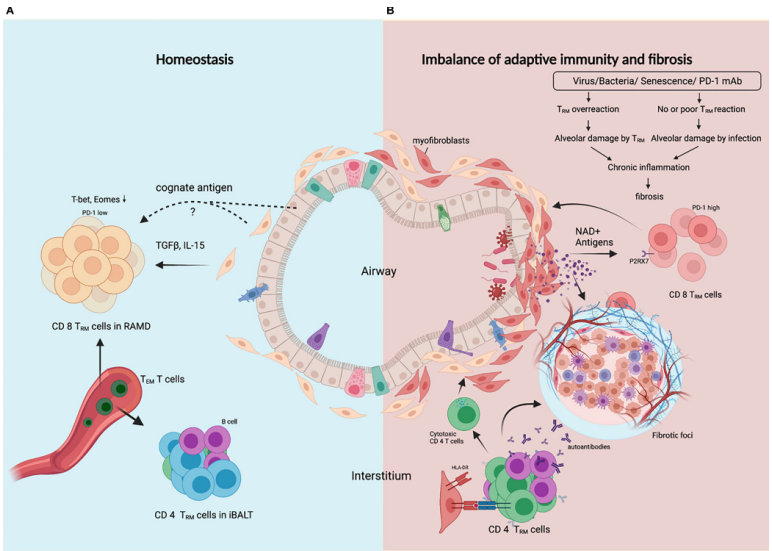

Figure 1.2: Pathogenesis [3]

Consequently the fibroblasts can be considered by the important sentinel cells capable of decreeing the passage from the acute inflammatory phase to the phase

of adaptive immunity, moreover they participate in the scar repair of the tissues. In addition to the action of the fibroblasts, the response of the T lymphocytes must also be considered.

Abnormalities in the behavior of lymphocytes can lead to processes of destruction and repair of the alveolar epithelium, if these processes are repeated over time there is an irreversible loss of functional lung tissue for gas exchange.

A characteristic factor in the histology of fibrosing ILDs is the aberrant accumulation of fibroblasts (fibroblastic foci) activated to give myofibroblasts, the production of extracellular matrix by myofibroblasts without its correct elimination over time leads to organ fibrosis.

The origin of the fibroblasts is varied, the main culprits are the resident fibroblasts, but the same cells of the alveolar epithelium if subjected for a long enough period to the action of the cytokine TGF-beta, can undergo metaplasia and give new fibroblasts.

Finally to summarize, from the macroscopic point of view the action of fibroblasts activated with the deposition of extracellular matrix determines the generation of fibrosis, fibrosis makes the organ more rigid, and less functional in gaseous exchanges In addition to the fibroblastic foci which, as mentioned, represent a characteristic sign of progressive fibrosing ILDs, another distinctive element of the histology of ILDs is the presence of tertiary lymphoid structures.

Tertiary lymphoid structures are often found within an infected tissue, near a tumor lesion and in inflamed tissue as is the same lung tissue in ILDs. Activated fibroblasts play a role in the organization of these tertiary lymphoid structures, fibroblasts subjected to the action of TNFα acquire an activated phenotype and become an organizing cell of the lymphoid tissue. A transient inflammation is not sufficient for the fibroblast to assume the role of organizing cells of the lymphoid tissue, in fact when the inflammation stops, even the activated fibroblasts undergo apoptosis. However, if the antigen that triggered the inflammation remains or if the inflammation becomes chronic then it is possible that lymphoid tissue-like mesenchyme will form.

Remember that underlying the processes just analyzed there is the action of numerous inflammatory cytokines, the action of TNFα has been mentioned, but underlying the formation of Bronchus-Associated Lymphoid Tissue there is the action of cytokines such as IL-17 , IL-13.

1.6 Pulmonary Function Testing

Detecting a restriction following a pulmonary function test may be a sign of the presence of interstitial lung disease. However, especially in the early stages of the disease, this element may not be present.
Spirometry is a physiological test that allows you to evaluate the volumes of air inhaled and exhaled over time. Spirometry combined with DLCO assessment are the two most widely used functional tests in the investigation of lung disease [4]. It is an extremely useful test both in the diagnosis of respiratory pathologies and during follow-up.
The execution of spirometry allows the pulmonary pathologies to be divided into two macrogroups: obstructive pathologies (COPD, asthma) and restrictive pathologies,there is also a group of pathologies with mixed patterns.
The measurement of lung volumes has several important variables starting from considering the TLC or the total lung capacity it represents the volume of air present in the lungs at the end of a maximal inspiration. Total lung capacity is considered the gold standard for the diagnosis of restrictive disease, a value lower than $< 80\%$ is in fact indicative of a possible restrictive disease affecting the parenchyma. FEV1 instead represents the volume of air exhaled in the first second after completing a maximal inspiration, FVC is an extremely important volume to be evaluated in spirometry, it is used to quantify the severity of the pathology, and it also has value in the follow-up.
FVC is an acronym that stands for forced vital capacity, it measures the volume of air that can be exhaled with maximal effort after the patient has inspired.
Another variable of spirometry always to be evaluated because it helps to distinguish between an obstructive and restrictive pathology is the tiffenau index or the ratio between FEV1 and FVC the forced vital capacity, its value is evaluated as a percentage on the theoretical expressed if the value is lower at $< 70\%$ we are faced with an obstructive deficit.
Diffusion is a parameter that evaluates the functionality of the capillary-alveolar membrane through which gaseous exchanges take place.
Diffusion assessed through in parameter DLCO is a measure that can quantify the ability to transfer gases in inhaled air to the bloodstream. Carbon monoxide is used to evaluate diffusion. CO is the preferred gas for performing the test given its affinity for hemoglobin the diffusion test consists of a ten-second apnea during which CO absorption per unit time is measured. Various factors influence the DLCO parameter, among them we can mention the ventilation, the gas mixture

used, the volume of gas inhaled and finally the intrinsic characteristics of the capillary alveolar membrane. The membrane is composed of three elements the alveolar epithelium, the interstitium and the endothelium. According to Fick's equation for the diffusion of gas:

$$V_g = \frac{K \cdot A \cdot \Delta P}{T} \tag{1.1}$$

- V_g = volume of gas transferred per unit time
- K = diffusion coefficient of the gas
- A = surface area for gas exchange
- ΔP = partial pressure difference of gas
- T = membrane thickness

It is clear from the formula that the factors influencing gas diffusion are the area of the capillary alveolus membrane, the thickness of the membrane, and the pressure gradient from one side of the membrane to the other; pathological processes that lead to a change in even one of these factors will result in a change in the DLCO parameter.
The DLCO paremeter value is always interpreted together with the other spirometry values. A high DLCO is associated with asthma, obesity or intraparenchymal hemorrhage.
Low DLCO values in a setting in which the other spirometry values are normal may be an indication of an early stage interstitial disease, or diseases of the pulmonary vessels.
We saw earlier that pulmonary pathologies can be divided into obstructive, restrictive or mixed.
As far as obstructive pathologies are concerned, they are those pathologies in which there is a disproportionate decrease in the maximum airflow from the lung expressed through the FEV1 parameter with respect to the forced vital force FVC.
In practical terms, a FEV1/FVC ratio of less than 0.70 is an index of obstructive pathology. Also the spirogram reflects in its shape an obstructive pathology; the shape qualitatively presents itself with a concave shape at the level of the flow-volume curve. The FEV11 value is used to quantify the severity of the disease.
The restrictive defects instead focus on our interest are characterized by having a

normal FEV1/FVC ratio in spite of a reduced TLC value below the fifth percentile or below the $< 80\%$ value predicted on the basis of the physical characteristics of the subject . A restrictive defect can also be suspected in the presence of a higher FEV1/FVC ratio approached to a lower FVC value of $< 80\%$. However, a standard spirometry can only give rise to the suspicion of a restrictive pathology, the suspicion must be confirmed with the execution of a global spirometry with which it is also possible to investigate the TLC value which is the gold standard in the diagnosis of the restrictive pathology.

1.7 Radiological Diagnosis

The pulmonary interstitium in physiological conditions is a virtual space between the walls of the alveoli, the spaces around the blood vessels and the small airways. When an interstitial lung disease is established, the interstitium loses its physiological architecture, and such pathological changes can be visualized by radiological techniques.

The use of high-resolution computed tomography has significantly increased the ability to discriminate between the various interstitial lung diseases, so much so that it has become the gold standard imaging technique used in the diagnosis and follow-up of these patients.

In clinical practice following the suspicion of interstitial lung pathology deriving from the characteristic symptoms complained of by the patient such as for example irritating cough, worsening dyspnea, the clinician prescribes the execution of HRCT.

The three main signs of pulmonary fibrosis that we can see on CT are: honeycomb cyst, traction bronchiectasis, and volume loss [25]. Characteristic honeycombing of the patterned UIP, it is defined on HRCT by the presence of clustered cystic spaces, with thick walls, with a homogeneous diameter usually between 3 and 10 mm, although it can also reach up to 25 mm.

