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TESI DI LAUREA

Clinical, radiological and hematological features of patients with progressive interstitial lung disease: a comparison with IPF patients

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ABSTRACT

Background

Interstitial lung diseases (ILD) are a heterogeneous group of disorders characterized by diffuse lung parenchyma damage. Among ILDs, some patients could develop progressive fibrosing interstitial lung disease (PF-ILD), showing poor response despite conventional treatment and leading to early mortality. Therefore, there is a growing interest in recognizing and applying biomarkers to predict disease course and response to the therapy.

Aim of the study

This retrospective and multicentric study explored whether clinical, radiological, and hematological features can predict disease progression in interstitial lung disease. The second goal is to evaluate and compare the cellular blood count at the time of the diagnosis between patients with idiopathic pulmonary fibrosis (IPF) and other ILDs.

Materials and methods

This study enrolled 119 ILDs patients and 147 patients with IPF. Based on recent guidelines, in the ILDs group, 43 were considered progressors and 76 non-progressors. For the whole population, demographics, clinical and radiological data were collected at three different time points: the time of diagnosis, one year before the last follow-up, and at the last follow-up. Complete blood counts at the diagnosis are gathered to evaluate neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Only for the IPF population, patients were enrolled in the University Hospital of Padua (n°92) and University Hospital of Palermo (n° 55).

Results

At diagnosis, monocytes count was significantly higher in patients with IPF compared with NP-ILD (0.67 vs. 0.59 x 10^9/L; p=0.008) and also in patients

with PF-ILD compared with NP-ILD (0.68 vs. 0.59 x 10^9/L; p=0.0007). In univariate analysis, age at the diagnosis (p=0.01), FVC%pred. at diagnosis (p=0.0001), complete blood count at the time of the diagnosis with monocyte level of >0.6 x 10^9/L (p=0.003), consolidations (p=0.005), and reticulations (p=0.005) at the HRCT and the presence of exposures (p=0.022) appear to be predictors of disease progression. In the multivariate analysis, FVC%pred. (p=0.002), complete blood count at the time of the diagnosis with a monocyte level of >0.6 x 10^9/L (p=0.036) and the finding of reticulations at the HRCT (p=0.04) are independent factors of disease progression in the ILD population. We also analyse the overall survival of patients diagnosed with IPF, PF-ILD, and NP-ILD at 10 years, and the results show a statistically significant difference (p<0.0001). Furthermore, even without a real statistically substantial difference (p=0.05) in the group of patients whose monocyte level is higher than 0.6 x 10^9 /L, the probability of survival at 10 years is lower.

Conclusion

Based on our findings, monocytes at diagnosis could also be a potential biomarker of progression in patients with ILDs. Further studies are needed to recognize potential progression markers and investigate the role of monocytes in developing lung fibrosis.

RIASSUNTO

Background

Le malattie polmonari interstiziali (ILD) sono un gruppo eterogeneo di patologie respiratorie, la cui eziologia può essere nota o sconosciuta, e sono caratterizzate dalla presenza di un diffuso danno del parenchima polmonare. All'interno dello spettro delle ILD si riconosce un sottogruppo di pazienti che mostra scarsa risposta ai trattamenti convenzionali e sviluppa una malattia progressiva (PF-ILD) il cui decorso può condurre a mortalità precoce. Attualmente vi è un crescente interesse rivolto al riconoscimento e all' applicazione di nuovi possibili biomarcatori per prevedere il decorso della malattia e la risposta alla terapia.

Scopo dello studio

Questo studio retrospettivo e multicentrico mira a valutare se le caratteristiche cliniche, radiologiche ed ematologiche possono predire la progressione delle patologie interstiziali del polmone. Il secondo obiettivo è valutare e confrontare l'emocromo al momento della diagnosi tra pazienti con fibrosi polmonare idiopatica (IPF) e pazienti con altre patologie interstiziali polmonari (ILD) che non siano IPF.

Materiali e metodi

In questo studio sono stati arruolati 119 pazienti con diagnosi di ILD e applicando definiti criteri di progressione, 43 di essi sono considerati progressivi e 76 non progressivi. Per l'intera popolazione sono stati raccolti dati demografici e clinici risalenti al momento della diagnosi, all'ultimo follow-up e ad un anno prima. Inoltre sono stati raccolti gli emocromi completi di formula leucocitaria al momento della diagnosi con lo scopo di valutarne le componenti (neutrofili, linfociti, monociti, eosinofili, basofili). Gli emocromi di questi pazienti con ILD sono stati confrontati con quelli di una popolazione di 147 pazienti con IPF, quest'ultimi arruolati dall'Ospedale Universitario di Padova (n°92) e da quello di Palermo (n° 55). Per questa popolazione i dati

demografici e clinici sono stati raccolti al momento della diagnosi e durante il follow-up.

Risultati

Abbiamo osservato che il valore della conta dei monociti al momento della diagnosi risulta significativamente più alta nei pazienti con IPF rispetto a NP-ILD (0.67 vs 0.59 x 10^9/L p=0.008), e anche nei pazienti con PF-ILD rispetto a NP-ILD (0.68 vs 0.59 x 10^9/L p=0.0007). Per quanto riguarda la progressione della malattia nell'analisi univariata l'età alla diagnosi (p=0.01), FVC%pred. ai test di funzionalità polmonare eseguiti alla diagnosi (p=0.0001), livello di monociti > 0.6 x 10^9/L al momento della diagnosi (p=0.003), riscontro di consolidamenti (p=0.005) e reticolazioni (p=0.005) all'HRCT e la presenza di esposizioni (p=0.022) sembrano essere predittivi di progressione di malattia. Nell'analisi multivariata FVC%pred. ai test di funzionalità polmonare al momento della diagnosi (p=0.002), livello di monociti > 0.6 x 10^9/L al momento della diagnosi (p=0.036) e riscontro di reticolazioni all' HRCT (p=0.04) sono fattori indipendenti di progressione di malattia nella popolazione con ILD. Analizzandola sopravvivenza globale a 10 anni dei pazienti con diagnosi di IPF, PF-ILD e NP-ILD i risultati mostrano una differenza statisticamente significativa (p<0.0001). Inoltre, anche se in assenza di una reale differenza statisticamente significativa (p=0.05) nel gruppo di pazienti il cui livello di monociti è superiore a 0.6 x 10^9/L la probabilità di sopravvivenza a 10 anni è più bassa.

Conclusioni

Sulla base dei nostri risultati, la conta dei monociti alla diagnosi potrebbe essere un potenziale biomarcatore di progressione in pazienti con diagnosi di ILDs. Anche se, è necessario sottolineare che per riconoscere potenziali marcatori di progressione e indagare il ruolo dei monociti nella fibrosi polmonare sono necessari ulteriori studi.

1. INTRODUCTION

1.1 Interstitial Lung Disease

Interstitial lung diseases (ILDs) are a heterogeneous group of acute and chronic conditions characterized by similar symptomatic presentation (1)(2). The term "interstitial" refers to the pathological process originating in the interstitial space (the region between the epithelium and the endothelium). Still, these disorders are also associated with relevant airway alterations and alveolar architecture (3).

Achieving an early and accurate diagnosis and predicting disease progression may be challenging because ILDs are characterized by various disease behaviours (4)(5).

Recently, the incidence of ILD has been reported between 1 and 31.5 per 100 000 person-years, and the prevalence is between 6.3 and 71 per 100 000 people. Thus, it is necessary to emphasize that the disease burden appears heterogeneous among the different countries, probably due to the different approaches to diagnosis and classification (1). Focusing on Europe, idiopathic pulmonary fibrosis (IPF) and sarcoidosis are the most prevalent ILDs (6).

The pathogenesis of ILD is highly variable and, for some aspects, still unknown: it is supposed that both inflammation and fibrosis contribute to the development of ILD, and more than 200 different etiologists are now recognized (7).

The definition of these diseases can be helpful in clinical management and treatment decisions (8). However, ILDs categorizing remains problematic for clinicians, radiologists, and pathologists (9).

1.2 Classification of Interstitial Lung Diseases

Diffuse parenchymal lung diseases are usually divided into known causes and unknown causes (1). ILDs of known cause include autoimmune ILDs and exposure-associated ILDs. Within the ILDs of unknown cause, there is the broad category of idiopathic interstitial pneumonia (IIPs)(1). (Figure 1.1)

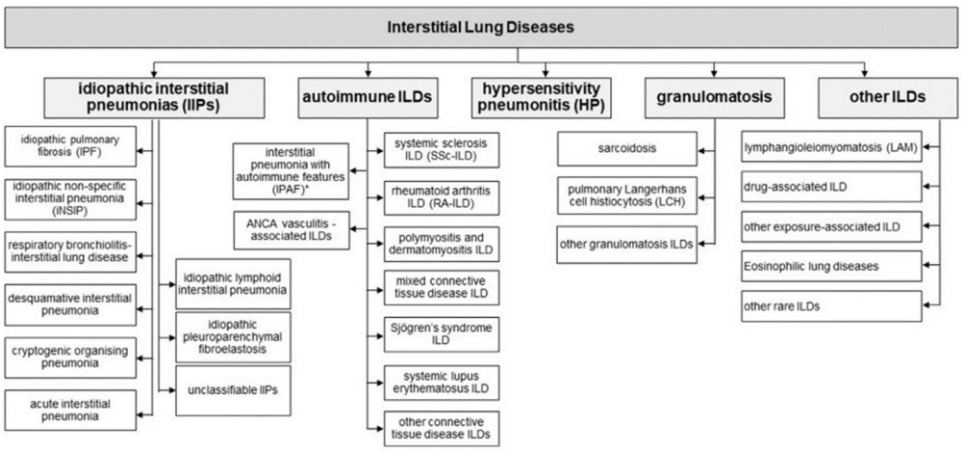

Figure 1.1: *Classification of ILDs,* adapted from Cottin and Valenzuela

1.2.1 ILD of known cause

The most common recognizable causes of ILD are occupational exposures (e.g. asbestosis, silicosis and berylliosis) and environmental agents, in particular organic (e.g. farmer's lung) or inorganic dust, medication, and radiations (3). An ever-increasing number of drugs can cause interstitial lung diseases, such as amiodarone, methotrexate, antibiotics, nonsteroidal anti-inflammatory drugs (NSAIDs), chemotherapeutic agents (e.g. bleomycin), nitro drugs (e.g. nitrofurantoin), biological agents, growth factors (e.g. colony-stimulating factors and interferons) and proteins (e.g. plasma fraction, intravenous immunoglobulins and anti-thymocyte globulin). Illicit medicaments and herbs can provoke acute lung damage and interstitial lung disease if not recognized and treated (9). In addition, also radiotherapy can induce lung toxicity, which may manifest acutely or chronically as pulmonary fibrosis (10). Within the ILDs of known causes, a very important sub-category is represented by those related to an underlying systemic disease: not rarely a pulmonary involvement complicates the course of connective tissue diseases (eg, polymyositis, dermatomyositis, rheumatoid arthritis, systemic lupus erythematosus, scleroderma, and mixed connective tissue disease) (3).

1.2.2 ILD of unknown cause

Idiopathic interstitial pneumonia (IIPs) comprise the following entities in order of frequency: idiopathic pulmonary fibrosis (IPF), nonspecific interstitial pneumonia (NSIP), cryptogenic organizing pneumonia (COP), acute interstitial pneumonia (AIP), respiratory bronchiolitis-associated-ILD (RBILD) and desquamative interstitial lung pneumonia (DIP) (12). Other rare idiopathic interstitial pneumonias are pleural parenchymal fibroelastosis (PPFE) and lymphoid interstitial pneumonia (LIP) (13). IPF and NSIP are generally characterized by chronic presentation, while COP and AIP may have the acute or subacute presentation (3). As of 2013, when there was an update of the international multidisciplinary classification of the IIPs, RB-ILD, and DIP are considered together in the term smoking-related idiopathic interstitial pneumonia (SR-IIP) (13) (14). In 2015, the term interstitial pneumonia with autoimmune features (IPAF) was also introduced to identify individuals with IIP and features suggestive of, but not definitive for, a connective tissue disease (CTD) (8). However, in most cases, IIPs remain unclassifiable (12). Although the categorization of IIPs, these pneumonias represent a spectrum of injuries that share common pathologic pathways that lead to volume loss and lung distortion. In most cases, patients can experience a decline in lung function with progressive symptoms, poor response to treatment, and reduced quality of life (9) (15).

1.2.3 other ILDs

In addition to ILDs of known and unknown causes, other interstitial lung diseases do not fit these categories completely, like Langerhans cell histiocytosis (LCH), eosinophilic pneumonias, lymphangioleiomyomatosis (LAM) and sarcoidosis which is a granulomatous lung disorder (1) (12). Another granulomatous interstitial lung disease is hypersensitivity pneumonitis (HP) which can develop, in susceptible and sensitized individuals, as a result of exposure to a large variety of inhaled antigens found in the environment which can determine an immune-mediated response (16).

1.3 Diagnosis

The diagnostic process of ILD starts with clinical suspicion. However, establishing an exact diagnosis in the field of ILDs could be very difficult and often requires a multidisciplinary approach, which is necessary to estimate the patient's prognosis and the individuation of the appropriate treatment (17). The global complexity of clinical diagnosis and management of ILDs justifies the critical role of multidisciplinary team discussion (MDD), the gold standard (17). Different reasons underlie the difficulty of classification and diagnosis: the absence of robust diagnostic criteria for some ILDs and a limited ability to differentiate specific ILD entities (18). Unfortunately, the diagnostic experience for patients with ILD may be characterized by relevant delays, exposure to invasive diagnostic procedures, frequent misdiagnosis, and consistent use of healthcare resources (19).

