UNIVERSITÀ DI SIENA 1240

Dipartimento di Biotecnologie Mediche

Dottorato in Genetica, Oncologia e Medicina Clinica

36° Ciclo

Coordinatrice: Prof.ssa Ilaria Meloni

Serum amyloid A: a new biomarker in Idiopathic Pulmonary Fibrosis

Settore scientifico disciplinare: MED/10
(Malattie dell'apparato respiratorio)

Candidata
Lucia Vietri
Università degli Studi di Siena e Università di Ferrara

Firma digitale del/della candidato/a
LUCIA
VIETRI
05.12.2023
10:48:22
GMT+01:00

Tutor
Elena Bargagli
Universita' degli Studi di Siena

Supervisore
Miriana d'Alessandro
Universita' degli Studi di Siena

Anno accademico di conseguimento del titolo di Dottore di ricerca
2022/2023

Università degli Studi di Siena
Dottorato in Genetica, Oncologia e Medicina Clinica
36° Ciclo

Data dell'esame finale
20/12/2023

Commissione giudicatrice
Prof.ssa Elena Bargagli, UniSi
Prof.ssa Sara Tomassetti, UniFi
Prof. Francesco Pistelli, UniPi

Supplente
Prof.ssa Ilaria Meloni, UniSi

“La resilienza disse al cuore:
ciò che tu chiami fragilità
lo trasformerò in coraggio”...

General index

Background

Idiopathic Pulmonary Fibrosis

Definition

Diffuse interstitial lung diseases (DPLDs) are a group of diseases that predominantly affect the lung parenchyma and can lead to fibrosis. In 2013 interstitial lung disease were divided in three groups: major, rare and unclassifiable. Further studies led to the new ATS classification published on February 2022, where ILDs are distinguished in (1-2):

- Idiopathic pulmonary fibrosis (IPF);
- other idiopathic interstitial pneumonia (IIP): idiopathic nonspecific interstitial pneumonia (iNSIP), cryptogenic organizing pneumonia (COP), idiopathic pleuroparenchymal fibroelastosis (PPFE), idiopathic lymphoid interstitial pneumonia (iLIP), idiopathic desquamative interstitial pneumonia (iDIP), acute interstitial pneumonia (AIP);
- Autoimmune-ILDs: rheumatoid arthritis (RA), systemic sclerosis (SSc), mixed connective tissue disease (MCTD), myositis, Sjogren, vasculitis, systemic lupus erythematosus (SLE);
- exposure related ILDs: hypersensitivity pneumonitis (HP), asbestosis, silicosis, drug related-ILDs, actinic pneumonitis, respiratory bronchiolitis associated interstitial lung disease (RB-ILD);
- ILD with cysts and/or airspace filling: Langerhans cell histiocytosis (LCH), lymphoproliferative ILD, pulmonary alveolar proteinosis (PAP), lymphangioleiomyomatosis (LAM).

IPF is defined as a chronic and progressive idiopathic interstitial pneumonia of unknown cause that occurs predominantly in adult males and is limited to the lung. This disease is characterized by a progressive functional worsening with the development of persistent cough. IPF accounts for approximately 25% of all ILDs and is differentiated by the presence of a radiological and histological UIP (Usual Interstitial Pneumonia) pattern (3). In addition to the presence of the characteristics described above, in order to be able to diagnose IPF, it is necessary to exclude other forms of idiopathic lung diseases and those with a known cause (secondary to connective tissue

diseases, occupational exposure or drugs). The median survival from diagnosis is about 3-5 years (4-6), but the natural history of the disease is variable from patient to patient and for this reason the prognosis is relatively unpredictable (7,8).

Epidemiology

The incidence of IPF in Europe and North America is estimated at between 2.8- 18 cases per 100,000 inhabitants per year; the prevalence is approximately 42.7 per 100,000 inhabitants; data from the rest of the world are less available, but the incidence seems lower in Asia and South America where it is calculated at 0.5-4.2 cases per 100,000 inhabitants per year (4,9). In Europe alone, approximately 40,000 new cases of IPF are diagnosed each year. In Italy the incidence is estimated at around 9.3 cases per 100,000 per year and the prevalence at around 31.6/100,000 (10). The prevalence of IPF increases with advancing age, with the highest incidence being after age 60. Males are more affected than women (3). IPF has an average life expectancy of 3-5 years after diagnosis if untreated. Some studies showed declining overall mortality of IPF from 2004 to 2017, which may be related to decreased prevalence of tobacco smoking and the introduction of antifibrotic therapies.