Cyst distribution typically is subpleural, basal, and peripheral. The cysts can be on a single layer or be stratified. These characteristic distribution of the cysts has been shown to have a positive predictive value of 90-100% for the histological UIP pattern.

Traction bronchiectasis and bronchiectasis are defined as an irregular bronchial and bronchial dilatation caused by fibrosis of the surrounding tissue.

Signs of pulmonary fibrosis must be visible to distinguish traction bronchiectasis

from other bronchiectasis. Background fibrosis around the dilated bronchi on CT is visualized as reticulations and groundglass areas.
Traction bronchiectasis are generally more present in the pulmonary periphery, where the bronchi have a lower cartilaginous component and are therefore more prone to undergo traction, exerted by the fibrous tissue, and to distortion.
Several studies analyzing prognostic markers for lung disease have revealed that the presence and severity of traction bronchiectasis are a predictive marker of poor outcome of the disease.
Volume loss is the least specific of the signs indicating pulmonary fibrosis on CT, however in some cases it can be extremely helpful in diagnosing pulmonary fibrosis, especially when reticulations and bronchiectasis are equivocal. A loss of volume in the lower and upper lobes can be identified by the no longer physiological position of the pulmonary fissures.
Ancillary signs of pulmonary fibrosis may be joints, small linear opacities due to thickening of the interlobular septa, and ground-glass opacities which, although they may also be found in other conditions, are often associated with fibrosis.
Frosted glass opacification is indicative of very fine fibrosis. In general, after performing HRCT, 5 possible radiological patterns of the disease are defined: two granular forms, hypersensitivity pneumonitis and sarcoidosis, and three idiopathic pneumonias, usual interstitial pneumonia, non-specific interstitial pneumonia and the fibrosing variant of organizing pneumonia. The information obtained with the CT can be further refined on the basis of the clinic and histology. The importance of identifying the UIP pattern lies in the fact that it is a pattern that is generally associated with Idiopathic pulmonary fibrosis, IPF among interstitial pneumonias is the most common form and also the one with the worst prognosis.

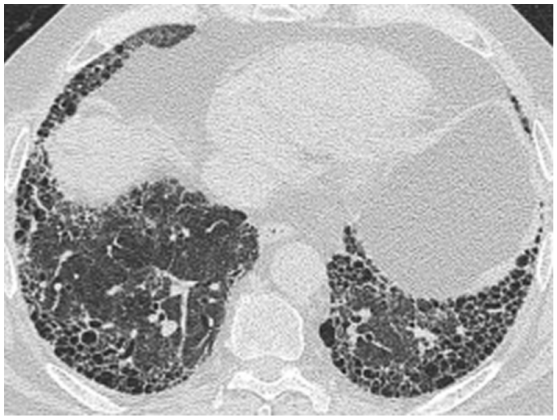

Figure 1.3: UIP pattern [25]

The American Thoracic Society and the European Respiratory Society agree in

stating that in order to make a diagnosis of the UIP pattern it is necessary to highlight the subpleural, basal level, the presence of reticulations, traction bronchiectasis and the presence of honeycombing cysts, the importance of the latter has been increasingly emphasized over time. As regards the pattern of non-specific interstitial pneumonia, it is characterized on HRCT to show the presence of ground-glass opacities that are higher in size than the crosslinks, furthermore honeycombing is minimal or absent in these forms.

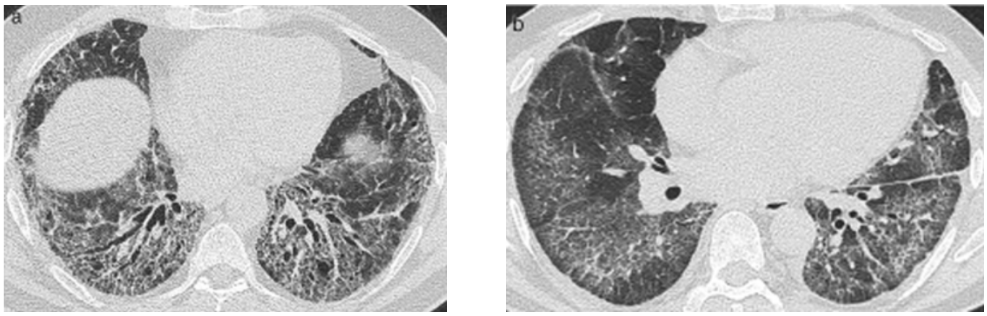

Figure 1.4: NSIP pattern [25]

There are cases in which it is difficult to distinguish between an NSIP pattern and a non-classical UIP form which may be a sign of IPF. In these cases it is useful for the diagnosis to be based on the age of the patient; IPF generally occurs in the elderly population. Organizing pneumonia usually manifests itself as a cluster. Opacities on CT are observed in the perilobular site, while consolidations are typically distributed in the peribronchovascular site, rarely it can also be present as an atoll sign. Hypersensitivity pneumonitis is another pattern observable, in its chronic form is manifested on CT by the presence of fibrosis and some other accessory signs. Fibrosis can concentrate mainly at the level of the upper or lower lobes or it can have a random distribution without favoring a specific area of the lung, therefore when the CT shows signs of diffuse, non-zonal fibrosis, the diagnostic hypotheses must be taken into account HP consideration.

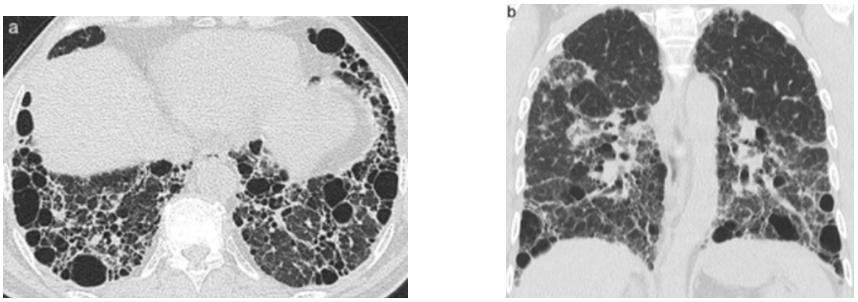

Figure 1.5: HP pattern [25]

In some cases where the fibrotic distribution is basal it may be difficult to distinguish a uip pattern from a hp pattern in these cases it is useful to evaluate the presence or absence of air trapping. Air trapping is a hallmark of hp due to the obliteration of the alveoli by granulation tissue. Finally, the last of the five possible patterns visible on CT, fibrosing sarcoidosis, is treated. Sarcoidosis in its fibrotic form appears as a fibrosis that radiates from the pulmonary hilum and then affects the posterior segments of the upper lobes.
In some forms of fibrosing sarcoidosis it is also possible to observe honeycomb cysts that have a random distribution with respect to the UIP pattern where they are predominantly basal, these honeycomb cysts can mix with emphysematoid bullae.

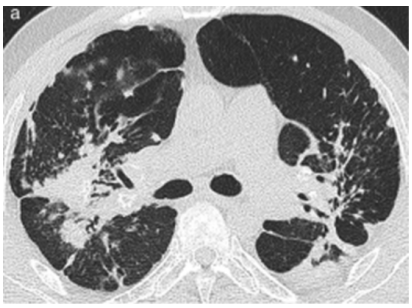

Figure 1.6: Sarcoidosis [25]

In addition to the five main patterns previously analysed, it is worth considering desquamative interstitial pneumonia DIP, which in addition to being a histological pattern has also been recognized as a pattern by HRCT, in DIP cases CT shows subpleural or basal ground-glass opacities that are multifocal or diffuse.

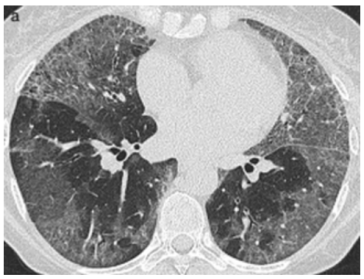

Figure 1.7: DIP pattern [25]

Despite the great advances made in the nosological field, studies show that approximately 10% of ILD cases remain unclassifiable after the collection of clinical radiological, probably this percentage is underestimated, and pathological data.
The percentage of cases that remain unclassified increases if we consider the population of end stage patients since the lung parenchyma is so compromised

that it is difficult to identify the initial disease pattern,in addition to this, it should be considered that very often patients with severe disease that compromises a large part of the parenchyma are not subjected to surgical biopsy due to the high risks it entails. The problem remains of the treatment and clinical management of these unclassifiable forms for which progression after one year is considered in order to remedy the lack of a diagnosis.

1.8 Bronchoalveolar lavage

Bronchoalveolar lavage (BAL) is a non-invasive procedure performed with a fiberoptic bronchoscope [28]. The bronchoalveolar lavage technique consists of instilling prewarmed saline solution at the level of the bronchus that is the object of our interest, and then proceeding to aspirate the fluid that will be subsequently analyzed.

Analysis of the cells and solutes recovered from the lower respiratory tract provides useful information for diagnosis and helps identify any inflammatory, infectious, and immunologic processes that are occurring at the alveolar level. Cytological analysis of BAL fluid is a routinely used method in the definition of lung diseases, particularly in the large group of interstitial lung diseases.