Diagnostic delay is a real problem for patients with ILD. It probably stems partly from its insidious onset and non-specific symptoms, which overlap with those of more common pulmonary and non-pulmonary diseases or could be generally attributed to the patients' aging (20). Another critical issue is the insufficient knowledge of ILD among primary care physicians and non-ILD experts (20). The results of the INTENSITY survey, conducted in 2018 that enrolled 600 patients with ILD show that 55% reported ≥ 1 misdiagnosis and 38% reported ≥ 2 misdiagnoses before the correct diagnosis, and the most common misdiagnoses were asthma (13.5%), pneumonia (13.0%), and bronchitis (12.3%) (19). The median time from the onset of the symptoms to the achievement of the correct diagnosis was 7 months (range, 0-252 months), with 43% of respondents reporting a delay of ≥ 1 year and 19% reporting a delay of ≥ 3 years (19). To avoid, or at least reduce, the diagnostic delay and improve clinical management, greater awareness is required from patients, general physicians, and specialists (20). To achieve this critical goal is essential to investigate persistent symptoms, to perform accurate physical examinations and chest imaging studies, and in the case of the suspect, refer the patient to ILD specialized centre (Figure 1.2) (20).

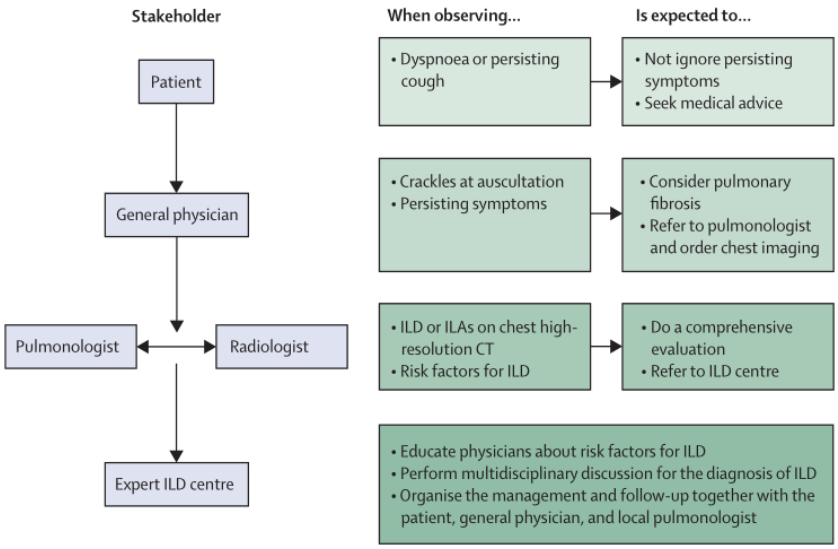

Figure 1. 2*: Potential solutions to diagnostic delays of ILD.* It is adapted from Spagnolo et al.

1.3.1 Medical history

As the first step, the sex and age of the patient are essential because some ILDs are more common in certain age groups or have a male or female predominance (21). The mean age of IPF, which affects males more than females, is around 65–70 years, and the incidence increases with age (21). In contrast, the majority of patients with sarcoidosis, connective tissue disease-associated ILD, lymphangioleiomyomatosis (LAM), and inherited forms of ILD (e.g., familial IPF) are between the ages of 20 and 40 (3). Two examples regarding the different gender prevalence are LAM, which primarily affects women, and exposure-related ILDs, which are more common among men (20)(22). Many occupational and environmental exposures are associated with an increased risk of ILD; however, the risk is higher among individuals with a family history of the disease (20). It is essential to investigate the personal and family history and to shed light on past medical history and situations of exposure to substances that may play a role in developing ILD, including cigarette smoking, drugs, and irradiation (3). The fund of a history of connective tissue disease, inflammatory bowel disease, or malignancy might be a clue to an associated ILD. It is also worth remembering that the condition and medications used for the treatment should consider (3). It is always

necessary to inquire whether the patient is a smoker or a former smoker and to quantify smoking exposure, for example, in terms of pack/years or cigarettes/die because a history of tobacco use can be related to interstitial and air space inflammation and fibrosis (23).

Furthermore, a family history of almost any type of ILD is essential because there is a genetic basis for the development of pulmonary fibrosis, and this explains the importance of supervising families with two or more members with pulmonary fibrosis (24). In connective tissue disease, patients should be evaluated for ILD at the time of the diagnosis and then periodically because it is known that some of them may develop lung involvement. However, precise data on method and time interval are scarce (20).

1.3.2 Clinical presentation and physical examination

ILD presentation is often characterized by non-specific and insidious symptoms, including (20):

- Dyspnea;
- Cough;
- Fatigue;
- Hemoptysis;
- Chest pain or discomfort;
- Extrapulmonary symptoms.

Dyspnoea, cough, and fatigue are the most frequently reported symptoms of ILDs (25). The term dyspnoea refers to the sensation of breathing discomfort, which is one of the most common and distressing symptoms experienced by patients and may be described as " difficulty of breathing", "shortness of breath, or "feeling of chest tightness," the spectrum of differential diagnoses to consider in case of dyspnea includes cardiovascular, pulmonary and neuromuscular diseases (26). The mMRC (Modified Medical Research Council) Dyspnoea Scale is used to assess the degree of functional disability due to dyspnea, and it is recommended by guidelines and used as an inclusion criterion or endpoint for clinical trials (27). Cough is the symptom responsible for

almost one in ten primary care consultations (28). Many patients with interstitial lung disease have a dry, hacking, and persistent cough, and evidence suggests that this symptom may be due to an increased cough reflex sensitivity. Still, cough could also result from more common disorders, such as gastroesophageal reflux disease or asthma, which need to be investigated (29). Focusing on fatigue in interstitial lung disease (ILD) is a common burdensome symptom that significantly impacts the quality of life, work productivity, and social relations (25). It is also essential to recognize extrapulmonary symptoms such as low-grade fever and arthralgia, which may be related to sarcoidosis, haematuria, eye dryness, and weight loss, which may be related to rheumatological diseases (22).

If the suspicion of ILD arises after the physical examination, it is essential to direct the patient to the most appropriate diagnostic path to assess the severity of the disease and provide clues to the underlying cause (20).

During a physical examination, the following assessments should be evaluated (30):

- **Vital signs**: blood pressure, heart rate, respiratory rate, and oxygen saturation (using a pulse oximeter) to assess the health and respiratory function of the patient;
- **Lung examination**:
    - **Inspection**: visually inspect the chest of the patient for signs of respiratory distress, including rapid breathing, use of accessory muscles, and chest retractions;
    - **Palpation**: palpation of the chest to assess for tenderness or masses;
    - **Percussion**: use percussion to assess for dullness or resonance, which can indicate the presence of fluid or air in the lungs;
    - **Auscultation**: using a stethoscope to listen to the breath sounds. In interstitial lung disease, the breath sounds may decrease, or crackles and wheezes may be present. Fine crackles

are heard during inspiration and are generally more prominent at the pulmonary bases and caused by the opening of small airways that are typically closed. At the same time, wheezes are almost always loudest during expiration (30). "Velcro-type" crackles on chest auscultation that sounds like velcro being ripped apart are considered a typical and early detectable acoustic finding of lung fibrosis, and their presence directly correlates with the extent of radiologic features of pulmonary fibrosis (7)(31). Such evidence provides grounds for further investigating lung sounds as an early identification tool in ILD (32).

- **Cardiac examination**: is usually normal except in more advanced stages of pulmonary fibrosis, when findings of pulmonary hypertension and cor pulmonale may become evident (3). Patients with interstitial lung diseases (ILDs) may develop pulmonary hypertension (PH), most with a pre-capillary pattern at invasive hemodynamic studies, being classified within groups 3 and 5 in the current classification of PH (33).
- In patients with advanced ILD can occur **cyanosis**, that is a bluish discoloration of the skin, mucous, and nail beds; it is the result of reduced oxygen level in the blood (>5 g of deoxygenated hemoglobin/dL) (22). Cyanosis means that some lung regions are ventilated poorly but are perfused either normally or less poorly (30).
- **Digital clubbing** is characterized by thickening the terminal segments of the fingers and toes. It results from the proliferation of connective tissue between the nail matrix and the distal phalanx, leading to a change in digital shape and appearance: swollen and convex distal phalanx (34) (35). Clubbing may indicate the presence of underlying interstitial lung disease (35).
- **Other extra-pulmonary signs** which can be recognized in ILD are erythema nodosum and lupus pernio, which are cutaneous manifestations of sarcoidosis, and Raynaud's phenomenon, which can

frequently be the presenting sign of connective tissue diseases (CTD) (36) (37) (38).

1.3.3 Laboratory studies

Laboratory studies are often used because they may be helpful for the diagnosis but are rarely specific in defining it. The laboratory evaluation of suspected ILD includes both simple tests but also, in selected cases, more specific ones (22) (39) (40):

- complete blood count with leukocyte formula;
- serum protein electrophoresis;
- hepatic and renal function;
- markers of inflammation;
- Precipitating antibodies;
- ACE;
- ANCA, ANA, ENA, RF, and other rheumatological markers according to the clinical suspicion;
- urine analysis.

Laboratory studies can be constructive in the workup for an underlying CTD-associated ILD or in the suspect of hypersensitive pneumonitis (22) (41).

1.3.4 Pulmonary function tests

Pulmonary function tests are a proper investigation in managing patients with previously diagnosed or suspected respiratory disease; they are helpful in diagnosis, assessing response to treatment, and monitoring disease progression. The knowledge of respiratory pathophysiology is essential for interpreting the results (42).

Spirometry is the most important and frequently used pulmonary function test and measures volume against time (Figure 1.3): patients are asked to take maximal inspiration and forcefully expel air for as long and as quickly as possible (43). To perform the test correctly, the patient should be seated erect,

with shoulders slightly back and chin somewhat elevated, and should be used a chair of the right height with arms (to prevent fall in case of syncope) (43).

The results include (42):

- Forced expiratory volume in one second (FEV1): the amount of air a person can exhale forcefully in one second;
- Forced vital capacity (FVC): the maximum amount of air a person can exhale forcefully after taking a deep breath;
- The ratio of the two volumes (FEV1/FVC): the percentage of the total air capacity that a person can exhale forcefully in one second;

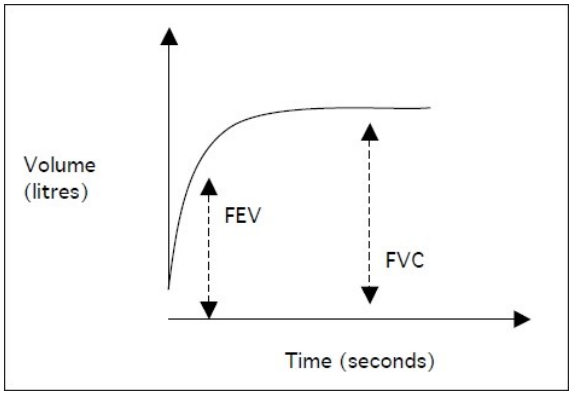

Figure 1. 3: *Normal spirometry,* adapted from Ranu et al.

In restrictive defects, the volumes are globally reduced (Figure 1.4), and the ratio FEV1/FVC is average or increased (42). If the FEV1/FVC ratio and the FVC are low, the patient has a mixed defect (restrictive and obstructive pattern) (44).

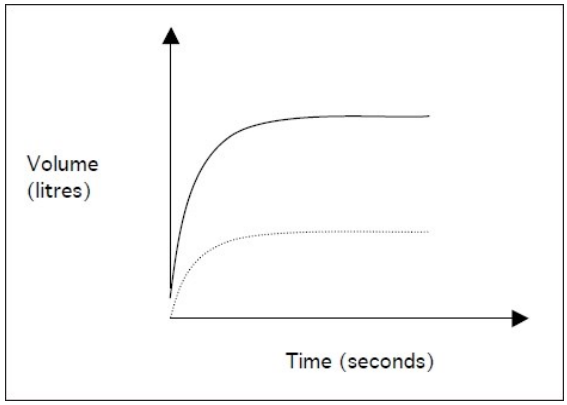

Figure 1. 4: Spirometry in restrictive lung disease adapted from Ranu et al.

In spirometry, a flow volume curve is the graphical representation of the volume of air that a person can exhale forcefully, plotted against the flow rate at which the air is exhaled. The flow volume curve begins with the patients inhaling deeply to their maximum lung capacity (opposing inspiratory limb) and then exhaling fully as possible (positive expiratory stem) (Figure 1.5)(42).

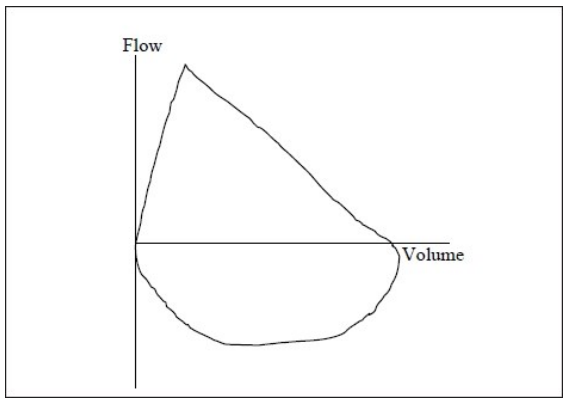

Figure 1. 5: Normal flow-volume curve, adapted from Ranu et al.

Knowing the typical morphology of a flow volume loop allows for recognizing any alterations indicative of pulmonary diseases. In particular, the figure below represents the curve's aspect in the case of restrictive deficit (Figure 1.6) (42).

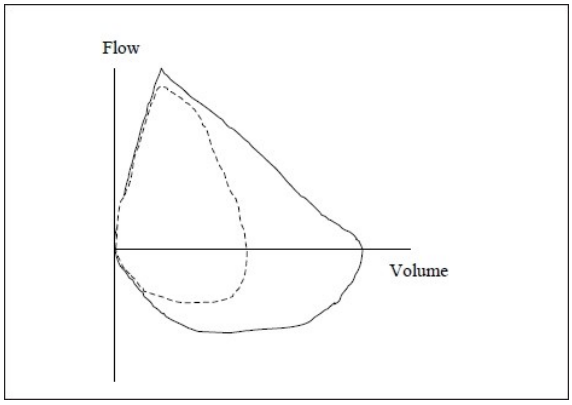

Figure 1. 6: Flow volume curve in restrictive lung disease, adapted from Ranu et al.