Etiopathogenesis

The etiopathogenesis of IPF is not fully understood. Historically, IPF was considered a chronic inflammatory disease with a gradual progression leading to fibrosis. Through recent studies and since anti-inflammatory therapy does not improve the outcome, this concept has been re-evaluated (11). In healthy lungs, type1 alveolar epithelial cells (AEC1) are the primary mediators of gas exchange. Type2 alveolar epithelial cells (AEC2) produce surfactant and serve as the primary progenitors for injured AEC1 cells. Injury to the alveolus requires a healthy functioning population of regenerative AEC2 cells. In IPF lungs, there are higher levels of apoptosis, senescence, abnormal differentiation of AEC2 cells. A combination of extrinsic and intrinsic factors, including aging, oxydative stress,

mitochondrial dysfunction and telomere shortening, leads to an inability of the AEC2 cells to repair the injured epithelium (50). According to the most accepted hypothesis, this lung disease is the result of repeated stimuli that act at the level of type II alveolar epithelial cells (AEC2s). These persistent pneumolesive stimuli would cause an aberrant communication between epithelial cells and fibroblasts with deposition of extracellular matrix and remodeling of the pulmonary interstitium; these cell accumulations are called *fibrobastic foci* (aggregates of mesenchymal cells within a myxoid appearing matrix) (12). A central role in the pathogenesis of the disease has the chronic dysregulation of type II alveolar epithelial cells (AEC2s). These cells contribute to the renewal of type I alveolar epithelial cells (AEC1s) during homeostasis or after lung injury (13,14). From the analysis of tissues where pulmonary fibrosis is present, abnormal AEC2s and loss of AEC1s were identified, with the presence of fibroblastic foci typically adjacent to hyperplastic or apoptotic alveolar epithelial cells (11).

Senescence, characterized by arrest of cell growth and a diminished replicative potential, predisposes lung to fibrosis by impairing regeneration of alveolar progenitor cells. Telomere shortening is the hallmark of aging and senescence (50). A study conducted in 2016 (15), showed that AEC2s present in areas of IPF fibrosis had impaired renewal capacity. The abnormal behavior of the epithelial cells is associated with an alteration of the new epithelial tissue. Activated alveolar epithelial cells produce numerous growth factors and cytokines, including TGF-β, and platelet growth factors with aberrant activation of myofibroblasts, which produce extracellular matrix (ECM) destroying pulmonary alveolar architecture. Fibrotic diseases are typically characterized by upregulation of TGF- β signaling, which promotes recruitment of fibrotic mesenchymal cells and inflammatory mediators, stimulating ECM deposition (50). TGF-β is secreted by epithelial, fibroblast and immune cells. Once secreted, various molecules lead to TGF-β activation and binding to receptors on target cells, initiating a fibrotic signaling cascade. In IPF, TGF-β is expressed by AECs and alveolar macrophages, and its expression localizes to fibroblastic foci.

TGF-β mediate myofibroblast differentiation and resistence to apoptosis, increasing collagen deposition. Myofibroblast activation is also induced by the coagulation cascade (Factor X) (53). IPF fibroblasts are resistant to apoptosis. This metabolic reprograming is mediated by TGF-β, leading to increased mTORC1 signaling that promotes apoptosis resistance and decreased autophagy in fibroblasts (54). Attenuation of TGF-β signaling may represent one of the mechanisms of action of the antifibrotic drug Pirfenidone (51-52).

Numerous evidences have documented how AECs are the main target of the mechanisms of accelerated cellular senescence and apoptosis that would induce pathological changes in IPF (16,17).

Role of T lymphocytes

The immune system uses distinct populations of T cells to respond to inflammation and fibrosis in health and disease. Some studies demonstrate that T helper 2 (Th2) cytokines promote fibrosis, whereas Th1 cytokines (e.g., IFN-γ, IL-12) promote inflammation. Th2 cytokines include IL-4, IL-5, IL-9, IL-13 and are important for fibrosis across multiple disease states. These cytokines promote differentiation toward myofibroblasts (58-60).