BAL alone, however, is not sufficient to discriminate which interstitial disease the patient has, so it is evaluated within a multidisciplinary team along with clinical, radiological, and sometimes pathologic data.

One of the reasons why the use of BAL is an unspecific technique in the diagnosis of interstitial lung disease is that the cellular patterns returned by BAL fluid analysis are limited while interstitial lung disease is extremely numerous, and therefore the data obtained are subject to great variability [29].

Therefore only in rare cases does the BAL lead to an unambiguous conclusion, most often the BAL goes to define as more likely some diagnoses instead of others. The above implies that to date the value of BAL in the diagnosis of ILD is still controversial.

1.9 Biopsy

Lung biopsy is a widely accepted and extremely useful technique for diagnosing lung disorders, the diagnosis is made by obtaining lung tissue samples using various techniques.

Lung biopsy, in addition to being indispensable for assessing the nature of lung nodules detected at CT, is also necessary in the diagnosis of pulmonary interstitial pathologies [6]. A definite diagnosis is also the first step towards the most appropriate treatment of these pathologies. Lung tissue samples can be obtained by various methods:

- Percutaneous Transthoracic Lung Biopsy: using CT guidance, a biopsy needle is inserted into the chest wall in the suspected area to obtain a tissue sample
- Open Lung Biopsy: a procedure carried out under general anaesthesia, consisting of removing a piece of lung tissue that will be analysed histologically and microbiologically. Surgical biopsy is the currently recommended standard for obtaining specimens necessary for histologic diagnosis of ILD; however, it is fraught with risks for patients with interstitial lung disease, so it is always a good idea to balance the risks and benefits before proposing it [18].
- Video-Assisted Thoracic Surgery (VATS): this is an endoscopic removal of lung tissue
- Transbronchial Biopsy: access in this case is transnasal or transoral through a flexible bronchoscope, and is the preferred technique for central lesions
- Cryobiopsy: consists of freezing and then removal using nitrous oxide.

New lesions of the lung parenchyma detected by high-resolution CT scan are assessed by a multidisciplinary team. A lung biopsy is necessary if the diagnosis leads to a change in the clinical management and treatment of the patient.
The indications for performing a biopsy are the detection of multiple nodules in patients without a history of malignant disease, solitary nodules found on X-ray and which cannot be diagnosed by CT scan or other less invasive techniques. A biopsy is also performed in the case of a hilar mass and if there are persistent focal infiltrates which have not been diagnosed by sputum, haemoculture or serology. Previous pneumonectomies, suspicion of a lesion with a vascular character, or the need for mechanical ventilation are three of the possible contraindications to performing a biopsy. Other possible contraindications are poor pulmonary function and coagulation abnormalities. Contraindications to obtaining samples by transbronchial biopsy are haemorrhagic diathesis, hypoxaemia, thrombocytopenia, uraemia and pulmonary hypertension.

VATS are contraindicated not only in cases of poor general condition, but also in cases where the patient is unable to withstand single lung ventilation.

Transbronchial biopsy remains a key technique in the evaluation of lung lesions. The technique after anaesthesia involves introducing a flexible bronchoscope either via the transnasal or transoral route. A deep inspection of the various lung segments is carried out and then the bronchoscope is introduced at the level of the bronchus which is directed towards the area affected by the lesion, previously visualised by CT scan, after having wedged the bronchoscope at the level of the bronchus of interest a forceps is introduced through the operative channel of the bronchoscope and slid until it meets some resistance. The forceps are then retracted and slightly opened and then sunk into the tissue until resistance can be felt again, the jaws close and the histopathological piece is retracted and placed in formalin for its subsequent analysis. Usually 4 to 6 samples can be obtained.

One of the limitations of transbronchial biopsy is the small amount of material obtained. As far as VATS are concerned, after anaesthesia is performed the patient is intubated with a double-lumen tube. The classic procedure involves three openings.

Complications that may be associated with performing the biopsy are haemoptysis, massive haemorrhage and gas embolism.

Complications that relate more specifically to transbronchial biopsy are allergic reactions to the anasthetic, laryngospasm, bronchospasm that can also be very serious, haemorrhage or pneumothorax can also occur. VATS has a higher safety profile than the open surgical technique, the problem of postoperative pain, the development of hypoxaemia or atelectasis, possible bleeding and wound infection remain.

The need for lung biopsy must be determined by a multidisciplinary team involving at least a pulmonologist, a radiologist and pathologist. In cases of interstitial disease, lung biopsy should be required if a clear uip pattern is not visible on CT.

Biopsy is also indicated in all patients who have a progressive course of disease, with atypical CT findings, with extrapulmonary pathology.

Transbronchial biopsy is a fairly safe technique, with low mortality rates compared to surgical biopsy, the technique is used for sampling of central lesions. To conclude, interventional pulmonology will increasingly equip itself with new safer techniques that allow for early diagnosis. Long there have been and still continue to be questions about the value of performing a biopsy [22]; unfortunately, there have been no robust studies on the change in therapy following the performance

of lung biopsy in the post anti-fibrosing therapy era.
In addition to the role that histologic diagnosis may play in the management of the patient's treatment, there is also the role that biopsy has in defining the patient's prognosis to consider, e.g., the finding of a UIP pattern suggests progressive disease, and these are then patients who benefit from follow-up and initiation of treatment.
Performing the biopsy from the perspective of drug treatment changes in only one-third of cases, but can still lead to other treatment recommendations such as finding a new antigen responsible for the disease or increased antigen remediation efforts of the patient's environment. These exhibits are sufficient to convince the physician of the usefulness of performing biopsy in doubtful cases.

1.10 Histological Diagnosis

Histologic classification plays a central role in the management of interstitial lung disease; it goes to separate the multiple forms of interstitial pneumonia into clinically relevant classes.
Some of the microscopic criteria that are studied to classify these entities are inflammation distribution, collagen distribution, and remodeling of organ architecture [20].
A certain diagnosis is the first fundamental step towards an effective treatment of the pathology and although high resolution CT has allowed great strides in the diagnosis of pathologies of the pulmonary interstitium, in cases of doubtful diagnosis, lung biopsy is the gold standard for arrive at a correct diagnosis.
As far as the techniques used are concerned, the transbronchial biopsy is widely exploited however with this technique it is not possible to collect sufficient material to arrive at a diagnosis of certainty, however it remains an excellent technique which allows to make a diagnosis with precision in the case of widespread pathologies of the lung parenchyma or in the case of pathologies affecting the peribronchial area such as, for example, organizing pneumonia.
The technique instead turns out to be not optimal in the diagnosis of pathologies such as desquamative interstitial pneumonia (DIP) and nonspecific interstitial pneumonitis (NSIP).
In these cases a diagnosis is reached by surgical biopsy, which although involves greater risks, it has in fact been observed in patients suffering from idiopathic pulmonary fibrosis an increase in mortality following the execution of the procedure,

it guarantees the recovery of a quantity of material suitable for the histological study necessary for the identification of the pathology.
A histopathological classification plays a key role in identifying and knowing various macro-categories of different pathologies for prognosis, treatment and natural history.
The histological components that are of interest in defining interstitial lung disease are the pattern and microanatomic distribution of inflammation, fibroblast proliferation, collagen deposition, and architectural remodeling.
The classification includes 7 histological patterns:Usual interstitial pneumonia which corresponds to a clinical diagnosis of idiopathic pulmonary fibrosis, Desquamative interstitial pneumonia (DIP),Respiratory bronchiolitis interstitial lung disease (RBILD),Diffuse alveolar damage (DAD) which corresponds to a clinical diagnosis of Acute interstitial pneumonia, Nonspecific interstitial pneumonia (NSIP), Organizing pneumonia (OP) and Lymphoid interstitial pneumonia (LIP).

1.10.1 Usual Interstitial Pneumonia

For the UIP pattern the main distinguishing feature and a main criterion of diagnosis is the finding of a heterogeneous picture, there are areas of diseased tissue mixed with areas of tissue with a preserved structure. areas affected by low-growing disease encompass inflammatory, fibrous, and honecombing elements, resulting in a characteristic "pacthwork" appearance.

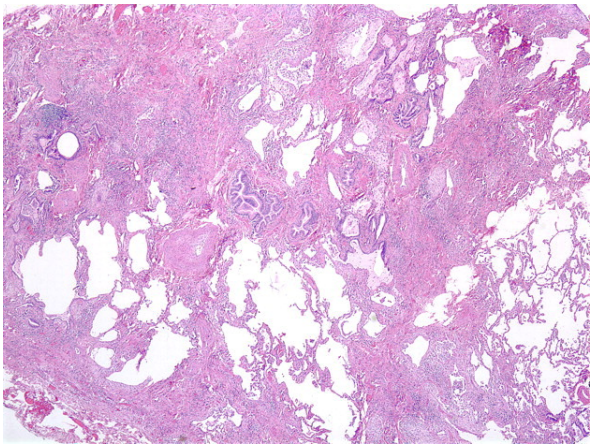

Figure 1.8: Histological presentation typical of the UIP pattern [20]

Fibrosis predominates over inflammation. Fibroblast foci are observed which are a sign of acute damage to the parenchyma often arising against a background dominated by chronic scarring, such foci consist of linearly arranged fibroblasts

and myofibroblasts within a pale matrix. Foci are not a characteristic feature of UIP but are nevertheless an important element for the diagnosis. The overlying epithelium consists of hyperplastic pneumocytes or non-ciliated bronchiolar cells. There is the formation of honeycomb cysts, these are bronchial tubes that have undergone delay, inside there are mucus and leukocytes, they are covered by columnar respiratory epithelium. other characteristic elements of the histological pattern UIP are the presence of scars and the hyperplasia of the smooth muscle cells which can sometimes be very accentuated.