It is essential to measure lung volumes if the previous tests have revealed the presence of some abnormalities. There are two different ways of measuring lung volumes: plethysmography and measurements derived from gas dilution (45).

Lung volume measurements include (Figure1.7) (42) (46):

- **Vital capacity** (VC): maximum volume exhaled after ultimate inspiration; can be measured during forced exhalation (FVC) or slow exhalation (SVC);
- **Functional residual capacity** (FRC): the importance of air remaining in the lungs at the end of a normal expiration (sum of RV plus ERV);
- **Residual volume** (RV): volume of air remaining in the lung after maximal expiration (about 500 ml)
- **Expiratory reserve volume** (ERV): the importance of air exhaled from end-tidal book (FRC) to the point of maximal exhalation (RV);
- **Inspiratory capacity** (IC): maximum inspiration from end-tidal volume (FRC) to total lung capacity;
- **Inspiratory reserve volume** (IRV): the importance of air inhaled during tidal breathing from end-inhalation to total lung capacity;
- **Total lung capacity** (TLC): volume of air in lungs at the end of maximal inspiration (usually calculated by the sum RV plus VC).

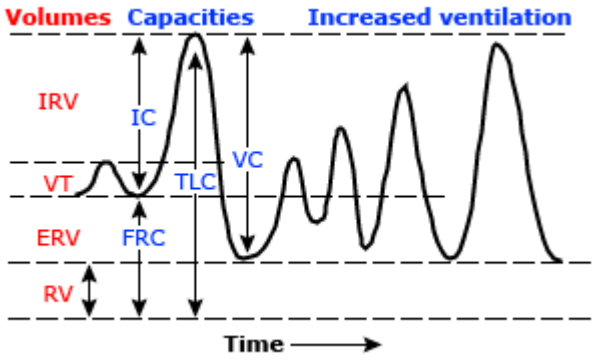

Figure 1. 7 Pulmonary function tests: lung volume and capacities adapted from UpToDate.

Restrictive impairment may be suspected from spirometry when the flow-volume curve shows a convex pattern, FVC is reduced, and FEV1/FVC is normal or increased. This type of impairment is characterized by a reduction of lung volumes and, in particular, TLC value below the 5th percentile (47).

Restrictive disorders can be divided into three groups (44):

- Interstitial lung diseases (ILDs);
- Disorders of the chest wall or the pleura, which limit the expansion of the lungs;
- Neuromuscular disorders decrease the respiratory muscles' ability to inhale and exhale.

Suppose the spirometry's results raise the suspicion of restrictive or mixed impairment. In that case, the patient should perform full PFTs with DLCO (Diffusing Capacity of the Lung for Carbon Monoxide), a quantitative measurement of how effectively gas is transferred from the alveoli to the blood (44). Therefore, the finding of a reduced value of DLCO results from impaired gas exchange due to diseases that decrease blood flow to the lungs or damage alveoli (44). To perform the test, the patients have to inhale a mixture of gas which includes helium (10%), carbon monoxide (0.3%), and others; then, they have to hold their breath for 10 seconds before exhaling (44). DLCO value must be adjusted for blood hemoglobin content (42). DLCO is usually reduced in case of interstitial lung disease (restrictive pattern),

emphysema (in all the other obstructive conditions, the DLCO value is preserved), and pulmonary vascular disease (e.g., pulmonary hypertension and thromboembolic diseases, which are characterized by the absence of significant restrictive or obstructive impairment)(46). In general, the severity of the DLCO reduction correlates with the prognosis only if DLCO is lower than 35 % of the predicted value and allows to identification an actual progression or regression in disease severity if a longitudinal change of 15 % is observed (48) (39). When there is a suspicion of ILD, a complete pulmonary function test (spirometry, lung volumes, diffusing capacity) and blood saturation should be obtained (49).

Blood oxygen levels can be assessed directly with arterial blood gas sampling or indirectly with a pulse oximeter. Blood saturation provides essential information on gas exchange and, consequently, on oxygen delivery to the tissues (42). Although diffusion abnormalities impair the diffusion of oxygen and CO2, hypercapnia is less common because CO2 is twenty times more soluble in water than oxygen and diffuses faster. Hypoxemia with hypercapnia can be found in the advanced stage of the disease (50).

Submaximal exercise testing is often conducted along the clinical pathway of diagnosed or suspected ILD (46). The three best-known are the six-minute walk test (6MWT), the incremental shuttle walk test (ISWT), and the endurance shuttle walk test (ESWT) (46). A multinational and multidisciplinary group of experts in exercise testing have defined these testing procedures, considering a systematic review of the measurement properties and interpretation of the 6MWT, ISWT, and ESWT in adults with respiratory disease (51).

1.3.5 Chest imaging studies

Focusing on imaging studies in ILD, the first step is chest radiography, followed by high-resolution computer tomography (HRCT). The first suspicion of ILD may arise from the detection of some alterations of chest X-ray, but generally, to obtain a specific diagnosis, HRCT is required (22)(3)(39).

Chest X-ray, which is rapidly available and cheap, is vital in the initial detection, follow-up, and differential diagnosis (40).

High-resolution computer tomography (HRCT) is considered the gold standard, providing greater diagnostic accuracy in ILD by filling the lack of specificity of the chest X-ray (40). The downsides of HRCT are that it is expensive and exposes the patients to more ionizing radiations than the X-ray (52). This type of computer tomography is high-resolution because it allows us to obtain volumetric imaging with slice thickness ≤1.5 mm (49).

HRCT can detect six typical ILD patterns (40):

- reticular pattern;
- nodular pattern;
- cystic pattern;
- consolidation;
- ground-glass opacities;
- thickened interlobular septa.

1.3.6 Bronchoalveolar lavage (BAL), transbronchial lung biopsy (TBLB), and surgical lung biopsy (SLB)

When non-invasive techniques are inconclusive, the next step is to consider other procedures to obtain tissue, cellular elements, or alveolar fluid. Minimally invasive and invasive procedures that could be useful in diagnostic confirmation are bronchoalveolar lavage (BAL), transbronchial lung biopsy (TBLB), and surgical lung biopsy (SLB).

Bronchoalveolar lavage (BAL) is performed with a flexible bronchoscope wedged into a segmental or subsegmental bronchus. Then saline is infused in five aliquots up to the total volume of 150-200 ml. After each saline instillation, BAL fluid is gently recovered, and the sample is pooled (52). BAL can help identify neoplastic cells and characteristic phenotypical and cytological profiles (54) (55). This procedure could also be an essential tool if lung biopsy is not feasible, to have still the possibility of reaching a specific diagnosis

(56). Various changes in individual cell constituents' relative and absolute numbers have been described in patients with ILDs (57). Generally, these changes can be only suggestive, but in some cases, the pattern may lead to a differential diagnosis or confirm the previously suspected diagnosis (54). For example, even if it is not specific, the founding of a marked lymphocytosis (>50% of total cells) can be characteristic of hypersensitivity pneumonitis (HP) (16). On the contrary, the increase of lymphocytes is unusual in pure fibrotic interstitial lung diseases such as IPF (16).

Transbronchial lung biopsies (TBLB) are obtained during flexible bronchoscopy using biopsy forceps that are passed through the channel of the bronchoscope (58). This procedure achieves the highest diagnostic yield in ILDs with centrilobular accentuation (39). The main complication of TBLB is bleeding; less frequent complications that may occur during the procedure are pneumothorax, hypoxemia, and cardiac arrhythmias. The rate of pneumothorax is reduced when fluoroscopic guidance is used (59). Frequently the combination of BAL with TBLB is sufficient to achieve a definitive diagnosis. However, in some cases, surgical lung biopsy (SLB) is still relevant, in particular in idiopathic interstitial pneumonia (IIP)(55).

When multidisciplinary team discussion is inconclusive and the etiological diagnosis remains unknown, surgical lung biopsy (SLB) should be considered. It is an invasive procedure with potential complications associated, including a not negligible risk of death (60). The surgical approach to lung biopsy allows a significantly larger tissue sample to be obtained than with the TBLB, and the artifacts are less frequent. Surgical lung biopsies may be performed through conventional limited thoracotomy (open lung biopsy, OLB) or through video-assisted thoracoscopic (VATS) (39). The risk of complications is higher if SLB is performed during acute exacerbation, in the case of immunocompromised patients and those with impaired lung function or pulmonary hypertension (61).

Another available technique is the transbronchial cryo-biopsy (cryo-TBLB) which allows for obtaining a larger size of tissue samples and increasing the diagnostic yield compared to forceps biopsies (61).

Therefore, within the diagnostic process of ILDs, it is necessary to analyze when an invasive procedure is valuable, what procedures are available, and in which order they should be scheduled (55).

1.4 Progressor and non-progressor patients

A new disease entity, namely progressive-fibrosing ILD (PF-ILD), has recently been proposed; it includes a subgroup of patients with ILD who show poor response despite conventional treatment chosen based on the underlying diagnosis (7). Progressive fibrosing interstitial lung disease (PF-ILD) describes a phenotypic subset of ILDs characterized by advanced lung fibrosis. (Figure 1.8)

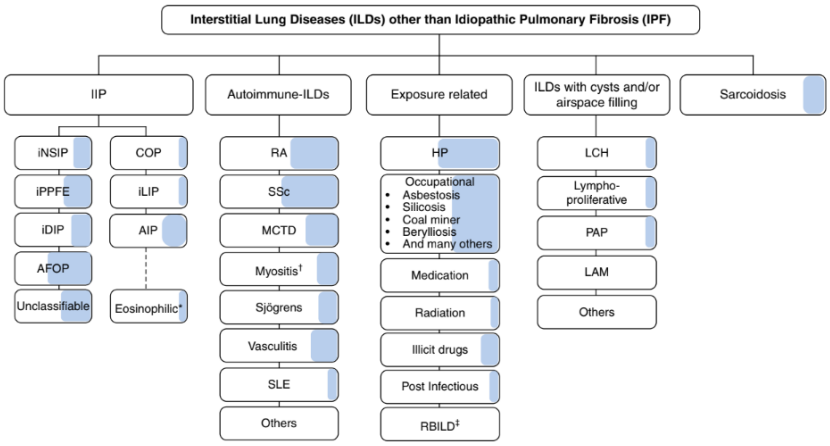

Figure 1.8: ILDs manifesting progressive pulmonary fibrosis (PPF). The shaded area represents the estimated proportion of patients with various types of ILD who display PPF—adapted from Raghu et al *(iNSIP: idiopathic Non Specific Interstitial Pneumonia; iPPFE: idiopathic PleuroParenchymal FibroElastosis; iDIP: idiopathic Desquamative Interstitial Pneumonia; AFOP: Acute Fibrinous and Organizing Pneumonia; COP: Cryptogenic Organizing Pneumonia; iLIP: idiopathic Lymphoid Interstitial Pneumonia; AIP: Acute interstitial Pneumonia; RA: Rheumatoid Arthritis; SSc: Systemic Sclerosis; MCTD: Mixed Connective Tissue Disease; SLE: Systemic Lupus Erythematosus; HP: Hypersensitivity Pneumonitis; RBILD: Respiratory Bronchiolitis ILD; LCH: Langerhans Cell Histiocytosis; PAP: Pulmonary Alveolar Proteinosis; LAM: Lymphangioleiomyomatosis,).*

Besides the IPF, which is the emblematic example of progressive phenotype, this pathological behavior can also be found in hypersensitivity pneumonitis (HP), idiopathic nonspecific interstitial pneumonia (NSIP), idiopathic PleuroParenchymal FibroElastosis (iPPFE), ILD associated with connective tissue diseases (CTD-ILD such as myositis-associated ILD, systemic sclerosis-associated ILD, and rheumatoid arthritis-associated ILD), and unclassifiable ILD (u-ILD). In contrast, it seems to be less typical of others, such as lymphoid interstitial pneumonia (LIP) or organizing pneumonia (OP)(63-64).

Several insults, including inflammation, organic and inorganic dust exposure, and autoimmunity, can trigger lung fibrosis (65). Such triggers provoke epithelial and vascular injuries that stimulate the inflammatory response and precipitate the activation of fibroblasts of the lung and the recruitment of those circulating in the blood (Figure 1.9) (63)(64). Fibroblasts differentiate into myofibroblasts, leading to excessive extracellular matrix deposition and the subsequent fibrotic remodelling of the lung parenchyma (63). The inflammatory response stimulates the production of further fibrosis mediators by lymphocytes and macrophages, generating an abnormal proliferative stimulus for fibroblasts; overall, this mechanism degenerates into progressive and uncontrolled lung fibrosis (63). Pro-fibrotic mediators involved in the disease pathogenesis include platelet-derived growth factor (PDGF), tumor necrosis factor-alpha (TNF-α), transforming growth factor beta (TGF-β) and matrix metalloproteinases (MMPs) (64) (63). It is necessary to underline that regardless of the initial cause, the aging processes and the genetic predisposition affect the fibrogenic response in the lung (64).

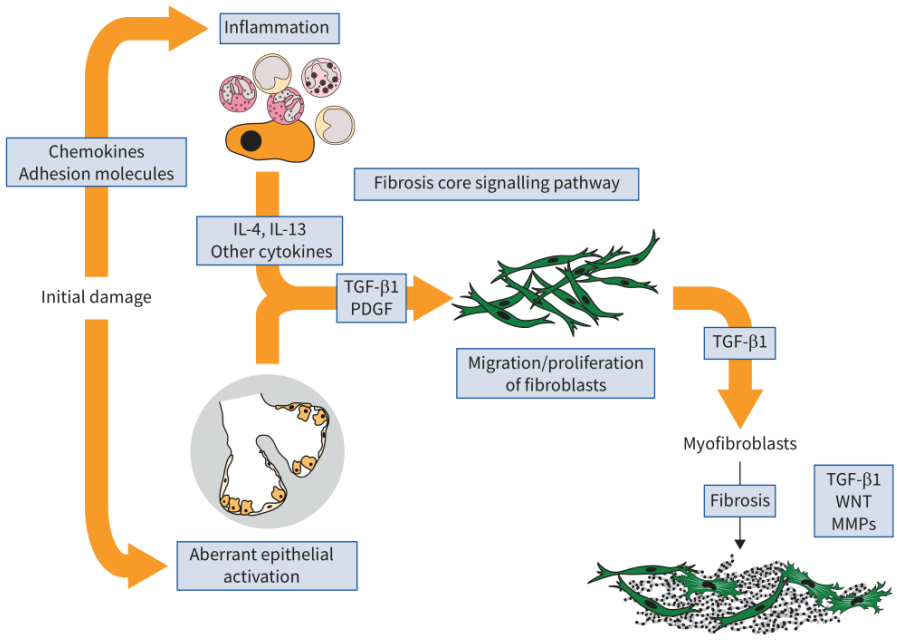

Figure 1.9: Mechanisms driving the progressive fibrosis phenotype in interstitial lung diseases, adapted from Selman et al.