Genetics and risk factors

Genetic susceptibility plays a role in the development of IPF.

IPF occurs both sporadically and in families, consistent with an underlying genetic predisposition. Familial pulmonary fibrosis (FPF), defined as having two or more family members with idiopathic interstitial pneumonia, represents about 5-20% of IPF cases. Also sporadic IPF have a family history of pulmonary fibrosis, suggesting that genetic variation is a key determinant for the development of IPF (50).

Thanks to the study of familial pulmonary fibrosis, rare genetic variants associated with surfactant (SFTPC, SFTPA2) and telomere (TERT, TERC, PARN, RTEL) alterations have been identified. However, the finding of these genetic modifications does not represent a direct cause of disease (3,18,19). The TOLLIP gene codes for proteins that mediate the innate and

adaptive immune response, while the MUC5B gene for a mucin that contributes to the production of mucus in the airways is important for maintaining homeostasis of the immune system. MUC5B *r35705950* is the most common genetic variant in IPF, which is present in 38% of patients with IPF and is associated with a 21-fold risk for disease in homozygous individuals. The pathologic mechanism of this mutation may be related to excess mucin production and impaired mucociliary clearance (50).

Single nucleotide polymorphisms of TOLLIP and MUC5B have been associated with survival of IPF (20–22). Loss of function of the MUC5B gene has been observed in both familial and sporadic forms: the altered variant of this gene has low penetrance and the isolation of this gene appears to be a contributing cause of IPF (20,21). It is assumed, therefore, that in the sporadic forms there may be a genetic susceptibility which, however, alone is not sufficient for the development of the disease, but the presence of further risk factors is also necessary.

According to the ATS/ERS/JRS/ALAT 2011 guidelines (3) risk factors for IPF include:

- Cigarette smoking: smoking is closely associated with the disease, particularly in subjects with a history of smoking greater than 20 pack/years in both sporadic and familial cases, however in the latter cases smoking leads to a more than doubled risk of developing IPF (23). A history of smoking is present in 41 to 83% of patients with IPF.
- Environmental exposure: a significant increase in the risk of developing IPF has been observed after exposure to metal and wood dusts; various professions can be associated with IPF: farmer, hairdresser, poultry farmer, cattle breeder.
- Gastroesophageal reflux: several studies suggest that gastroesophageal reflux, associated with presumable microaspiration, is a risk factor for IPF.
- Infections: various studies have investigated a possible role of viral agents in the etiology of IPF; previous EBV, CMV, HCV and herpes virus infections have been hypothesized as possible risk factors, but without any direct experimental demonstration.

Clinical presentation

Patients typically present with nonspecific symptoms such as worsening dyspnea, first on exertion and then also at rest, associated or not with a nonproductive cough. Physical examination of the chest is characterized by "*velcro-like*" crackles, present during the inspiratory phase. In the initial stages of the disease they are typically found at the level of the lung bases, then as the disease progresses they also tend to affect the upper fields. However, these auscultatory noises lack specificity as they are also present in other forms of interstitial diseases.

Clubbing fingers are another clinical sign. In the advanced stages of the disease, signs of cyanosis are evident. The natural history of the disease can predict different trends: the majority of patients present a slow and progressive worsening of dyspnea and respiratory function, some patients remain stable while others develop rapid disease progression (3,24,25). It is not possible to predict the course of the disease at the time of diagnosis.

Complications

The clinic of IPF can change both due to the progression of the disease and the occurrence of complications.

Pulmonary hypertension (PH): signs of pulmonary hypertension can occur with split second heart sound and with signs of chronic cor pulmonale (jugular turgor, hepato-jugular reflux, hepatomegalia and dependent edema) (26,27). The PH is present in 8-15% of patients with IPF and up to 60% of those with end-stage disease and is associated with increased mortality (0.7 years) (28). PH is more frequent in the combined radiological pattern emphysema and fibrosis. We have to suspect PH if there is a decline in DLCO out of proportion to the decline in FVC with any clinical worsening. Treatment with inhaled treprostinil has been shown to improve exercise capacity (119).