1.10.2 Desquamative Interstitial Pneumonia

Regarding the histology of desquamative interstitial pneumonia, the most characteristic feature of histology is the uniform filling of the distal air spaces by pigmented alveolar macrophages, among them multinucleated cells, eosinophils and lymphocytes are occasionally found.
These clusters are cohesive between them. Macrophages are distinctive and have abundant cytoplasm containing finely granular powdery brown pigment. Definite diagnosis of DIP requires that there be concurrent interstitial pneumonitis. In this pathology the septa are thickened by a scant inflammatory infiltrate and lined by uniform and plump cuboidal pneumocytes.

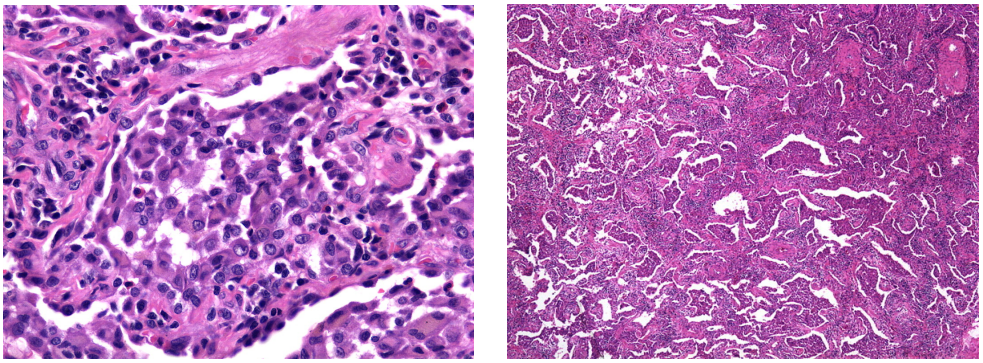

Figure 1.9: Histological presentation typical of DIP pattern [20]

1.10.3 Nonspecific interstitial pneumonitis

Nonspecific interstitial pneumonitis (NSIP) can be either idiopathic or secondary to systemic connective tissue disease. It is currently defined as a disease distinct from other idiopathic interstitial pneumonias due to the important implications this has for prognosis and treatment. NSIP is found in 15-35% of biopsies performed for interstitial lung disease.

In the past the diagnosis of NSIP was predominantly a diagnosis of exclusion, it was diagnosed when the lung biopsies did not show distinctive elements such as to allow the diagnosis of a specific disease.

Today, however, it has been clarified that NSIP has characteristic morphological elements that allow it to be identified as a distinct disease.

The NSIP patterns present a uniform expansion of the alveolar septa due to inflammation or fibrosis, the heterogeneity which instead characterizes the uip pattern is not visible. NSIP has a spectrum of morphological manifestations, there are more cellular forms, and others in which only paucicellular fibrosis prevails.

The forms with prevalent cellularity have septa uniformly thickened by an infiltrate made up of lymphocytes and plasma cells.

Granulomas are rare, if they are present in considerable numbers the diagnosis of NSIP should be questioned. The extent of interstitial fibrosis is highly variable, manifesting as a uniform accumulation of collagen with consequent expansion of the alveolar septa, peribronchiolar interstitium, and interlobular septa or visceral pleura.

The fibrotic forms, on the other hand, are characterized by the presence of dense collagenous tissue and the inflammation is very minimal.

Smooth muscle hyperplasia tends to be less extensive than in the UIP pattern, and findings characteristic of organizing pneumonia can often be found, but must be focal.

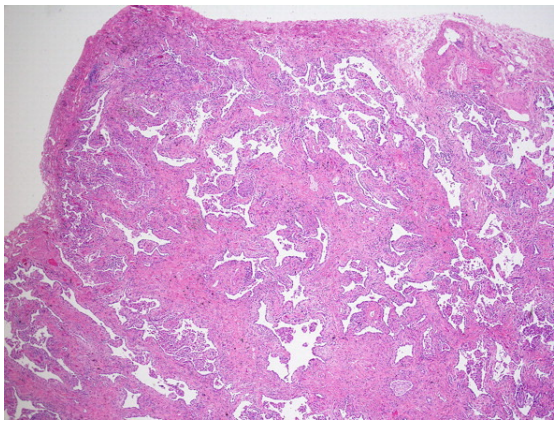

Figure 1.10: Histological presentation typical of the NSIP pattern [20]

1.10.4 Organizing Pneumonia

In the histological pattern of organizing pneumonia, a rich fibrous exudate is detected which from the alveoli also occupies the alveolar ducts and respiratory bronchioles. the characteristic lesions have a macroscopically polypoid appearance, the inflammatory infiltrate is present and consists predominantly of lymphocytic macrophages and eosinophils.

1.10.5 Hipersensisivity pneumonia

The histologic pattern of hypersensitivity pneumonitis can differ greatly depending on whether one is dealing with an acute or subacute form, in which the central histopathologic feature is noncaseous granulomas, or a chronic form where the dominant feature is fibrosis [21].
The pattern seen in cases of chronic HP may be similar to the UIP pattern, so there are patches of subpleural fibrosis, granulomas consisting of epithelioid cells are absent or present in small numbers, giant cells are seen in the interstitium.
A characteristic feature is the presence of bridging fibrosis between the peribronchiolar and perilobular areas. Autopsy findings demonstrated both upper and inferior lobe contraction.

1.11 Multidisciplinary Approach

Correctly classifying the ILD is of paramount importance since follow up, treatment and even prognosis are closely related to the ILD afflicting the patient. But arriving at a definitive diagnosis is not always easy given that there is often overlap between the different forms of ILD.
In addition to the overlap between the different forms of ILD, it must be considered that many patients suffering from connective tissue pathologies are prone to develop pulmonary involvement associated with the systemic disease, and pulmonary involvement has an extremely negative impact on the prognosis of the patient.
Here is that a multidisciplinary approach has become increasingly necessary in the panorama of pulmonary interstitial diseases.
The multidisciplinary team generally involves pulmonologists, lung expert radiologists, pathologists, and very often also the figure of the rheumatologist.
Although it is now clear that a multidisciplinary approach is needed in the diagnosis of ILDs, multidisciplinary pathways are extremely heterogeneous between different centers and between different countries, and the best strategy to apply has yet to be established and validated. It is important to emphasize that the multidisciplinary approach does not detract from the importance of performing the biopsy and proper radiologic follow-up, the multidisciplinary approach goes on to define which cases are most worthy of histologic investigation, where the biopsy is much more informative than an HRCT [14].
An Interstitial Lung Diseases Multidisciplinary Group (ILD MDG) is a specialized team of healthcare professionals who work together to diagnose, treat, and manage patients with interstitial lung diseases (ILDs). ILDs are a group of lung disorders that primarily affect the interstitium, which is the tissue that surrounds and supports the air sacs (alveoli) in the lungs. By bringing together experts from different disciplines, the ILD MDG aims to provide comprehensive and coordinated care to patients with ILDs.
The primary goals of an ILD MDG include:

- Accurate diagnosis: ILDs encompass a wide range of diseases, including idiopathic pulmonary fibrosis, sarcoidosis, connective tissue disease-associated ILD, and others. These conditions often have overlapping symptoms and require careful evaluation and diagnostic testing. The ILD MDG collaborates to ensure accurate and timely diagnosis through a thorough evalu-

ation of clinical symptoms, lung function tests, imaging studies (such as high-resolution computed tomography), and sometimes lung biopsies.

- Treatment planning: Once a diagnosis is established, the ILD MDG works together to develop an individualized treatment plan for each patient. Treatment options for ILDs may include medications to reduce inflammation, immunosuppressants, oxygen therapy, pulmonary rehabilitation, and, in some cases, lung transplantation. The ILD MDG considers the specific needs and preferences of the patient to determine the most appropriate treatment strategy.

- Long-term management: ILDs are often chronic conditions that require long-term management and monitoring. The ILD MDG provides ongoing care to help patients manage their symptoms, optimize lung function, and improve their overall quality of life. This may involve regular follow-up visits, adjustments to treatment plans, and collaboration with other healthcare providers involved in the patient's care, such as primary care physicians or specialists from other disciplines.