To define PF-ILD, it is necessary to satisfy at least two of the following three criteria occurring within the last year with no alternative explanation (66):

1) Worsening of respiratory symptoms;

2) Physiological evidence of disease progression (either of the following):

- Absolute decline in FVC ≥ 5% predicted within one year of follow-up;
- The total decline of DLCO (corrected for Hb) ≥ 10% in one 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;

- The increased extent or increased coarseness of reticular abnormality;
- New or expanded honeycombing;
- Increased lobar volume loss.

Patients who require lung transplantation or die from ILD's evolution are considered progressive (65).

Regarding pulmonary function, a decline in the diffusion capacity for carbon monoxide (DLCO) has been proposed as a progression criterion of ILD. Still, it has a controversial role since this parameter also is reduced in the case of pulmonary hypertension and emphysema. DLCO may be considered a sign of progression when associated with FVC decline or worsening of fibrosis at HRCT (7) (67).

The progression of fibrosis can also be suggested by a decline in the 6 minutes walk distance (6MWD) (4).

Several factors have been recognized to predispose to an increased risk of progression of fibrosis, and these include older age, diagnosis of IPF, rapid disease progression, extensive traction bronchiectasis on HRCT, no regression or stabilization with initial therapy, and short telomere syndrome (4).

Patients with IPF and PF-ILD have comparable outcomes: progressive decline in lung function, symptoms' worsening, end-stage fibrosis, and early mortality (63). Idiopathic pulmonary fibrosis (IPF) is the emblematic example of inexorable disease progression and poor prognosis. Still, progressive fibrosing has been recently extended to a range of underlying ILD diagnoses (4). This development about PF-ILD other than IPF opens the discussion on the importance of an appropriate diagnostic process according to the international guidelines and fore need for an accurate definition of disease progression to have the possibility of undertaking antifibrotic therapy, as a second-line treatment in progressor patients (Figure 1.10) (67) (66).

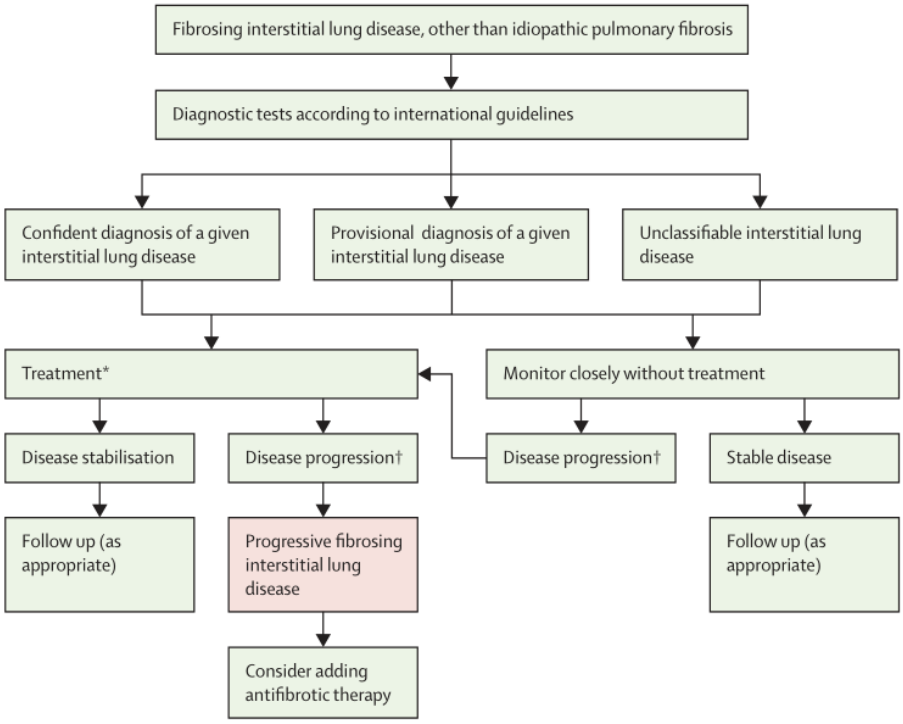

Figure 1.10 Diagnosis and management of PF-ILD, adapted from George et al.

1.5 Treatments

Attention is ongoing on the therapeutic possibilities for patients with ILDs (68). Most ILDs were presumed inflammatory, at least in their early phases, and thus likely to respond to corticosteroids and other immunosuppressives, representing the first-line treatment. Still, it is known that inflammation and fibrosis can underlie the pathological process, and so the effect of the drugs is aimed to counteract both of them. (69). Immunomodulatory drugs are used in ILDs associated with connective tissue diseases (CTD-ILD), hypersensitivity pneumonitis (HP), non-specific interstitial pneumonitis (NSIP), and other presumptive inflammatory diseases.

In contrast, antifibrotics are mainly used in IPF because it does not respond to immunosuppressive therapy. Immunomodulatory therapy includes corticosteroids, azathioprine, cyclophosphamide, mycophenolate mofetil, rituximab, and tocilizumab (69). If disease progression is observed despite the first-line treatment, antifibrotic drugs should be considered a second-line therapy to

counter progressive fibrosing (69). Before defining a PF-ILD phenotype, it is recommended to set an immunosuppressive therapy that can be useful, for example, in stabilizing the course of CT-ILD and HP (4). The administration of mycophenolate mofetil, azathioprine, or cyclophosphamide can get the stabilization of lung involvement in many cases of CT-ILD; on the contrary, in IPF, the immunosuppressive therapy can be dangerous. Therefore, decisions regarding drug therapy must be carefully evaluated case-by-case (4). In the context of ILDs, the need to intervene with pharmacological treatment is found in patients with progressive fibrosis. This portion of patients experiences worsening lung function, a decline in quality of life, and early mortality (70).

Nintedanib is an intracellular inhibitor of tyrosine kinases, which inhibits the processes involved in the progression of lung fibrosis. It has been approved for treating IPF and systemic sclerosis-associated interstitial lung disease (SSc-ILD). Still, many efforts have recently been made to make patients with PF-ILD other than IPF and SSc-ILD eligible for Nintedanib (71). The INBUILD trial suggests that nintedanib, regardless of the underlying ILD diagnosis, reduces the rate of ILD progression, as measured by FVC decline, in patients who have a chronic fibrosing ILD and show a progressive phenotype despite appropriate management (72). More in detail, regarding the primary endpoint of the INBUILD trial, in the overall population, the adjusted rate of decline in the FVC over the 52-week period was −80.8 ml per year in the nintedanib group and −187.8 ml per year in the placebo group (between-group difference, 107.0 ml; 95% confidence interval, $P<0.001$) (71). Nintedanib is administered orally, and the most common adverse events are gastrointestinal, mainly diarrhea, nausea, weight loss, and an increase in alanine aminotransferase and aspartate aminotransferase (71). The recommended dosage is 150 mg twice daily, except for patients who do not tolerate the higher dose or have mild hepatic impairment, for which a reduced dosage of 100 mg twice daily is recommended (73). Nintedanib presents a manageable tolerability profile in patients with PF-ILDs in clinical trials and real-world studies (73).

Pirfenidone is the other antifibrotic drug approved for the treatment of IPF. Similarly to nintedanib, it is orally administered (74). In addition to the anti-fibrotic effect obtained by suppressing growth factors, pirfenidone also has an anti-inflammatory role (74). The recommended daily dose of pirfenidone is 801 mg three times per day, which can eventually be reduced or suspended in the case of short-term side effects that generally occur early and have short-term (75). The most common adverse events of pirfenidone are gastrointestinal (e.v. nausea, diarrhea) and skin-related (rash) (75).

A conceptual framework for the treatment of ILDs (Figure 1.11) (69):

- Patients with IPF should be treated with antifibrotic drug (red bar),

Among the patients with other ILDs:

- Stable patients only need to be monitored (white arrow),
- In the case of progressive phenotype (PF-ILD), patients may benefit from immunomodulatory therapy as first-line therapy (blue arrow) or antifibrotic therapy as a second-line treatment (red arrow). The combination of immunomodulatory and antifibrotic drugs (purple arrow) could be considered only in particular cases, but further evidence should be obtained.

The decisions about drug treatment require an assessment of the risk-benefit profile and need to be individualized, considering drug access, comorbidities, and patient preferences (39).

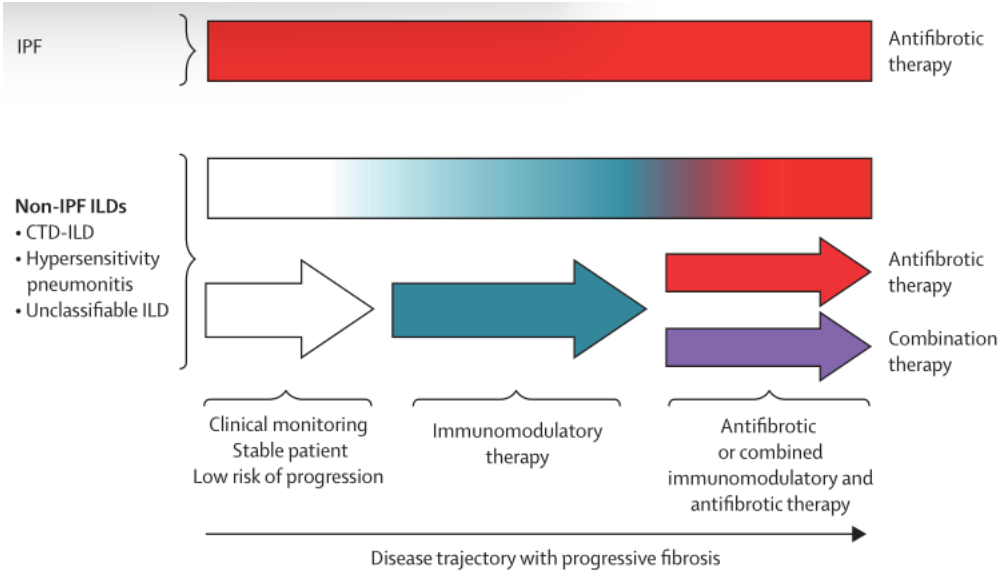

Figure 1.11: Framework for treating ILD, adapted from Johannson et al.

The last chance in case of irrepressible fibrosis progression despite pharmacological treatment is lung transplantation, which can be a life-extending option; currently, among the most common indications for lung transplantation are interstitial lung diseases (ILDs) (76). Nonpharmacological treatment of patients with ILDs includes the management of comorbidities, of which gastroesophageal reflux (GERD) and pulmonary hypertension are frequent (69). The prevalence of GERD or esophageal dysmotility is higher in patients with ILDs compared to those without them, and further studies are needed to establish whether treatment of GERD, either pharmacologically (proton pump inhibitors PPI) or surgically (laparoscopic fundoplication), can affect the interstitial lung disease progression, complications, or other significant outcomes (such as delay in lung transplantation) (77). Several specific drugs have been tested for the treatment of pulmonary hypertension due to interstitial lung disease; among these, the use of inhaled Treprostinil has recently shown an improvement in exercise capacity from baseline, assessed with the help of a 6-minute walk test, as compared with placebo (78).

ILDs also require non-pharmacological treatments such as symptom management, pulmonary rehabilitation, preventive strategies, end-of-life planning, and education and support for caregivers and patients (69).

Supplemental oxygen therapy should be considered for patients with ILD in case of severe resting hypoxemia or isolated exertional hypoxemia in the context of exercise limitation or symptoms to improve gas exchange (69). Supplemental oxygen is the only treatment, other than lung transplantation, capable of enhancing hypoxemia that persists despite optimal medical management of the underlying disease (79).

1.6 Idiopathic pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is the most common disease among idiopathic interstitial pneumonia (IIPs) and represents the prototype of progressive fibrosis (80). The clinical course of IPF is characterized by increasing symptoms, mainly dyspnea and cough, and poor quality of life. However, at the time of diagnosis, the progression of the single patient is difficult to predict, and the prognosis is generally poor (21). The available antifibrotic treatments help reduce the risk of acute exacerbations and improve overall survival, but they can only slow the fibrosis progression (6). Despite the recent advantages in disease management and drug treatment, the median survival of IPF remains between 2-5 years, and some studies suggest that it is worse than many cancers that affect people with similar demographic data (81) (82). The diagnosis of IPF is reached more frequently in male and smoker patients over 60 years old, and in about 3% of the cases, a familial clustering is identified (83). About 40,000 new diagnoses of IPF are reached in Europe every year, and the incidence is expected to increase because of the global population aging (84). The diagnosis of IPF could be challenging and still too frequently obtained after significant diagnostic delay. To avoid waste of time and ensure a better prognosis for the patients, diagnosis and management of IPF require a multidisciplinary team of experts (81). One of the reasons that can explain the diagnostic delay is that, generally, the first symptoms of IPF are dyspnea and non-productive cough, which are non-specific and can be attributed to other respiratory disorders, smoking, aging, or comorbidities (81). The clinical suspicion of ILD arises when bibasilar “velcro” crackles and digital clubbing are found on physical examination (32) (35). In the case of newly detected ILD, the suspicion of a diagnosis of IPF, which is by

definition an idiopathic disease, arises when other specific diagnoses of ILD are excluded on the basis of the tests performed (85).