Cardiovascular diseases: patients with IPF have an increased risk of developing acute cardiovascular disease (29,30) and pulmonary thromboembolism (31); in fact, it has been shown that in this disease there is a pro-thrombotic state four times higher than in health patients (32).

Lung cancer: the analysis of studies carried out in the USA and UK showed a prevalence of lung cancer in patients with IPF ranging from 4 to 40%; a large study found that patients with IPF without cancer (n:1571) had a longer survival than patients with IPF and lung cancer (n:114) (28). Lung tumors in patients with IPF develop preferentially in the periphery immediately adjacent to fibrotic areas, with different histologic distribution and immunohistochemical features compared with non-IPF associated lung tumors. IPF and lung cancer share many pathogenic similarities including genetic and epigenetic markers. It has been suggested that specific germline mutations predispose toward both IPF and lung cancer, leading to imbalance between oncogenes and tumor suppressor genes and ultimately carcinogenesis within fibrotic lungs. Genetic and epigenetic alterations lead to abnormal activation of common transduction pathways, including Wnt/β-catenin, mediating metaplasia and hyperproliferation in alveolar type II epithelial cells. TERT (telomerase reverse transcriptase) and TERC (telomerase RNA component), lead to shortened telomeres and genomic instability and epithelial cells senescence. Telomeres consist of nucleotide repeats at chromosome ends that buffer against loss of genetic information during normal cell division. Telomere shortening has associated with sporadic IPF (25%) and familial IPF (37%) (124).

Some studies also have shown that lung fibroblasts in IPF patients overexpress PD-L1 (programmed death ligand 1), that is utilized by cancer cells to escape the surveillance of the immune system. It could be block by specific monoclonal antibodies (as has been recently established in treatment of advanced non-small cell lung cancer-NSCLC). IPF patients on Pirfenidone treatment have a lower incidence of lung cancer development and also reduced rates of perioperative mortality and acute exacerbation when underwent surgical interventions (76-77, Fig1).

Gastroesophageal reflux: the prevalence is 0-94%; the median survival is higher for patients undergoing GER therapy and for those undergoing Nissen fundoplication (33).

Depression: present above all in the advanced stages of the disease and correlated with the increase in dyspnea (34).

Sleep apnea syndrome: some studies have shown reduction of the REM phase, episodes of nocturnal desaturation associated with frequent awakenings (35).

Low body weight: a reduction in BMI is associated with increased mortality (36,37).

Diabetes mellitus: the correlation with IPF is not yet known (38,39).

Cardiovascular diseases and pulmonary hypertension may be a consequence of the involvement of the endothelial cells in IPF. Fibrotic lung demonstrated vascular abnormalities (vascularity is decreased within the fibrotic foci with large, dilated vessels in areas of honeycombing) (55). The lack of vessels in areas of fibrosis leads to increased pulmonary vascular resistance and ultimately PH. It remains unclear whether these vascular abnormalities contribute to fibrosis or constitute an adaptive response to fibrosis. Several mechanisms have been proposed for abnormal vascular patterns in IPF, including consequences of Hypoxic vasoconstriction, microvascular injury due to antibodies against the endothelium (56). TGF- β and endothelin-1 (ET1) are important angiogenic factors. ET1 induces vasoconstriction and vascular smooth muscle cell growth; although antagonists are used in PH, clinical trials of endothelin receptor antagonists have not shown success in IPF patients with early stage disease (57).

Acute exacerbation (AE)

The natural history of IPF is quite heterogeneous, from chronic stable symptoms to progressive respiratory failure or acute exacerbation. The incidence of AE of IPF is 5-10% per year (65). However, the incidence varies according to ethnicity. Japanese patients are more susceptible to AE of IPF. Therefore, some genetic regulatory factors may be related to AE (66-67).

These are very serious events, burdened by high mortality rates (median survival 3-4 months after AE). The diagnosis of AE-IPF was defined by the following criteria (40):

- previous diagnosis of IPF
- unexplained worsening of dyspnea within the previous 30 days
- presence of ground-glass type alterations on chest HRCT

- exclusion of known causes of respiratory distress (lung infections, pulmonary embolism, heart failure).

However, the clinical conditions of the patient often do not make it possible to perform tests such as bronchoscopy and therefore the exclusion of all known causes is not always possible.