Overall, an ILD MDG plays a crucial role in the comprehensive care of patients with interstitial lung diseases, ensuring that they receive accurate diagnoses, appropriate treatments, and ongoing support.
ILD MDGs typically consist of healthcare professionals from various specialties. This can include pulmonologists, radiologists, pathologists, rheumatologists, and thoracic surgeons. The specific composition may vary depending on the resources and expertise available in a particular medical institution or center.
Collaboration and communication among team members are vital in ILD MDGs. The team members work closely together, sharing information, expertise, and insights to reach a consensus on the diagnosis and treatment plan for each patient. Regular meetings, such as multidisciplinary conferences, are held to discuss challenging cases, review imaging studies, pathology findings, and discuss treatment options.
The diagnostic process for ILDs can be complex and requires a thorough evaluation. ILD MDGs utilize a combination of clinical assessment, imaging studies (such as high-resolution computed tomography scans of the chest), pulmonary function tests, and sometimes lung biopsies to establish an accurate diagnosis.
The multidisciplinary approach ensures that all available information is considered, leading to more precise diagnoses and reducing the risk of misdiagnosis.

ILD MDGs play a crucial role in determining the most appropriate treatment strategies for patients with ILDs. The team takes into account various factors, including the specific ILD subtype, disease severity, the presence of underlying conditions (such as autoimmune disorders), and individual patient characteristics. Treatment options may involve medications aimed at reducing inflammation and fibrosis, managing symptoms, oxygen therapy to improve breathing, and pulmonary rehabilitation programs to enhance lung function and overall well-being. ILD MDGs prioritize patient-centered care, considering each patient's unique circumstances and preferences. They aim to involve patients in the decision-making process, educate them about their condition.

Many ILD MDGs actively engage in research activities to advance knowledge and improve patient outcomes. They may participate in clinical trials investigating new therapies or diagnostic approaches for ILDs.

By bringing together the expertise of different specialists, ILD MDGs provide comprehensive, evidence-based care to patients with interstitial lung diseases. The multidisciplinary approach optimizes diagnosis, treatment, and management strategies, ultimately improving patient outcomes and quality of life.

Chapter 2

Aim of the Study

The study we conducted is a retrospective and single-centre study focusing on patients followed up for interstitial disease in the University Hospital of Padua . The aim of our study is to understand if there are any histological variables found during the examination of the biopsy specimen, such as to lead the clinician to suspect that he is dealing with a progressive fibrosing interstitial lung disease. The aim therefore is to understand if there are any histological factors that suggest the presence of a progressive ILD at an early stage. Currently, there is no specific histological biomarker that can reliably detect progressive lung disease in clinical practice. In our study, several histological features were evaluated for their high predictive value.

- Fibroblastic foci are made up of aggregates of activated fibroblasts which are believed to play a fundamental role in the fibrosis process, there have been studies that have highlighted how a high number of fibroblastic foci correlates with progressive disease and lower survival of patients
- The extent of the fibrotic area was evaluated and quantified, which also seems to correlate with the rate of progression and severity of the disease
- Inflammatory cells pattern
- Fibrosis
- Architectural distortion

So the first goal of our study is to evaluate histological features that suggest a diagnosis of progressive fibrosing ILD. The second goal is to assess contextually whether there are clinical and radiological features that point toward a diagnosis of progressive fibrosing ILD.

The third goal to quantify the presence of proinflammatory cytokines in tissue samples.

Chapter 3

Materials and Methods

3.1 Study design and population

The population of our study is patients with interstitial lung disease referred to the University Hospital of Padua (division of Pulmonology), the patients were enrolled retrospectively. The study enrolled 48 patients , who were diagnosed with interstitial lung disease from march 2016 to March 2022. This study is performed following the declaration of Helsinki and is approved by the ethics committee of the University Hospital of Padua (n°428/AO/17) clinical, radiological, demographic and histological data were obtained at the time of Diagnosis. Patients were further evaluated during their last follow-up, and at one year prior to detect any progression of pathology.

3.2 Selection of the study sample

The study initially involved collecting available data for 215 patients with interstitial lung disease followed by the Division of Pulmonology at the University Hospital of Padua. Patients with sarcoidosis stage less than 4, with cystic disease,with IPF, and those with lung disease secondary to connective tissue disease were not enrolled in the study.
From the initial 215 patients, 64 were excluded from the study because they had not performed lung biopsy, an essential element to be enrolled in the final study, the remaining patients are 85. The number was further reduced to 48 patients because 34 patients were excluded, due to the presence of cancer in the histological specimens analyzed.
Other patients were excluded because they performed only bronchial biopsy or

because the materials of biopsy were insufficient for the analysis.
Finally, 48 patients were enrolled in the study who met all criteria.

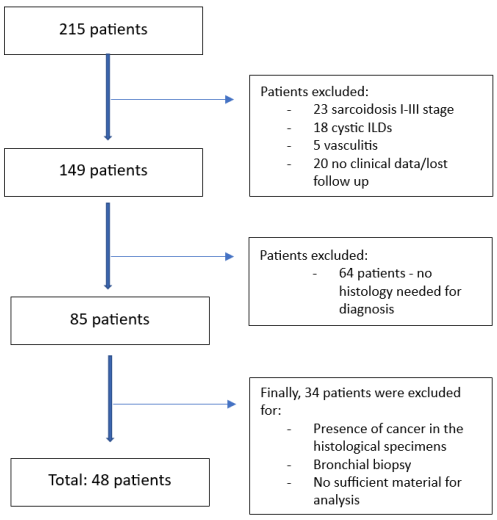

Figure 3.1: Selection of the study sample

3.3 Definition of patient progression criteria

Patient progression was assessed by applying the criteria found in the guidelines published in 2022 by Raghu and all [23]. According to these guidelines, a patient can be defined as progressive if 2 of the 3 criteria that will now be explained are present. The criteria are:

1. Worsening of respiratory symptoms
2. Physiologic evidence of disease progression assessed by worsening spirometry (either of the following):
   - Absolute decline in FVC $> 5\%$ predicted within 1 year of follow-up
   - Absolute decline in DLCO (corrected for Hb) $> 10\%$ predicted within 1 year of follow-up
3. Radiological evidence of disease progression (one or more of the following):
   - Increased extent or severity of traction bronchiectasis and bronchiolectasis
   - New ground-glass opacity with traction bronchiectasis

- New fine reticulation
- New or increased honeycombing
- Increased lobar volume loss

However, the use of the DLCO parameter as a criterion for defining progression is still the subject of much controversy, since it is a parameter that can be influenced by pulmonary hypertension, the presence of emphysema, so it is generally defined as a criterion for progression if it is associated with a decline in FVC or in association with radiological worsening, and so it was also chosen to do in our study i.e. the decline in DLCO was considered significant only if it was associated with the decline in FVC.
Progressive patients are considered those who have gone through transplantation and those who have died from lung disease.
For the purposes of our study, those who did not meet 2 of the 3 criteria proposed in the guidelines but were placed on antifibrosing drug treatment and those who went on to receive lung transplant are considered progressive.
The patients enrolled according to the data in our possession were divided into two groups, the first group are the progressive patients while the second group gathers the non-progressive patients. For the entire enrolled population, the following were collected at diagnosis:

- Clinical data (sex,age,age at diagnosis, BMI)
- Exposure to pneumotoxic agents (radiotherapy, chemotherapy, drugs, organic and inorganic dusts)
- Symptons (fever,asthenia, dyspnoea exertional, dyspnoea at rest, cough, hemoptysis, chest pain)
- Comorbidities (cardiovascular, oncological, metabolic, gastroenterological paying attention to patients suffering from reflux, and other pneumological comobirdities different from the one for which the patients were enrolled)
- HRCT scan
- Smoking history and pack years
- Blood test (Hemoglobin, White Blood Cells count, Neutrophils, Lymphocytes, Monocytes, Eosinophils, Platelets, red blood cells)

- Spirometry
- BAL

All patients included in our study underwent lung biopsy (transbronchial biopsy or VATS). Bronchoalveolar lavage was also performed for some patients. During the last follow-up evaluation, each patient was re-evaluated with:

- Spirometry
- HRCT
- Subjective assessment of worsening symptoms

For each of these parameters we checked whether there had been a worsening compared to the data collected in the previous year. Other data that were collected during the follow-up:

- Need for oxygen therapy on exertion or rest
- Steroid therapy
- Antifibrosing agent therapy

The data we collected covers a time frame from January 2016 to May 2023.