In the case of suspected IPF, the evaluation steps include (85) (86):

- Obtaining detailed medical history (including information about environmental and occupational exposure, pharmacological treatments, and chest radiation) and careful physical examination;
- Serological testing can be helpful in the differential diagnosis in the field of ILDs;
- Define the severity of functional impairment through the pulmonary function tests (PFT);
- High-resolution computer tomography (HRCT) to define patterns and distributions of alterations;
- Multidisciplinary discussion.

Usual Interstitial Pneumonia (UIP) is the typical radiologic and histopathologic pattern identifier of IPF (82). On HRCT, the UIP pattern is characterized by the presence of honeycombing (subpleural cystic airspaces delimited by defined walls), traction bronchiectasis (dilatation of the bronchi), and traction bronchiolectasis (dilatation of the bronchioles), which may be associated with fine reticulation and ground-glass opacification (85). In a compatible clinical context, founding a radiological UIP pattern with a mostly peripheral, basal, and bilateral distribution allows for the diagnosis of IPF (82). In the case of patients with HRCT patterns of probable UIP or indeterminate UIP, it is suggested to perform, if it is possible, the surgical lung biopsy (SLB) or, eventually, the analysis of the BAL (Figure 1.12) (85).

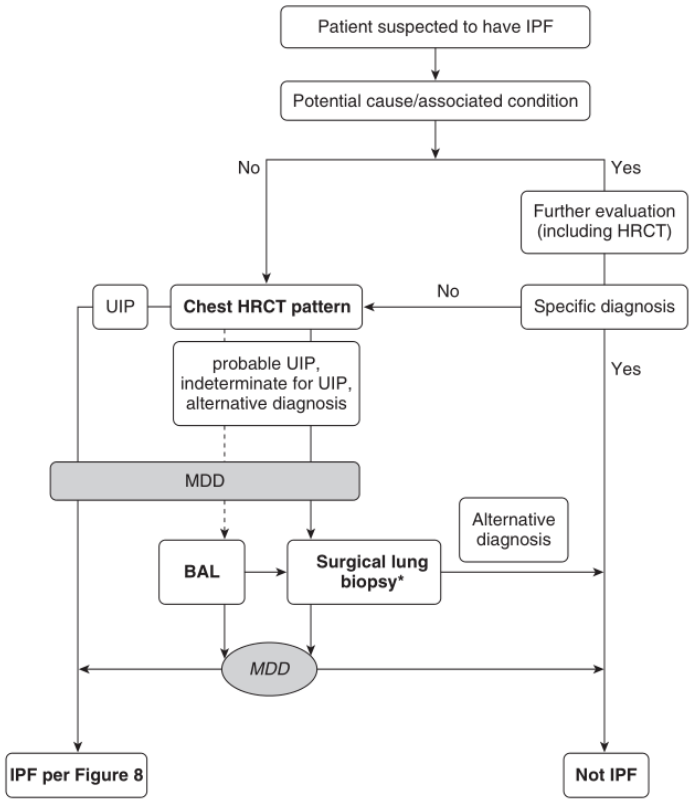

Figure 1.12: Diagnostic algorithm for idiopathic pulmonary fibrosis (IPF), adapted from Diagnosis of Idiopathic Pulmonary Fibrosis. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline.

Guidelines indicate the possibility of performing a surgical lung biopsy if HRCT is insufficient for a conclusive diagnosis of IPF. Still, it is necessary to carefully select patients who can face this invasive procedure, which presents a high risk of complications and mortality (82). The histopathological features of the UIP pattern are the finding of patchy dense fibrosis, the presence of inflammatory infiltrate of lymphocytes and plasma cells, and the hyperplasia of bronchiolar epithelium and type 2 pneumocytes (85) (Figure 1.13).

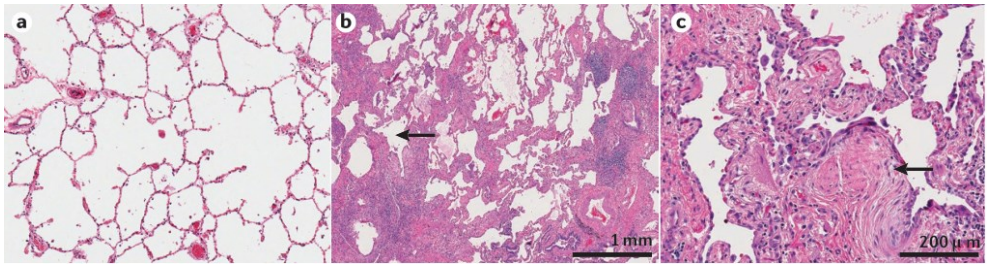

Figure 1.13: Histopathological features of UIP. ***a)*** *Normal lung histology of a terminal bronchiole, respiratory bronchiole, alveolar duct and alveoli. Magnification ×10.* ***b)*** *Low-power hematoxylin and eosin-stained section obtained from a surgical lung biopsy in a patient with UIP.* ***c)*** *High-power hematoxylin and eosin-stained section obtained from the same lung biopsy as in panel b, adapted* from Martinez et al.

Because of the inflammatory infiltrate, IPF has been considered an inflammatory disease. Still, over time, evidence suggested the critical role of the epithelium, which activation induced by subclinical injuries, the production of factors and cytokines that stimulate fibroblast migration, proliferation, and differentiation into myofibroblasts (82) (84). Some genetic factors, such as MUC5B, may predispose a greater susceptibility to this aberrant reparative response that can result in progressive fibrosis and respiratory dysfunction (83) (84).

Patients with IPF require supportive care, including supplemental oxygen, pulmonary rehabilitation, and prevention of acute exacerbations and pulmonary infections (87). Antifibrotic drugs should be considered for these patients to slow the disease progression and reduce the acute exacerbations; instead, immunosuppressive treatments are not indicated (69). Only a minority of patients with IPF meet the eligibility criteria and get a lung transplant, the only cure for this disease (84).

1.7 Blood count and monocytes

In suspected ILD cases, routine laboratory evaluation includes complete and differential blood counts, which are inexpensive and easily accessible (49). Laboratory tests are part of the diagnostic workup, but there is growing interest in recognizing and applying biomarkers to predict disease course and response to the therapy (20). In some progressive ILDs, the monocyte count has been reported as a potentially relevant biomarker. Still, it is necessary to

consider that it may be affected by ongoing infections or medications (88) (Figure 1.14).

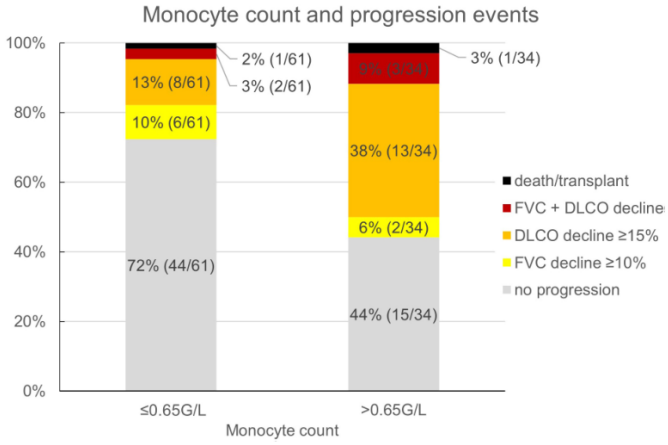

Figure 1.14: Monocyte count and fraction (number) of progression events (FVC: Forced Vital Capacity: DLCO: Diffusion Lung CO) Adapted from From Shao et al.

Prognostic biomarkers are urgently needed in patients with idiopathic pulmonary fibrosis (IPF), a lethal disease (89). In a retrospective analysis of ASCEND, CAPACITY, and INSPIRE trials, in which patients with IPF have been enrolled, the founding of elevated monocyte count (> 0,60 x 10^9cells/L) has been associated with increased risks of progression, hospitalization, and mortality over 1 year (90). According to other recent studies, it is known that the white blood count and the monocyte count, at the baseline, negatively correlate with lung function; moreover, the monocyte count is an independent predictor of progression during the first year of antifibrotic treatment and that in patients with a recent diagnosis of IPF the lymphocyte to monocyte ratio (LMR) lower than 4,18 it is associated with shorter survival (91).

Focusing on systemic sclerosis (SSc), it has been found that the white blood cells and the monocyte and neutrophil counts are higher in patients with lung involvement (ILD) than in patients without it, and also that the monocytes count is higher in patients with progressive lung fibrosis than non-progressors (92).

The findings of a multivariate analysis that considered interstitial lung abnormalities (ILA) have brought to light that the values of monocytes, monocyte

to lymphocyte ratio (MLR), neutrophil to lymphocyte ratio (NLR), and systemic inflammatory response (SIRI: (monocytes x neutrophils)÷ lymphocytes) are associated with radiological progression of lung disease (93). Therefore, the application of biomarkers could be helpful also in stratifying the risk of patients with early-fibrotic ILA and selecting which ones deserve closer and careful monitoring over time. Indeed, some early-fibrotic ILAs can progress to IPF or other PF-ILD (93).

More evidence is currently needed to establish robust markers to improve the management of ILDs, particularly in the heterogeneous group of PF-ILD other than IPF (88).

The macrophage populations inside the lungs contribute to the immune defense and inflammation; however, if the inflammation triggers persist and the inflammatory response is not adequately limited, pathological fibrosis may develop, leading to lung impairment and respiratory failure (94). Both alveolar macrophages and monocyte-derived macrophages can polarize into two different states: classically activated phenotype (M1), which is mainly involved in pro-inflammatory response and prevail in the stage of lung injury, and the alternatively activated phenotype (M2), which contributes to anti-inflammatory response and plays a role in the repair process (95). The development of lung fibrosis is a complex and not yet fully understood pathological process characterized by persistent lung injuries that lead to monocytes recruitment and subsequent aberrant polarization into M2 macrophage whose abnormal activity can produce an excess of TGF-β1 that, together with overexpression of cytokines (in particular IL-4 and IL-10) contributes to the differentiation of fibroblast into myofibroblast (94). The polarization, proliferation, and apoptosis of macrophages and monocytes derived macrophages are closely intertwined with that of fibroblast, myofibroblast, and alveolar cells; therefore, improving the knowledge about the micro-environment and the crosstalk between the various cells could be a challenging opportunity to make progress in drug therapy (94).

2. AIM OF THE STUDY

This retrospective and multicentric study considers whether clinical, radiological, and hematological features could predict progression in patients with ILD. The second goal is to evaluate and compare the blood count at the time of the diagnosis between patients with idiopathic pulmonary fibrosis (IPF) and other ILDs that are not IPF.

3. MATERIALS AND METHODS

3.1 Study design and population

In this study, 119 patients with ILDs, referred to the University Hospital of Padua, and 147 patients with IPF, referred both to the University Hospital of Padua (n°92) and University Hospital of Palermo (n°55), are retrospectively enrolled. Data are collected from the beginning of 2017 up to May 2023. This study is performed following the declaration of Helsinki and is approved by the University of Padua ethics committee (n° 428/AO/17). Demographic and clinical data are obtained at the time of the diagnosis and during the follow-up, particularly one year before and at the last follow-up; the complete blood counts are collected at the time of the diagnosis. Focusing on the ILD population, some exclusion criteria were applied for patient selection: diagnosis of cystic diseases (Langerhans cell histiocytosis (LCH), lymphangioleiomyomatosis (LAM)), interstitial lung involvement in patients with rheumatologic or autoimmune diseases (CTD-ILD), sarcoidosis (except for sarcoidosis at the fibrosing stage (IV)). Regarding the IPF population, patients with concomitant lung cancer were excluded.

3.2 Definition of disease progression

3.2.1 ILD population

The following data on the ILD population were collected at the time of the diagnosis:

- Clinical data (gender, date of birth, age at diagnosis, specific diagnosis, Body Mass Index (BMI), exposures to occupational or environmental agents including both organic and inorganic substances, drug and radiation exposure);
- Smoking history (current-smokers and former smokers) and pack-years;

- Comorbidities (cardiovascular, oncological, metabolic, gastrointestinal, and pneumological diseases);
- Symptoms (dyspnea at rest, exertional dyspnea, cough, chest pain, asthenia, and fever);
- Blood test (Haemoglobin, White Blood Cells Count, Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils);
- Pulmonary Function Tests (PFTs);
- HRCT.

Other data are obtained during the follow-up:

- Drug treatment (corticosteroids and antifibrotic drugs);
- Long-term oxygen therapy at rest or under exertion;
- Pulmonary Function Tests (PFTs);
- Follow-up HRCT;
- Date of death or lung transplant.

To define PF-ILD it is necessary to satisfy at least two of the following three criteria occurring within the last year with no alternative explanation (66):

1) Worsening of respiratory symptoms;

2) Physiological evidence of disease progression (either of the following):

- Absolute decline in FVC ≥ 5% predicted within one year of follow-up;
- The absolute decline of DLCO (corrected for Hb) ≥ 10% expected within one 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;

- The increased extent or increased coarseness of reticular abnormality;
- New or improved honeycombing;
- Increased lobar volume loss.

In this study the decline in DLCO was considered significant only if it was associated with the decline in FVC. In this study patients who needed antifibrotic therapy, were transplanted or on the transplant list, or died due to the evolution of pulmonary fibrosis were also considered progressive.

3.2.2 IPF population

The following data on the IPF population were collected at the time of the diagnosis:

- Clinical data (gender, date of birth, age at diagnosis, date of the first drug administration, Body Mass Index (BMI));
- Smoking history (current-smokers and former smokers) and pack years;
- Comorbidities (cardiovascular, metabolic, and GERD);
- Blood test (White Blood Cells Count, Neutrophils, Lymphocytes, Monocytes);
- Pulmonary Function Tests (PFTs);
- Prescription of Nintedanib or Pirfenidone.

Other data are obtained during the follow-up:

- Long-term oxygen therapy at rest or under exertion;
- Pulmonary Function Tests (PFTs);
- Date of death or lung transplant.

IPF represents the prototype of progressive fibrosis, but even within this group of patients, it is possible to differentiate between slow and fast progressors. The parameter to which reference is made for this differentiation is the

delta FVC/year, calculated based on the FVC values of two spirometry, and patients are classified as fast progressors when this delta is greater than 5%.

3.2.3 Blood tests

Complete blood counts at diagnosis were collected for the ILD and IPF populations.

3.3 Statistical analysis

Descriptive statistics are applied to summarize the demographic and clinical features of patients: continuous variables are described as median value and range (min-max), whereas categorical variables as absolute (n) ad relative values (%). Mann-Whitney U test is used for quantitative variables, and Fisher's exact test is used for categorical variables. Correlation coefficients between data are calculated using the non-parametric Spearman's rank method. Overall survival was defined as the 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 analyzed 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.

4. RESULTS

4.1 Clinical features of the study populations

Considering the overall population, patients are predominantly male (67%), with a median age at the diagnosis of 68 years (range: 30-87) and a median BMI of 27.4 Kg/m^2 (range: 17.7 - 38.9). More than half of the patients are former smokers (56 %), and 7% are current smokers; the median value of pack-years is 11.1 (range: 0 -160). Other demographic and clinical data of these 266 patients are shown in Table I.

The population comprises 119 patients with ILD and 147 patients with IPF. These two subgroups differ in age at diagnosis: IPF patients are older at the time of diagnosis compared to ILD patients [70 years (46-84) vs. 63 years (30-87); $p < 0.0001$]; they also differ in male prevalence, which is higher in the IPF patients (80% vs. 50%; $p < 0.001$). The two subgroups (ILD vs. IPF) showed no differences regarding BMI (27.1 kg/m^2 vs. 27.5 kg/m^2 p=0.79) and the number of current smokers (8.4% vs. 6% p=0.48). However, the number of pack-years is significantly lower in the ILD population (0 vs. 17; p=0.0002). Regarding comorbidities, patients with IPF present more frequently with metabolic comorbidities (50% vs. 30%; p=0.0008). However, cardiovascular ones (69% vs. 64%; p=0.36) and GERD (40% vs. 34%; p=0.31) have no differences. Moreover, no statistically significant difference was found regarding the values of FVC (L) (2.61 vs. 2.63 liters; p=0.38), FVC%pred (80% vs. 78%; p=0.16), and DLCO (59% vs. 53%; p=0.07) at diagnosis.

Complete blood counts at the time of diagnosis reveal no statistically significant difference between ILD and IPF patients regarding WBC (7.30 vs. 8.24 x 10^9/L p=0.28), neutrophils (n°) (4.23 vs. 4.58 x 10^9/L; p=0.29), neutrophils (%) (60.3 vs. 57.9 p=0.16), lymphocytes (%) (28.35 vs. 29.5; p=0.10), monocytes (n°) (0.62 vs. 0.67 x 10^9/L p=0.19), and monocytes (%) (8.3 vs. 8.29; p=0.7. However, the lymphocytes (n°) differ between the two subgroups (1.8 vs. 2.25 x 10^9/L; p=0.003).

	Overall (266)	*ILD (119)*	*IPF (147)*	*p-value*
Age at diagnosis – years	68 (30-87)	63 (30-87)	70 (46-84)	**<0.0001**
Sex – Male n° (%)	178 (67%)	60 (50%)	118 (80%)	**<0.0001**
BMI – (Kg/m^2)	27.4 (17.7-38.9)	27.1 (17.7-38.9)	27.5 (19.4-38.3)	0.79
Pack-Years	11.1 (0-160)	0 (0-160)	17 (0-100)	**0.0002**
Current smoker – n°(%)	19 (7%)	10 (8.4%)	9 (6%)	0.48
Former smoker – n°(%)	149 (56%)	53 (44%)	96 (65%)	**0.0008**
Comorbidities				
• *Cardiovascular – n° (%)*	178 (67%)	76 (64%)	102 (69%)	0.36
• *Metabolic – n° (%)*	109 (41%)	36 (30%)	73 (50%)	**0.002**
• *GERD – n° (%)*	99 (37%)	40 (34%)	59 (40%)	0.31
Pulmonary Function Tests				
• *FVC (L)*	2.6 (0.99-5.03)	2.61 (0.99-5.03)	2.63 (1.12-4.61)	0.38
• *FVC (%)*	79 (31-148)	80 (31-148)	78 (40-140)	0.16
• *DLCO*	55 (18-126)	59 (18-126)	53 (19-116)	0.07
Complete Blood Count				
• *WBC (x 10^9/L)*	7.92 (2.8-17.55)	7.30 (2.8-16.57)	8.24 (2.9-17.55)	0.28
• *Neutrophils (x 10^9/L)*	4.4 (0.78-14.9)	4.23 (0.94-14.55)	4.58 (0.78-14.9)	0.29
• *Neutrophils (%)*	58.26 (26.9-89.5)	60.3 (33-89.5)	57.9 (26.9-86.7)	0.16
• *Lymphocytes (x 10^9/L)*	2.14 (0.46-5.87)	1.8 (0.46-5.87)	2.25 (0.68-5.3)	**0.003**
• *Lymphocytes (%)*	28.82 (5.6-58.7)	28.35 (5.6-58.7)	29.5 (6.9-58.1)	0.10
• *Monocytes (x 10^9/L)*	0.64 (0.11-1,72)	0.62 (0.11-1.29)	0.67 (0.25-1.72)	0.19
• *Monocytes (%)*	8.3 (2.4-26.1)	8.3 (2.9-17.5)	8.29 (2.1-26.1)	0.74

*Table I: **Demographics and clinical features of the overall patient with ILD and IPF.** (BMI: Body Mass Index, GERD: Gastroesophageal reflux disease, FVC: Forced Vital Capacity, DLCO: Diffusion Lung CO, WBC: White Blood Cells. Values are expressed as numbers and (%) or median and range, as appropriate. To compare demographics between ILD and IPF, the chi-square test and Fisher's t-test for categorical variables, and Mann–Whitney t-test for continuous variables were used)*

4.2 ILD population

Patients with different specific diagnoses are included within the ILD population, the most frequent being (Figure 4.1): OP (23,5%); HP (18.5%), Unclassifiable (16.8%), Drug-related-ILD (10.1%); Smoking-related-ILD (8.4%) and NSIP (8.4%); regarding further details on the distribution of diagnoses between PF-ILD and NP-ILD refer to table II.

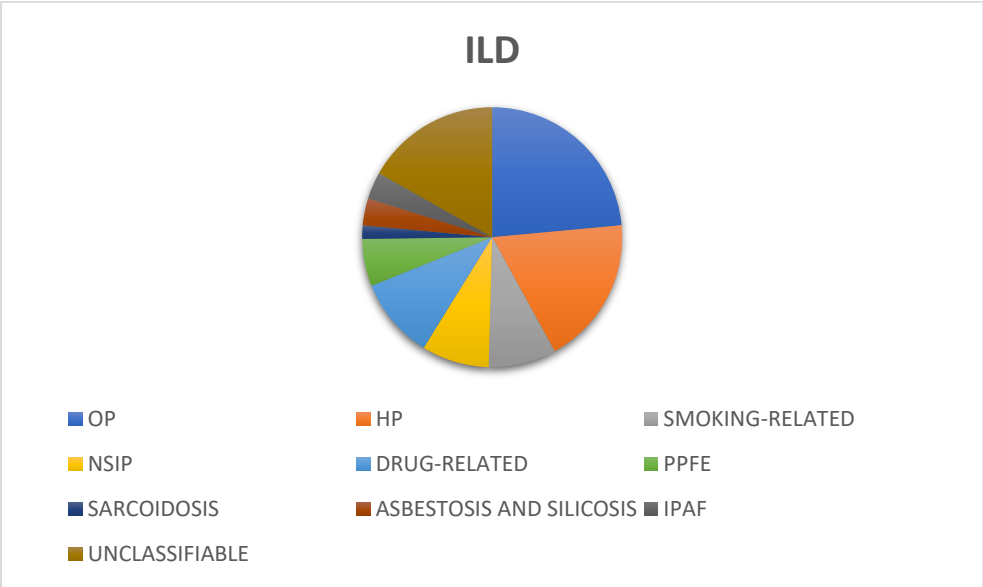

Figure 4. 1: Distribution of the diagnosis in the ILD population. (OP: Organizing Pneumonia; HP: Hypersensibility Pneumonia; NSIP: Non Specific Interstial Pneumonia; PPFE: PleuroParenchymal FibroElastosis; IPAF: Interstitial Pneumonia with Autoimmune Features.)

Given the application of defined progression criteria, of the 119 patients diagnosed with ILDs, 43 are considered progressors (progressive fibrosing ILD; PF-ILD) and 76 non-progressors (Non-Progressive ILD; NP-ILD).

Considering the ILD population (Table III), patients with PF-ILD are younger at the diagnosis in comparison with NP-ILD (59 vs. 65.5 years; p=0.008), the two subgroups do not differ in sex (58% vs. 67%; p=0.43), BMI (26.9 vs. 27,9; p=0.46), pack-years (6 vs. 0; p=0.44), use of corticosteroid therapy (74% vs. 63%; p=0.53), but other environmental exposures are more frequently in PF-ILD (69% vs. 48%; p=0.04).

	ILD (119)	*PF-ILD (43)*	*NP-ILD (76)*
OP – n° (%)	28 (23.5%)	4 (9.3%)	24 (31.6%)
HP – n° (%)	22 (18.5%)	13 (30.2%)	9 (11.8%)
Unclassifiable – n° (%)	20 (16.8%)	7 (16.3%)	13 (17.1%)
Drug-related – n° (%)	12 (10.1%)	2 (4.7%)	10 (13.1%)
Smoking-related – n° (%)	10 (8.4%)	5 (11.6%)	5 (6.6%)
NSIP – n° (%)	10 (8.4%)	5 (11.6%)	5 (6.6%)
PPFE – n° (%)	7 (5.9%)	4 (9.3%)	3 (3.9%)
Asbestosis and Silicosis – n° (%)	4 (3.4%)	1 (2.3%)	3 (3.9%)
IPAF – n° (%)	4 (3.4%)	1 (2.3%)	3 (3.9%)
Sarcoidosis (IV stage) – n° (%)	2 (1.7 %)	1 (2.3%)	1 (1.3%)

Table II: **Prevalence of different specific diagnoses in the ILD population.** (PF-ILD: progressive-fibrosing ILD, NP-ILD: non-progressive ILD, OP: Organizing Pneumonia, HP: Hypersensitivity Pneumonitis, NSIP: Non-Specific Interstitial Pneumonia, PPFE: Pleuroparenchymal Fibroelastosis, IPAF: interstitial pneumonia with autoimmune features). Values are expressed as numbers and (%).

There is no statistically significant difference regarding comorbidities and symptoms at the time of diagnosis with two exceptions: dyspnea on exertion (76% vs. 53%; p=0.02) more frequent in the case of PF-ILD, and fever (2% vs. 16%; p=0.03), which on the contrary is more frequent in case of NP-ILD. Focusing on HRCT PF-ILD reported more frequent reticulations (89% vs. 62%; p=0.03), while NP-ILD reported more frequently the presence of consolidation (34% vs. 3% p=0.001).

	PF-ILD (43)	*NP-ILD (76)*	*p*
Age - years	59 (30-75)	65.5 (37-87)	**0.008**
Sex – Male n° (%)	25 (58%)	51 (67%)	0.43
BMI - (kg/m²)	26.9 (18.3-37.5)	27.9 (17.7-38.9)	0.46
Pack-years	6 (0-90)	0 (0-160)	0.44
Current smoker – n° (%)	3 (7%)	7 (9%)	0.99
Former smoker – n° (%)	21 (50%)	32 (43%)	0.56
Exposure	29 (69%)	36 (48%)	**0.04**
Comorbidities			
• *Cardiovascular – n° (%)*	30 (70%)	46 (61%)	0.33
• *Metabolic – n° (%)*	11 (25,6%)	25 (33%)	0.53
• *GERD – n° (%)*	18 (42%)	22 (29%)	0.16
• *Pneumological – n° (%)*	11 (26%)	19 (25%)	0.99
Symptoms			
• *Dyspnea at rest – n° (%)*	3 (7%)	8 (11%)	0.74
• *Dyspnea on exertion– n° (%)*	32 (76%)	40 (53%)	**0.02**
• *Cough – n° (%)*	23 (55%)	43 (57%)	0.84
• *Chest pain – n° (%)*	2 (5%)	5 (7%)	0.99
• *Fever – n° (%)*	1 (2%)	12 (16%)	**0.03**
• *Asthenia – n° (%)*	1 (2%)	8 (11%)	0.15
HRCT			
• *GGO*	10 (27%)	24 (35%)	0.4
• *Reticulations*	33 (89%)	42 (62%)	**0.03**
• *Consolidations*	1 (3%)	23 (34%)	**0.001**
• *Bronchiectasis*	10 (27%)	14 (21%)	0.63
• *Honeycombing*	8 (22%)	7 (10%)	0.15
Corticosteroids therapy	32 (74%)	51 (67%)	0.53

Table III: **Demographics and clinical features of the ILD population**. (PF-ILD: progressive-fibrosing ILD, NP-ILD: non-progressive ILD, BMI: Body Mass Index, GERD: Gastroesophageal reflux disease, HRCT: High-Resolution Chest Tomography, GGO: ground-glass opacity). Values are expressed as numbers and (%) or median and range, as appropriate. To compare demographics between PF-ILD and NP-ILD, the chi-square test, Fisher's t-test for categorical variables, and Mann–Whitney t-test for continuous variables were used).

Regarding pulmonary function tests at the time of diagnosis (Table IV), all the values are significantly lower in the PF-ILD in comparison with NP-ILD: FVC (L) (2.32 vs. 3.12 litres; p<0.0001; FVC%pred. (72% vs. 90%; < 0.0001); FEV1 (L) (2.13 vs. 2.57 litres; p=0.009); FEV1%pred. (79 vs. 96 p=0.0001); TLC (L) (3.56 vs. 4.53 litres; p= 0.0001); TLC%pred. (57.5% vs. 82%; p<0.0001); DLCO%pred. (49.5% vs. 65%; p=0.0001).