3.4 Histological Analysis

Histological analysis was performed by the pathological anatomy section of the University Hospitalof Padua. Of the 215 patients initially enrolled in the study, 48 were selected based on the availability of slides and tissue blocks, which had a sufficient amount of residual tissue for further analysis to be performed.
37 patients had transbronchial biopsy, for 7 of them only VATS was available, and for 4 of them both transbronchial biopsy and VATS were available. Tissue specimens on which analysis was performed included formalin-fixed, paraffin-embedded transbronchial biopsies and specimens from video-assisted thoracic surgery.
A representative slide of H&E and Massons trichomes from each specimen was evaluated for adequacy and digitally scanned. The slides were analyzed by exploiting QuPath v.0.4.3 software. The evaluators of the slides were blinded, i.e., they were unaware of whether or not the patient had progressive ILD. The histological features analyzed were as follows:

- Lung parenchyma
- Lymphoid aggregates
- Follicles with germinal centres
- Granulomas
- Inflammatory cells (granulocytes, lymphocytes, plasma cells)
- Pigmented intra-alveolar macrophages
- Increase in alveolar macrophages
- Pattern of inflammation (can select more than one)
- Organising pneumonia
- Fibroblastic foci
- Fibrosis
- Pattern of fibrosis (can select more than one)
- Final diagnosis

Fibrosis was assessed with Image P in QuPath using a Masson scanend stained slide. The percentage of blue staining was calculated as a percentage of the total stained area using the Color Threshold function.
In addition to histological analysis, a molecular analysis was also performed, which is still ongoing and is aimed at going to identify which cytokines are most highly expressed in the context of lung tissue affected by interstitial lung disease. Molecular analysis aims to identify which genes for cytokine transcription are expressed in the analyzed tissue samples and BAL fluid.
RNA was extracted from each available sample collected. Cytokine gene expression was assessed using TaqMan™ Array Human Cytokine Network (Applied Biosystems) through conversion to cDNA, real time PCR and probes for 28 cytokine network associated genes.

3.5 Statistical analysis

Descriptive statistics are used to summarise the characteristics of patients.
Categorical variables are described as absolute (n) and relative values (%), whereas continuous variables as median and range (min-max).
Fisher's exact test is used for categorical variables, instead Mann Whitney U test is used for quantitative variables.
A Cox regression was used to evaluate the correlation between mortality and the presence of honeycombing patterns. Continuous variables are dichotomized based on the median value for univariate and multivariate regression.
Overall survival was defined such as the time interval between diagnosis and death/lung transplant or between diagnosis and the patient's last follow up. This parameter was estimated using the Kaplan Meier method, reporting its median and the 95% confidence interval.
All data are analysed using SPSS software version 25.0 (New York, NY, US: IBM Corp. USA) and GraphPad Prism V8 (GraphPad Software, La Jolla, CA, USA). P-values < 0.05 were considered statistically significant.

Chapter 4

Results

4.1 Clinical characteristics of the study population

Taking into consideration the general population the patients are predominantly male sex (64%), the mean age is 62.1 years, the mean BMI (Body mass index) found is 28.6 kg/m^2.
Always going to consider the whole population, smoking habit was assessed and of the enrolled population, 56% have smoked in their lifetime, 6% are still smokers while 38% have never smoked. Pack-years were also assessed with an average result of 10 pack-years.
Patients exposed to possible pneumotoxic agent are 60% of the total.
The population was divided into two groups based on evidence of disease progression, patients with progressive disease are 15 while non-progressive patients are 33. Within the group of progressive patients those with smoking history are 73% versus 48% found in the non-progressives, also the number of pack years we find in the progressives is higher (17) than that found in the non-progressives group.
Going to assess exposure to pneumotoxic substances in the two different groups 73% of progressive patients had exposure to pneumotoxic agent versus 54% of non-progressives.
Concerning the comorbidities affecting the study population, 34% of non-progressive patients had a second pulmonary comorbidity (different from the one that led them to be enrolled in the study) compared with 2% found in progressive patients (p=0.004). Other clinical and demographic characteristics are summarized in Table 4.1.

	Overall (n.48)	**ILD Progressive (n.15)**	**ILD Non-Progressive (n.33)**	**p**
Age - years	62.1 (36.5-81.3)	63.2 (49.4-69.1)	61.6 (36.5-81.3)	0.83
Sex - male n° (%)	31 (64 %)	9 (60 %)	22 (66%)	0,80
BMI (kg/m^2)	28.6 (17.7-41.3)	29.4 (23.3-37.5)	28.6 (17.7-41.3)	0.26
Smoking:				
- Never	18 (38%)	3 (20%)	15 (45%)	0.12
- Active	3 (6%)	1 (6%)	2 (6%)	0.99
- Former	27 (56%)	11 (73%)	16 (48%)	0.13
- Pack/Years	10 (0-90)	17 (0-90)	2 (0-80)	0.15
- Other Exposition	29 (60%)	11 (73%)	18 (54%)	0.34
- Symptoms at diagnosis				
- Dyspnea at rest	29 (60%)	10 (66%)	19 (57%)	0.75
- Cough	29 (60%)	8 (53%)	21 (63%)	0.52
- Dyspnea on effort	4 (8%)	1 (6%)	3 (9%)	0.99
- Thoracic Pain	6 (12%)	2 (12%)	4 (12%)	0.99
- Asthenia	4 (8%)	1 (7%)	3 (9%)	0.99
Comorbidities:				
- CVD	35 (74%)	12 (80%)	23 (72%)	0.72
- Oncologic	9 (19%)	2 (13%)	7 (22%)	0.69
- Metabolic	15 (32%)	6 (40%)	9 (28%)	0.50
- Gastrointestinal	18 (38%)	7 (47%)	11 (34%)	0.52
- Pneumological	14 (30%)	3 (2%)	11 (34%)	**0.004**

Table 4.1: Differences between progressor and non-progressor patients

4.2 Respiratory function and radiological haracteristics of the study population

The results of spirometry performed at diagnosis show that between the progressive group and the non-progressive group there is a statistically significant difference in FVC values, expressed in liters at diagnosis, in the progressive group the average FVC at diagnosis is lower than the FVC values found in the non progressives also at diagnosis (2.42 L progressive vs. 3.37 L non progressive, p=0.004).

The same trend in TLC values was also found, with lower values in progressive patients than in non-progressive patients (3.83 L progressive vs.4.65 L nonptogressive, p=0.03).

Regarding the HRCT evaluation obtained at diagnosis, it is observed that consolidations are more present in non-progressive patients than in progressive patients, although they do not reach statistical significance (20% progressive vs. 53% non-progressive p=0.11).

A fairly significant gradient is also found for the distribution of bronchiectasis, bronchiectasis being more prevalent in progressive patients than in non-progressive patients, although it does not reach statistical significance (33% progressive vs. 18% non-progressive p=0.28).

Other characteristics analyzed are shown in Table 4.2.

	Overall (n.48)	**ILD Progressive (n.15)**	**ILD Non-Progressive (n.33)**	**p**
Spirometry At Baseline:				
- FVC (L)	2,82 (1.52-5.03)	2.42 (1.52-3.76)	3.37 (2.02-5.03)	**0.004**
- FVC (%)	86 (46-129)	79 (48-108)	89 (46-129)	0.19
- FEV1 (L)	2.51 (1.53-4.46)	2.25 (1.83-4.46)	2.8 (1.53-3.89)	0.11
- FEV1 (%)	93 (46-130)	92 (58-119)	96 (46-130)	0.41
- TLC (L)	4.31 (2.54-8.12)	3.83 (2.54-7.75)	4.65 (3.19-8.12)	**0.03**
- TLC (%)	79.5 (46-109)	75 (47-105)	82 (46-109)	0.06
- DLCO	63.5 (30-105)	59 (33-105)	67 (30-95)	0.23
CT scan:				
- Reticulation	12 (26%)	3 (2%)	9 (3%)	0.73
- Bronchiectasis	11 (24%)	5 (33%)	6 (18%)	0.28
- Consolidation	19 (42%)	3 (20%)	16 (53%)	0.11
- Groundglass	17 (38%)	5 (33%)	12 (40%)	0.99

Table 4.2: Differences at respiratory function tests and imaging between progressors and non-progressors

4.3 Serological characteristics and bronchoalveolar lavage

The results obtained from serological analysis were not statistically significant. Monocyte values were essentially the same in the two groups (p=0.78). White blood cell values are slightly higher in progressors than in non-progressors ($7.69 \cdot 10^9/L$ progressive vs. $6.93 \cdot 10^9/L$ non progressive, p=0.41).
The results achieved from the analysis of the data obtained from BAL also did not reach statistical significance. Macrophages reaching higher levels in progressors than in non-progressors are reported.
Other characteristics analyzed are shown in Table 4.3 and 4.4.