	PF-ILD	***NP-ILD***	***p***
FVC (L)	2.32 (0.99-4.18)	3.12 (1.14-5.03)	**<0.0001**
FVC (%)	72 (31-108)	90 (43-148)	**< 0.0001**
FEV1 (L)	2.13 (0.89-4.46)	2.57 (1.02-4.30)	**0.009**
FEV1 (%)	79 (32-119)	96 (39-154)	**0.0001**
TLC (L)	3.56 (1.41-7.75)	4.53 (1.84-8.12)	**0.0001**
TLC (%)	57.5 (30-105)	82 (38-109)	**<0.0001**
DLCO	49.5 (18-105)	65 (21-126)	**0.001**

Table IV: **Pulmonary Function Tests (PFTs) at the time of diagnosis** (FVC: Forced Vital Capacity, FEV1: Forced expiratory volume in one second, TLC: Total lung capacity, DLCO: Diffusion Lung CO. Values are expressed as median and range. To compare the PFTs between PF-ILD and NP-ILD Mann–Whitney t-test for continuous variables was used)

To evaluate the possible progression of the disease, the worsening of symptoms, delta FVC/year, and HRCT of the last follow-up compared with that of the previous year were assessed (Table V). The comparison between PF-ILD and NP-ILD shows a statistically significant difference in worsening of symptoms (71% vs. 10% p<0.0001), HRCT (61% vs. 10% p<0.0001,) and delta FVC/year calculated based on the FVC values of two spirometry (51% vs 12% p<0.0001).

	PF-ILD	***NP-ILD***	***p***
Evaluation at the last follow-up			
• *Worsening of symptoms*	29 (71%)	7 (10 %)	**<0.0001**
• *Worsening of HRCT*	23 (61%)	7 (10%)	**<0.0001**
• *Worsening of FVC*	21 (51%)	7 (12%)	**<0.0001**

Table V: **ILD definition of progression** (HRCT: High-Resolutionn Chest Tomography; FVC: Forced Vital Capacity. Values are expressed as numbers and (%). To compare progression data between PF-ILD and NP-ILD, the chi-square test and Fisher's t-test for categorical variables were used)

4.3 Blood tests in the study population

Complete blood counts of the diagnosis time were collected for ILD and IPF populations. Focusing on the ILD population (Table VI), no differences between PF-ILD and NP-ILD regarding the RBC, Hgb, WBC, neutrophils, lymphocytes, eosinophils, and basophils are reported. Still, the value of monocyte results is significantly higher in PF-ILD than in NP-ILD (0.68 vs 0.59 x 10^9/L p=0.0007).

	PF-ILD	***NP-ILD***	***p***
RBC (x 10^{12}/L)	4.67 (3.29-5.94)	4.68 (2.43-7.43)	0.44
Hgb (g/L)	143.5 (99-174)	140 (89-168)	0.07
WBC (x 10^9/L)	7.91 (4.68-16.01)	7.06 (2.81-16.57)	0.08
Neutrophils (x 10^9/L)	4.54 (1.99-12.81)	4.06 (0.94-14.55)	0.10
Neutrophils (%)	61.2 (36.9-89.5)	59.4 (33.5-87.8)	0.37
Lymphocytes (x 10^9/L)	1.99 (0.60-4.8)	1.78 (0.46-5.87)	0.38
Lymphocytes (%)	28.15 (5.60-46.20)	28.45 (5.8-58.7)	0.44
Monocytes (x 10^9/L)	0.68 (0.29-1.29)	0.59 (0.11-0.97)	**0.0007**
Monocytes (%)	8.8 (3.10-13.70)	8.10 (2.90-17.5)	0.21
Eosinophils (x 10^9/L)	0.11 (0.001-0.82)	0.14 (0.01-0.65)	0.57
Eosinophils (%)	1.45 (0.001-7.7)	2.10 (0.10-13.4)	0.25
Basophils (x 10^9/L)	0.03 (0.001-0.16)	0.03 (0.001-0.06)	0.63
Basophils (%)	0.40 (0.001-1.5)	0.40 (0.001-1.1)	0.80

Table VI: **Complete Blood Count at the time of diagnosis of ILD population** (RBC: Red Blood Cells Count, Hgb: haemoglobin; WBC: White Blood Cells Count. Values are expressed as median and range. To compare CBCs between PF-ILD and NP-ILD Mann–Whitney t-test for continuous variables was used).

Between IPF and NP-ILD, a statistically significant difference was found for both monocytes (0.67 vs. 0.59 x 10^9/L; p=0.008) and lymphocytes (2.25 vs. 1.78 x 10^9/L; p=0.0002), as shown in Table VII

	IPF	***NP-ILD***	***p***
WBC (x10^9/L)	8.24 (2.9-17.55)	7.06 (2.81-16.57)	0.004
Neutrophils (%)	57.9 (26.9-86.7)	59.4 (33.5-87.8)	0.43
Neutrophils (x10^9/L)	4.58 (0.78-14.9)	4.06 (0.94-14.55)	0.06
Lymphocytes (%)	29.5 (6.9-58.1)	28.45 (5.8-58.7)	0.33
Lymphocytes (x10^9/L)	2.25 (0.68-5.3)	1.78 (0.46-5.87)	**0.0002**

Monocytes (%)	8.29 (2.1-26.1)	8.10 (2.90-17.5)	0.67
Monocytes (x10^9/L)	0.67 (0.25-1.72)	0.59 (0.11-0.97)	**0.008**

Table VII: **Complete Blood Count of patients with IPF and NP-ILD.** (WBC: White Blood Cells Count. Values are expressed as median and range. To compare CBCs between IPF and NP-ILD Mann–Whitney t-test for continuous variables was used).

On the contrary, no significant difference was found between IPF and PF-ILD (Table VIII).

	IPF	***PF-ILD***	***p***
WBC (x10^9/L)	8.24 (2.9-17.55)	7.91 (4.68-16.01)	0.99
Neutrophils (%)	57.9 (26.9-86.7)	61.2 (36.9-89.5)	0.10
Neutrophils (x10^9/L)	4.58 (0.78-14.9)	4.54 (1.99-12.81)	0.51
Lymphocytes (%)	29.5 (6.9-58.1)	28.15 (5.60-46.20)	0.07
Lymphocytes (x10^9/L)	2.25 (0.68-5.3)	1.99 (0.60-4.8)	0.06
Monocytes (%)	8.29 (2.1-26.1)	8.8 (3.10-13.70)	0.21
Monocytes (x10^9/L)	0.67 (0.25-1.72)	0.68 (0.29-1.29)	0.22

Table VIII: **Complete Blood Count of patients with IPF and PF-ILD.** (WBC: White Blood Cells Count). Values are expressed as median and range. To compare CBCs between IPF and PF-ILD Mann–Whitney t-test for continuous variables was use).

Regarding the value of monocytes, the results of our study show a statistically significant difference between IPF and NP-ILD and between PF-ILD and NP-ILD. At the same time, there is no statistically significant difference between IPF and PF-ILD (Figure 4.2).

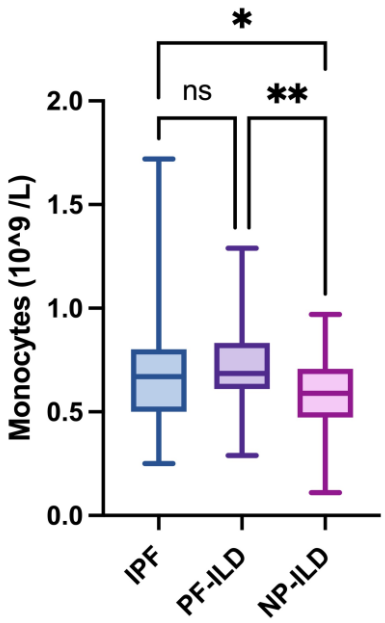

Figure 4.2: Levels of monocytes in IPF, PF-ILD, and NP-ILD. Horizontal bars represent median values; the bottom and top of each box plot 25th and 75th (PF-ILD vs. NP-ILD p=0.0007; IPF vs. NP-ILD p=0.008).

We further analyzed the probability of survival at ten years by dividing the PF-ILD population according to the value of monocytes, greater or less than 0.6 x 10^9 /L. In the group of patients whose monocyte level is higher than 0.6 x 10^9 /L, the probability of survival at 120 months is about 50%, instead in the group in which this level is lower than 0.6, the likelihood of survival is 100% (p=0.05) (Figure 4.3).

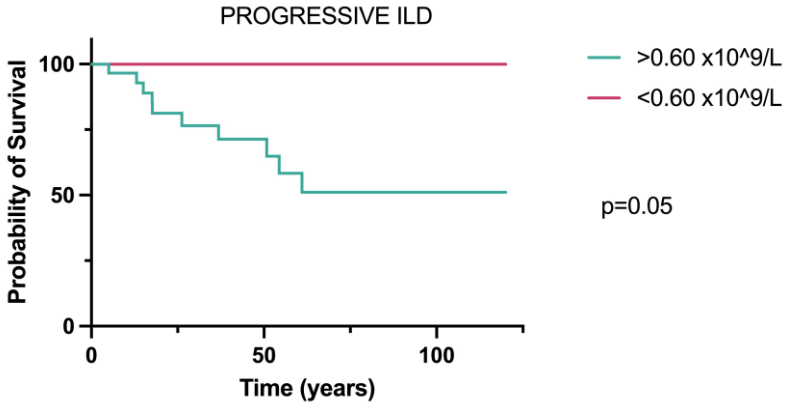

Figure 4.3: Probability of survival associated with monocyte level >0.6 x 10^9/L or <0.6 x 10^9/L.

4.4 Prognostic factors of radiological progression and disease progression

Logistic regression was performed to detect predictors for radiological progression and disease progression in the ILD population. In the univariate analysis, age at diagnosis (p=0.02), male sex (p=0.026), FVC%pred. at the diagnosis (p=0.001), monocyte level > 0.6 x 10^9/L (p=0.03) and the presence of consolidations at HRCT (p=0.045) are predictors of radiological progression. In multivariate analysis, the male sex (p=0.002) is an independent predictive factor of radiological progression in the population diagnosed with ILD. (Table IV).

	Univariate OD (0.95 CI)	***p***	***Multivariate OD (0.95 CI)***	***p***
Age – years	0.96 (0.92-0.99)	**0.02**	0.95 (0.89-1.01)	0.078
Sex – Male	2.68 (1.13-6.39)	**0.026**	8.39 (2.13-33.02)	**0.002**
Pulmonary Function Tests at diagnosis				
• *FVC (%)*	0.96 (0.94-0.98)	**0.001**	0,97 (0.94-1.01)	0.10
Monocytes (<0.6/>0.6 x 10 ^{9}/L)	2.94 (1.09-7.88)	**0.03**	1.74 (0.41-7.35)	0.45
HRTC at diagnosis				
Consolidations – yes	0.21 (0.04-0.96)	**0.045**	0.32 (0.04-2.73)	0.30

Table IV: **Predictive factors of radiological progression in patients diagnosed with ILD.** (FVC: Forced Vital Capacity, HRCT: High-Resolution Chest Tomography. Values are expressed as numbers and (%) or median and range as appropriate)

Concerning disease progression in the univariate analysis, age at the diagnosis (p=0.01), FVC%pred at the Pulmonary Function Tests at the diagnosis (p=0.0001), complete blood count at the time of the diagnosis with monocyte level of > 0.6 x 10^9/L (p=0.003), finding of consolidations (p=0.005) and reticulations (p=0.005) at the HRCT and the presence of exposures (p=0.022) appear to be predictors. In the multivariate analysis FVC%.pred. at the

Pulmonary Function Tests at the diagnosis (p=0.002), complete blood count at the time of the diagnosis with monocyte level of >0.6 x 10^9/L (p=0.036), and the finding of reticulations at the HRCT (p=0.04) are independent factors of disease progression in the ILD population.

	Univariate OD (0.95 CI)	***p***	***Multivariate OD (0.95 CI)***	***p***
Age – years	0.96 (0.93-0.99)	**0.01**	0.98 (0.92-1.04)	0.456
Sex – Male	1.47 (0.68-3.18)	0.33	-	-
Pulmonary Function Tests at diagnosis				
• *FVC (%)*	0.95 (0.93-0.97)	**0.0001**	0.94 (0.91-0.98)	**0.002**
Monocytes (<0.6/>0.6 x 10 ^{9}/L)	3.85 (1.58-9.33)	**0.003**	4.17 (1.09-15.89)	**0.036**
HRTC at diagnosis				
• *Consolidations – yes*	0.5 (0.01-0.42)	**0.005**	0.000 (0.000-0.001)	0.998
• *Reticulations – yes*	5.1 (1.60-16.08)	**0.005**	8.82 (1,1-70,8)	**0.04**
Exposures – yes	2.57 (1.14-5.76)	**0.022**	3.58 (0.97-13.26)	0.056

Table V**: Predictive factor of disease progression in patients diagnosed with ILD.** (FVC (%): Forced Vital Capacity, HRCT: High-Resolution Chest Tomography. Values are expressed as numbers and (%) or median and range as appropriate)

4.5 Correlations

Based on the results from univariate and multivariate analysis, we performed different correlations using the Spearman correlation test.

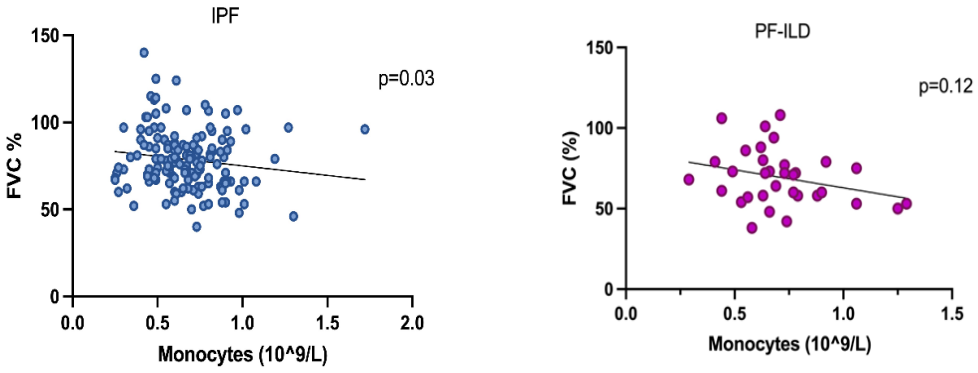

Figure 4.4: Correlations analysis between FVC (%) and monocytes (x10^9/L), in the IPF (p=0.03) and PF-ILD (p=0.12) population.

We found that FVC%pred at the time of the diagnosis negatively correlated with the level of monocytes (x 10^9/L) in patients with IPF (p=0.03). The correlation in the PF-ILD population shows an interesting trend but not a statistically significant difference (p=0.1), as reported in Figure 4.4.

4.6 Survival

Furthermore, we analyse the probability of survival at 120 months of patients with a diagnosis of IPF, PF-ILD, and NP-ILD, and the results show a statistically significant difference (p<0.0001). Patients diagnosed with IPF have the lowest survival, followed by those with PF-ILDs, while NP-ILDs are the group with the best probability of survival.

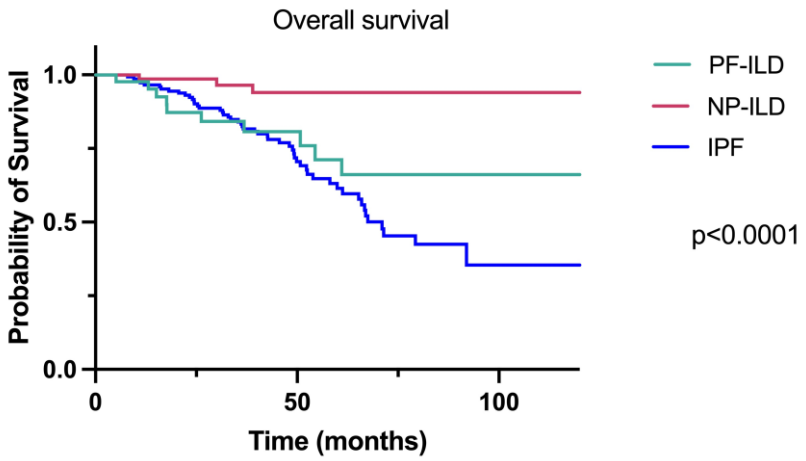

Figure 4.5: Overall Survival comparing patients with progressive interstitial lung disease (PF-ILD), non-progressive interstitial lung disease (NP-ILD), and idiopathic pulmonary fibrosis (IPF). Kaplan-Meier test and Long-rank test were used (p<0.0001).

5. DISCUSSION

In this retrospective study, we evaluate the clinical, radiological, and hematological features of patients with ILDs and compare this population to one with IPF. Furthermore, we focused on the potential role of a complete blood count in predicting disease progression. We enrolled 119 patients with ILDs, referred to the University Hospital of Padua, and 147 patients with IPF, referred both to the University Hospital of Padua (n°92) and the University Hospital of Palermo (n°55). Within the ILD population, 43 patients are considered progressive (PF-ILD) and 76 non-progressive (NP-ILD). IPF patients are older at the time of diagnosis: 70 years (range: 46-84) compared to ILD patients 63 years (range: 30-87) ($p < 0.0001$); they also differ in male prevalence, which is higher in the IPF patients (80% vs. 50% $p <0.001$) and in smoking history, in particular, 65% of patients with IPF were former smoker, instead only 44% of those with ILD ($p=0.0008$) and also the number of pack-years is significantly higher in IPF population (17 vs. 0; $p=0.0002$). These demographic data regarding the IPF population agree with the literature (83) (85). Regarding comorbidities, there are no differences between cardiovascular ones (69% vs. 64% $p=0.36$) and GERD (40% vs. 34% $p=0.31$) but patients with IPF present more frequent metabolic comorbidities (50% vs. 30% $p=0.0008$). The literature data regarding the comorbidities in IPF report that hypothyroidism and type 2 diabetes mellitus (DMT2) are the most frequent among the metabolic ones, and their treatment is recommended (96). The fact that patients with IPF more frequently have metabolic comorbidities may be explained by the fact that they are older patients. The two groups are similar in terms of Pulmonary Function Tests (PFTs) and Complete Blood Count at diagnosis, except for lymphocytes, which are higher in patients with IPF (2.25 vs. 1.8; $p=0.003$). Specifying that the lymphocyte values of the populations under study fall within those that define the normal range of this parameter. This result is not confirmed in the literature, as no studies show an association with a higher lymphocyte level in patients with IPF.

Concerning the 119 patients with ILD, 43 have a diagnosis of PF-ILD (36%) and 76 (64%) of NP-ILD. Among the entire ILD population, 36% of the

patients are classified as progressive, which agrees with the estimated progression rates in the literature (15). The results show that advanced patients are younger at the diagnosis compared to the non-progressive (59 vs. 65.5 years; p=0.008), and in the latter, exposures to substances that could be pneumo-toxic appear to be less frequent (48% vs. 69%; p=0.04). Retrospective studies have recognized older age as a risk factor that increases the likelihood of progression and mortality (15). In our research, data collection referred to age at diagnosis, but disease progression is defined by the last year of follow-up. Therefore, it can occur even years after diagnosis. A possible interpretation of this result may be that patients who reach a diagnosis at a young age may have a long duration of disease which gradually leads to progression; however, this assumption needs more investigation. With the term exposures, we have referred to organic (e.g. hay) and inorganic (e.g. silica dust) substances, drugs (e.g. amiodarone), and anything potentially pneumotoxic with which patients has come into contact during their life. In the case of PF-ILD, the fact that the exposures are significantly higher could be explained by the fact that in some patients, the progression of the disease is caused by the exposure to the pneumo-toxic substances that have not been interrupted, resulting in a persistent harmful stimulus for the lungs. The two groups are similar regarding sex, smoking history, BMI, coexisting comorbidities, and corticosteroid therapy.

Moreover, the symptoms at the diagnosis do not differ between PF-ILD and NP-ILD except for dyspnea on exertion (76% vs 53%; p=0.02) which is more frequent in the case of PF-ILD, and fever (2% vs 16%; p=0.03), which on the contrary is more frequent in case of NP-ILD. Dyspnea is a very impactful symptom in patients. Its worsening constitutes a criteria for defining disease progression; even in the case of IPF, patients are affected by progressive exertional dyspnea (15)(97). Worsening dyspnea on exertion is an aspect that patients with PF-ILD and IPF share. Fever can be one of the onset symptoms of an acute interstitial disease which, if adequately treated, can resolve without further sequelae or progression. The HRCT of PF-ILD reported more frequent reticulations (89% vs. 62%; p=0.03), while NP-ILD reported more

regularly the presence of consolidation (34% vs. 3%; p=0.001). The literature reports that a higher extent of reticulation on HRCT appeared to be associated with the risk of disease progression, and therefore in agreement with what we found in this study (98). Regarding the Pulmonary Function Tests at the time of diagnosis, all the values are significantly lower in the PF-ILD compared to NP-ILD. These results agree with the literature identifying lower FVC and DLCO at baseline as risk factors for disease progression (15). In some cases, progressive patients arrive at diagnosis already respiratory compromised, which may be in part due to a potentially avoidable diagnostic delay. Diagnostic delay is a real problem for patients with ILD that probably stems in part from its insidious onset and non-specific symptoms, which overlap with those of more common pulmonary and non-pulmonary diseases, and in part from the scarce knowledge of ILD among primary care physicians and non-ILD experts (20). Based on progression criteria, patients were accurately identified as progressive or non-progressive and to confirm this the data comparing PF-ILD and NP-ILD show a statistically significant difference in worsening of symptoms (71% vs. 10%; p<0.0001), HRCT (61% vs.10%; p<0.0001) and delta FVC/year (51% vs. 12%; p<0.0001).

Regarding complete blood counts at the time of diagnosis, it was observed that the value of monocytes results significantly higher in PF-ILD compared to NP-ILD (0.68 vs. 0.59 x 10^9/L p=0.0007). Furthermore, between IPF and NP-ILD a statistically significant difference was found for monocytes (0.67 vs 0.59 x 10^9/L; p=0.008) and lymphocytes (2.25 vs. 1.78 x 10^9/L; p=0.0002). Instead, no significant difference was found regarding monocytes between IPF and PF-ILD (p=0.22). These results are showed in Figure 4.2. The literature confirms that in IPF the founding of elevated monocyte count (> 0,60 x 10^9/L) is associated with increased risks of progression, hospitalization and mortality over 1 year (90). We further analyzed the probability of survival at ten years by dividing the PF-ILD population according to the value of monocytes, greater or less than 0.6 x 10^9 /L (the threshold was selected on the bases of previous studies (90). In the group of patients whose monocyte level is higher than 0.6 x 10^9 /L, the probability of survival at 120 months is about

50%, instead in the group in which this level is lower than 0.6 the probability of survival is 100%, (p=0.05) as shown in Figure 4.3. The p-value is not significant, but the results is still relevant.

Both in univariate and multivariate analysis, male sex (p=0.002) is an independent predictive factor of radiological progression in the whole population with diagnosis of ILD. Moreover, our study shows that in univariate analysis, age at the diagnosis (p=0.01), FVC%pred. at diagnosis (p=0.0001), complete blood count at the time of the diagnosis with monocyte level of >0.6 x 10^9/L (p=0.003), consolidations (p=0.005), and reticulations (p=0.005) at the HRCT and the presence of exposures (p=0.022) appear to be predictors of disease progression. In the multivariate analysis FVC (%) at the Pulmonary Function Tests at the diagnosis (p=0.002), complete blood count at the time of the diagnosis with monocyte level of >0.6 x 10^9/L (p=0.036), and the finding of reticulations at the HRCT (p=0.04) are independent factors of disease progression in the ILD population. This could be an essential point to underlying since the literature reports that the monocyte count is an independent predictor of IPF progression during the first year of antifibrotic treatment (91).

In our correlations analysis, the results show that FVC (%) at the time of the diagnosis negatively correlated with the level of monocytes (x 10^9/L) in patients with IPF (p=0.03). The same correlation in the PF-ILD population shows an interesting trend but not a statistically significant difference (p=0.1). Our results agree with some IPF studies reporting that the white blood count and the monocyte count at the baseline negatively correlate with lung function (91).

Furthermore, we analyze the overall survival at 120 months of patients diagnosed with IPF, PF-ILD, and NP-ILD, and the results show a statistically significant difference (p<0.0001). Patients diagnosed with IPF have the lowest survival, followed by those with PF-ILDs, while NP-ILDs are the group with the best probability of survival. Despite the recent advantages in disease management and drug treatment, the prognosis of IPF remains poor (82). Our

results confirm that patients with IPF have the worst survival compared to the whole population. However, focusing on patients diagnosed with PF-ILD, the probability of survival at 10 years is not suitable. Even though IPF is the prototype of progressive disease, patients diagnosed with PF-ILD have comparable outcomes with patients with IPF: progressive decline in lung function, worsening symptoms, end-stage fibrosis, and early mortality (63).

The results of our study may therefore suggest that elevated monocyte values may be found not only in patients with IPF but also in patients with PF-ILD and that monocyte count could become, in the future, a progression biomarker of interstitial lung diseases. The potential future application in the setting of some progressive ILDs has already been reported in the literature (88). Although the development of lung fibrosis is a complex and not yet fully understood pathological process, it is known that the persistence of lung injuries leads to the recruitment of monocytes, which differentiate into macrophages whose aberrant activity produces an excess of grow-factors and cytokines (in particular IL-4 and IL-10) which in turn stimulate the differentiation of fibroblast into myofibroblast (94). The possibility of applying the monocyte count as a progression biomarker is exciting as it would be a question of performing a blood test that is readily available to all patients and inexpensive; on the other hand, it is necessary to remember that the monocyte count may be affected by ongoing infections or medications (88). These results need further studies and investigations.

Our study has some limitations. First, it is a retrospective study in which patients with a diagnosis of ILDs were enrolled only by the University Hospital of Padua. Instead, IPF patients were enrolled at the University Hospital of Padua and the University Hospital of Palermo. Second, our population does not include large numbers of patients, and not all the data were found for all patients involved in the study. Third, the study consists of patients with progressive disease treated with antifibrotics and patients not treated with these drugs. Further studies are therefore needed to overcome these limitations and provide new research perspectives.

The fact that patients with ILD may have a progressive disease phenotype, which resembles the progression of IPF, is a fair receipt but very relevant concerning the diagnosis, management, and prognostic evaluation of these patients. This development about PF-ILD other than IPF opens the discussion on the importance of an appropriate diagnostic process according to the international guidelines and on the need of an accurate definition of disease progression to have the possibility of undertaking antifibrotic therapy as a second-line treatment in progressor patients (67) (66). More evidence is needed to establish robust markers to improve the management of ILDs, particularly of PF-ILD other than IPF (88). It would be a brilliant future perspective to develop a multivariate index capable of assessing the risk of progression based on symptom presentation, respiratory function, complete blood count, and radiological features. Exploring new research horizons and enhancing knowledge in this field is necessary to pursue these results.

6. CONCLUSION

In conclusion, we observed that the value of monocyte counts at the time of the diagnosis results significantly higher in patients with IPF compared with NP-ILD and patients with PF-ILD compared with NP-ILD. We also found that FVC%pred. at diagnosis, monocyte count > 0.6 x 10^9/L, and the finding of reticulations at the HRCT are independent factors of disease progression in the ILD population. We also analyze the overall survival of patients diagnosed with IPF, PF-ILD, and NP-ILD at 10 years, and the results show a statistically significant difference in survival between the three groups. Furthermore, even if in the absence of a real statistical significance (p=0.05) in the group of patients whose monocyte level is higher than 0.6 x 10^9 /L, the probability of survival at 10 years is about 50%, while in the group in which this level is lower than 0.6 x 10^9 /L the probability of survival is 100%. Based on our findings, monocyte count at the diagnosis could be a potential biomarker of progression also in patients with PF-ILD, not only in those with IPF, even though further studies are needed to recognize potential markers of progression and to investigate the role of monocytes in the development of lung fibrosis.

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