	Overall (n.48)	**ILD Progressive (n.15)**	**ILD Non-Progressive (n.33)**	**p**
Blood Test:				
- WBC	7.22 (2.81 – 16.57)	7.69 (4.79 – 16.01)	6.93 (2.81 – 16.57)	0.41
- Neutrophils VA	4.41 (0.94 – 14.5)	4.46 (2.6 – 12.8)	4.08 (0.94 – 14.54)	0.65
- Monocytes VA	0.63 (0.11 – 1.06)	0.64 (0.41 – 1.06)	0.63 (0.11 – 0.97)	0.78
- Eosinophils VA	0.13 (0.00 – 0.82)	0.17 (0.00 – 0.82)	0.12 (0.00 – 0.33)	0.74
- Basophils VA	0.03 (0.00 – 0.50)	0.03 (0.00 – 0.16)	0.03 (0.00 – 0.50)	0.39
- Plateled VA	218 (143 – 432)	215 (153 – 368)	222 (143 – 432)	0.54

Table 4.3: Differences in blood tests between progressor and non-progressor

	Overall (n.48)	**ILD Progressive (n.15)**	**ILD Non-Progressive (n.33)**	**p**
BAL:				
- Neutrophils	7.5 (2 – 90)	5 (2 – 90)	10 (2 – 30)	0.78
- Lymphocite	10 (5 – 60)	10 (5 – 25)	10 (5 – 60)	0.81
- Macrophages	80 (10 – 90)	80 (80 – 90)	80 (10 – 90)	0.34

Table 4.4: Differences at BAL between progressor and non-progressor patients

4.4 Histological analysis

The results obtained from histological analysis did not yield statistically significant results but revealed significant gradients for some variables. Pigmented macrophages show to be slightly higher in progressor than in non-progressors (60% progressive vs. 45% non-progressive, p=0.53).
Among the variables evaluated was the increase in alveolar macrophages, a moderate to severe increase, from this it was found that macrophages appear to be increased in progressive patients compared to non-progressive patients (47% progressive vs. 24% non-progressive, p=0.17).
Analysis of the lymphocyte distribution variable, found that airway centered distribution is more present in non-progressive patients than in progressive patients (6% progressive vs. 27% non-progressive, p=0.14).
Assessment of inflammation distribution pattern showed no differences between the two groups.
Evaluation of the presence of OP pattern (organizing pneumonia)revealed, although not reaching statistical significance, OP pattern is more present in non-progressive patients than in progressive patients (33% progressive vs. 64% non-progressive, p=0.07).
Going to assess the localization of fibrosis, subjects belonging to the progressive group showed to have a greater centrilobular distribution comparing them with the non-progressive group (40% progressive vs.15% non-progressive, p=0.17). The other distribution patterns (interstitial ,alveolar, microhoneycombing) did not show significant differences between two groups.
Evaluating the pattern of distribution of fibrosis, evaluated as patchy or extensive, it was found that the patchy distribution is more frequently present in non-progressors than in progressors (47% progressive vs. 55% non-progressive p=0.13).
Among the variables taken into analysis, the presence in the tissue sample of bronchial metaplasia, anthracosis, the presence of foamy and pigmented macrophages were evaluated. The results show that there are no substantial differences.
Although the data does not reach statistical significance, the presence of bronchial metaplasia seems to be more incident in progressive patients (40% progressive vs. 24% non-progressive p=0.31). The presence of anthracosis occurs more in non-progressive patients, but also in this case, the statistical significance was not reached (53% progressive vs. 73% non-progressive p=0.21).

	Overall(n.48)	ILD Progressive (n.15)	ILD Non-Progressive (n.33)	p
Lymphoid aggregates	0 (0 – 22)	0 (0 – 22)	0 (0 – 9)	0.19
Follicles with GC	1 (2%)	1 (6%)	0 (0%)	0.31
Granulomas	0 (0 – 11)	0 (0 – 1)	0 (0 – 11)	0.79
MNGC	4 (8%)	1 (6%)	3 (9%)	0.99
Pigmented	24 (50%)	9 (60%)	15 (45%)	0.53
Interalveolar pigmented	25 (52%)	9 (60%)	16 (48%)	0.55
Increased Alveolar M0c				
- Not increased	19 (40%)	3 (20%)	16 (48%)	0.11
- Mild	14 (29%)	5 (33%)	9 (27%)	0.74
- Moderate	10 (21%)	4 (27%)	6 (18%)	0.70
- Severe	5 (10%)	3 (20%)	2 (6%)	0.31
Increased Alveolar M0c >34%	15 (31%)	7 (47%)	8(24%)	0,17
Total Inflammation cells	1406 (50 – 6437)	1397 (83 – 2909)	1409 (50 – 6437)	0.57
Lymphocytes distribution				
- No significant	5 (10%)	2 (13%)	3 (9%)	0.64
- Interstitial	38 (79%)	12 (80%)	26 (79%)	0.99
- Airway centered	10 (20%)	1 (6%)	9 (27%)	0.14
Inflammation distribution				
- Patchy	31 (64%)	10 (66%)	21 (63%)	0.99
- Extensive	16 (33%)	4 (27%)	12 (36%)	0.74
Fibroblast foci (No.)	0 (0 – 8)	0 (0 – 8)	0 (0 – 6)	0,56
OP	26 (54%)	5 (33%)	21 (64%)	0.07
%Fibrosis	38.74 (5.85 – 93.57)	36.07 (9.53 – 56.9)	43.6 (5.8 – 93.57)	0.40
Location Fibrosis				
- Interstitial	41 (85%)	13 (87%)	28 (85%)	0.99
- Alveolar	22 (46%)	6(40%)	16(48%)	0.76
- Subpleural	4 (8%)	1 (7%)	3 (9%)	0.99
- Centrilobular	11 (23%)	6 (40%)	5 (15%)	0.17
- Microhoneycombing	8 (16%)	3 (20%)	5 (15%)	0.69
Fibrosis distribution				
- Patchy	25 (52%)	7 (47%)	18 (55%)	0.13
- Extensive	23 (48%)	8 (53%)	15 (45%)	0.61
SRIF	1 (2%)	1 (6%)	0 (0%)	0.13
Wall thikening	6 (12%)	2 (13%)	4 (12%)	0.99
Thrombi	1 (2%)	1 (6%)	0 (0%)	0.31
Bronchiolar metaplasia	13 (27%)	6 (40%)	8 (24%)	0.31
Anthracosis	32 (67%)	8 (53%)	24 (73%)	0.21
BM thikening	3 (6%)	0 (0%)	3 (9%)	0.54
Foamy macrophages	8 (16%)	1 (7%)	7 (21%)	0.40
Pigmented macrophages	2 (4%)	0 (0%)	2 (6%)	0.99

Table 4.5: Histological differences between progressor and non-progressor patients

4.4.1 Prognostic Factors for mortality

To detect predictors of mortality, logistic regression has been performed.
In univariate analysis microhoneycombing (p=0.057), spirometry worsed (p=0.066), symptoms at last follow up worsed (p=0.007), increased alveolare macrophage (p=0,053), have been associated with the survival of patients.
In multivariate analysis it was found that microhoneycombing is independent predictors of mortality (p=0.046).

	Univariate OD	p	Multivariate OD	p
Microhoneycombing	6.873 (0.943-50.096)	0.057	22.488 (1.060- 477.158)	**0.046**
Spirometry worsened	8.447 (0.868-82.171)	0.066	4.549 (0.096-215.051)	0.441
Symptoms at last follow-up worsened	20.213 (2.235-182.771)	**0.007**	11.722 (0.769-178.560)	0.076
Increased Alveolar Macrophages >34%	6.313 (0.975- 40.900)	0.053	25.098 (0.291- 2164.044	0.156

Table 4.6: Cox regression

4.5 Survival

The study also evaluated the difference in ten-year survival between progressive and non-progressive patients, obtaining a statistically significant value as a result (p=0.02).

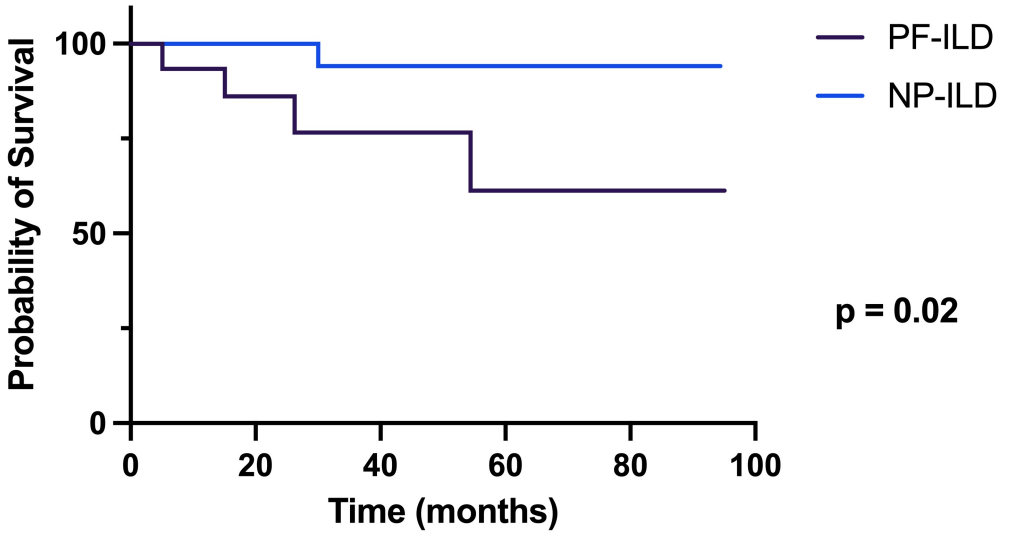

Figure 4.1: Difference in survival between progressives and non-progressors

Chapter 5

Discussion

This retrospective study aims to evaluate the role of histological investigation in patients with interstitial lung disease followed in our hospital.

From the total pool of 215, 48 patients was recruited for the study. This pool was divided into two categories, patiets with progressive ILDs fetures and patient with non progressive features. The progressive group consisted of 15 patients while the non-progressive group had 33 patients. Patients in whom disease progression was found to be 31% of the total, the result found is in line with what the literature shows.

The two groups were shown to be similar as the age of the patients, there were no substantial differences in sex distribution between the two groups.

Looking at the general population, the male sex is the most affected (64% of the total), another result in line with what has been said in the literature that male sex is a risk factor for the development of disease.

Patients' smoking history was also assessed, patients were divided into never-smoking patients, former smoking patients and active-smoking patients, the number of pack-years between the two populations were also assessed. While not reaching statistical significance the number of non-progressive patients among the non-smokers is higher than the progressives (45% non progressives vs. 20% progressive p=0.12).

In ex-smoking patients, on the other hand, we see the reverse trend, in the progressive patient group there is a higher number of ex-smoking patients than non-progressive patients (73% progressive vs. 48% non-progressive, p=0.13), this fact while not reaching statistical significance in our study, is a result that aligns with what has been reported in the literature that smoking is a risk factor for pulmonary fibrosis.

Another interesting element that sees a substantial difference between the two groups, is the exposure to pneumotoxic substances; the number of subjects exposed to pneumotoxic substances is higher in patients with progressive disease compared to patients with stable disease (73% progressive vs. 54% non progressive, p=0.34), a result in line with the literature which sees exposure to toxic substances as one of the major causes of the development of interstitial lung pathology [30].

As regards the symptoms recorded at diagnosis, there were no substantial differences between the two groups.

Comparing the two groups from the perspective of commorbidities afflicting patients, there were more patients with gastroenterological commorbidities in the progressive group than in the non-progressive group, however, the figure did not reach statistical significance (47% progressive vs. 34% non progressive, p=0.52). For pulmonological comorbidities the figure reached statistical significance, pulmonological comorbidities appear to be more in the non-progressor patient group (2% progressive vs. 34% non-progressive, p=0.004).

No differences were found between the two groups for patients with gastroesophageal reflux, nor for metabolic disorders.

What we know from the literature about the comorbidities that most afflict patients with ILD fibrosing is that they are patients commonly suffering from gastroesophageal reflux, which is a recognized risk factor for IPF, chronic obstructive pulmonary disease, and metabolic disorders, extremely common is diabetes [31].

Turning now to consider the data collected at diagnosis at respiratory function tests and from the performance of HRCT. Differences were observed between the two groups in FVC levels at diagnosis, progressive patients at initial spirometry having lower FVC values, expressed in liters, than non-progressive patients (2.42 L progressive vs. 3.37 L non progressive, p=0.004). The same trend is confirmed in the assessment of TLC in which progressive patients record lower values than non-progressors (3.83 L progressive vs. 4.65 L non progressive, p=0.03).

The data we found substantially overlaps with what is reported in the literature, in fact many studies see a low value of FVC as one of the main predictive factors of progressive disease [36].

Analysis of HRCT scans showed that there do not appear to be important differences in the distribution of reticulations between progressors and non-progressors. Reticulations in the literature represent a risk factor for disease progression and are also an expression of the severity of the disease itself.

A fairly significant gradient is also found for the distribution of bronchiectasis, bronchiectasis being more prevalent in progressive patients than in non-progressive patients although it does not reach statistical significance (33% progressive vs. 18% non-progressive p=0.28), bronchiectasis in the literature is also recognized as a factor most associated with aggressive pathology.

There was a greater presence of consolidations in non-progressors than in progressors, although the data did not reach statistical significance (20% progressive vs. 53% non progressive, p=0.11).

According to the literature, consolidations are more present in organizing pneumonia, which only in a quarter of cases develop chronic fibrosis while in most cases with steroid therapy they resolve, albeit slowly [33] [34].

The serological data collected did not show significant differences between progressive and non-progressive patients.

The monocytes between the two groups of patients do not seem to differ substantially although it now seems to be established that high values of monocytes are found in patients with more aggressive pathologies [35]. Although not reaching statistical significance, progressing patients tend to have higher levels of white blood cells while remaining within normal levels.

The data collected from the execution of the bronchoalveolar lavage were also analyzed but did not return statistically significant results.

In the histological evaluation, several varibials were studied. An increase in alveolar macrophages was found in non-progressive patients compared to progressive patients the finding did not reach statistical significance(47% progressive vs. 24% non progressive p=0.17).

The distribution pattern of lymphocytes was also evaluated, whether there was an interstitial or airway centered distribution, the interstitial pattern did not show significant differences between the two groups, while going to analyze the airway centered pattern pointed out a discrepancy between progressive and non-progressive patients, the airway centered pattern is in fact more present in the non progressive population than in the progressive population although it did not reach statistical significance (6% progressive vs. 27% non progressive, p=0.14).

The figure that went to assess the distribution of inflammation whether it was focal or extensive did not highlight any particular differences between the two groups under study.

The presence of an OP pattern revealed an interesting result, namely, that this pattern is more present in non-progressive patients although the data did not

reach statistical significance (33% progressive vs. 64% non progressive, p=0.07). Datum that seems to align with what we derive from the scientific literature according to which only a quarter of patients experience progressive disease.

The study also evaluated the location of the pulmonary fibrosis as a variable, we went to evaluate whether the distribution was more alveolar or centrilobular pattern or assumed a microhoneycombing pattern, as a result there do not seem to be differences in terms of location of the fibrosis between progressive and non-progressive, only the centrilobular pattern showed a higher incidence in progressive patients however not even this data was able to reach significance (40% progressive vs. 15% non-progressive, p=0.17).

In addition to the localization of the fibrosis, the distribution pattern of the fibrosis itself was also evaluated, it was evaluated whether the distribution was only focal patchy or if it extensively involved the parenchyma. The results obtained show that non-progressive patients generally have a patchy pattern of (47% progressive vs. 55 non-progressive, p=0.13), the data is not statistically significant but it aligns with what we know from the literature.

Finally, the presence of bronchial metaplasia, anthracosis, foamy macrophages and pigmented macrophages was evaluated. As a result, a greater incidence of anthracosis was observed in patients with non-progressive disease (53% progressive vs. 73% non progressive, p=0.21), the same tendency was found for the presence of foamy macrophages. However, neither data failed to reach statistical significance.

The presence of bronchial metaplasia has a higher incidence in progressive patients than in non-progressive patients, but even this data has not reached significance (40% progressive vs. 24% non progressive, p=0.31).

From the univariate Cox regression analisys, it emerged that there is statistical significance regarding worsening of symptoms at last follow-up (p=0.007).

Moreover, the presence of microhoneycombing reaches nearly statistical significance (p=0.057).

The multivariate analysis instead highlighted how the finding of microhoneycombing pattern is an independent factor of mortality (p=0.046).

The presence of honeycombing is characteristic of idiopathic pulmonary fibrosis, a disease that serves as a prototype to all fibrosing interstitial diseases of the lung, IPF is a disease that by its nature is progressive with an inauspicious prognosis. With the information available to us to date we know that progressive fibrosing ILD has a similar course to IPF.

So for patients to have microhoneycombing at the level of the histologic specimen subjects them to the same progression of pathology that we will find in IPF probably [37].

Finally, going to analyze the ten-year survival of the patients, a statistically significant difference was found between progressive and non-progressive patients (p=0.02).

Our study obviously suffered from some limitations. First, it is a retrospective and single-center study, and this goes to limit the strength of our results. Second, the small population probably did not allow us to achieve statistical significance for some of the variables we evaluated. Thirdly the imaging examinations were not evaluated by a radiologist experienced in lung pathology, and as a for fourth limitation of our study brings together non-homogeneous patients for pathologies diagnosis and treatment given that the study covers a long period of time.

Last limitation, the study suffers from a bias namely that only patients in good general condition and with not too low respiratory function can access biopsy because of the risks that the procedure inevitably carries.

Chapter 6

Conclusions

Significant finding that emerged from our analysis was that a low FVC value at diagnosis goes to identify those patients most at risk of disease progression if this is confirmed as early as the performance of a respiratory function test alone the clinician can identify those patients who will need close follow-up and early therapy.
At univariate analysis significant data were shown regarding worsening of symptoms at the last follow-up p=0.007. The results of the analysis we performed showed that the presence of microhoneycombing in histological specimens at multivariate analysis is an independent mortality factor for patients p=0.046 and thus is a data point defining a group of patients at higher risk for disease progression.
In addition, our analyses performed to evaluate the 10-year survival of patients diagnosed with progressive ILD and non-progressive ILD showed a statistically significant result, so the importance of identifying patients with progressive phenotype because the disease impacts their survival is reiterated, and this further reinforces our convience that it is necessary to find predditive factors of progressive patholgy .
Finally, the results obtained from molecular analysis of the samples remain to be evaluated, assess for which genes correlated with cytokine expression RNA is expressed in the sample. Analysis of the samples is ongoing. Being able to identify a group of cytokines more highly expressed in the group of progressive patients with different pathologies would go a long way toward confirming the presence of a common fibrosing phenotype would lay the foundation for early diagnosis of fibrosing pathology and perhaps also lead to changes in treatment choices for these patients.

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