Recently the AE-IPF criteria have been re-discussed (41):

- previous or concurrent diagnosis of IPF
- worsening of shortness of breath for less than a month
- presence of ground-glass changes and/or consolidative areas on HRCT-chest superimposed on a UIP pattern
- deterioration not explained by heart failure, fluid overload, pulmonary embolism or pneumothorax.

The term idiopathic has been eliminated and the time interval for the development of exacerbation is requested "lasting less than a month".

The acute exacerbation is therefore considered as an acute event probably triggered by multiple triggering factors, such as, for example, infectious factors, drug toxicity, aspiration, after procedures and post-operatively.

Studies have reported that reduced pulmonary function, especially forced vital capacity (FVC), never smoking status and baseline Krebs von den Lungen-6 (KL6) are crucial risk factors that predict an AE of IPF (62).

In the chemistry panel, lactate dehydrogenase (LDH) is a simple and sensitive marker that predicts the short-term prognosis of AE of IPF patients (63). Other studies demonstrate that a serum ferritin level above 500 ng/mL predicts poor prognosis in AE of IPF (64).

In chest radiographs, a new bilateral diffuse shadow that is superimposed on the lower-lobe reticular shadow is the typical finding in AE of IPF patients.

Akira et al. (68) proposed that the CT findings of AEs of IPF should be divided into three patterns, consisting of peripheral, multifocal and diffuse infiltrates. They also evaluated several follow up CT scans. In survivors with the peripheral pattern, the majority of GGO and consolidation regressed back to baseline levels of abnormality. In survivors with multifocal scan findings, GGO and consolidation disappeared with corticosteroid therapy. In contrast, survivors with the diffuse pattern demonstrated significant extension of

GGO and consolidation. In multivariate analysis, the diffuse CT pattern was the strongest predictor of mortality.

The 2018 Japanese IPF treatment guidelines suggested that IPF patients with AE should be treated with corticosteroids, including pulse therapy (pulse therapy is defined as discontinuous/intermittent intravenous infusion of very high doses of corticosteroids). Steroid pulse therapy is typically administered for three consecutive days. Weekly pulse therapy may sometimes be repeated once or twice. Prolonged pulse therapy may often be complicated by opportunistic infections such as pneumocystis pneumonia and viral infections (69). When there is a partial response with prednisolone, somebody try treatment with chronic immunosuppressants, such as intravenous cyclophosphamide (70). However, this treatment strategy is not supported by robust evidence.

Recently, novels therapies have been reported to have possible value in treating AE of IPF:

1) PMX-DHP (polymyxin B-immobilized fiber column), that was originally introduced to treat sepsis and septic shock. It has a great response in AE if administered within 3 days after disease onset;

2) rhTM (recombinant human soluble thrombomodulin), which has anti-inflammatory effects and mitigates the coagulation cascade. Several reports have shown that intravenous administration of rhTM for six consecutive days improved the survival rate of AEs of IPF patients (71-73);

3) nintedanib, with an important role in acute and chronic phase of IPF;

4) high-flow-nasal cannula (HFNC): reduced tachypnea and improves in minute ventilation (74).

Acute exacerbation of IPF is usually associated with a poor prognosis. It accounts for 40% of IPF deaths (75). The mean survival of AE of IPF is less than one year. Therefore, the prevention of AE is crucial.

Radiology

In patients suspected of having IPF, chest CT should be done thin-slice and with the patient in the prone position. It is desirable that the examination be performed in exhalation to evaluate the presence of air-trapping from

bronchiolar involvement. The radiological pattern associated with IPF is that of "Usual Interstitial Pneumonia" (UIP) and is characterized by the presence of reticular opacities on HRCT-thorax, often associated with traction bronchiectasis and honeycombing which typically has a basal-subpleural distribution.

The alterations present on HRCT-chest allow us to describe three types of UIP patterns (3):

- UIP pattern: basal and subpleural predominant fibrotic lesions, with heterogeneous distribution. Presence of honeycombing with or without traction bronchiectasis;
- Probable UIP pattern: basal and subpleural predominant fibrotic lesions, with heterogeneous distribution. Interstitial cross-links with traction bronchiectasis or bronchiolectasis;
- Indeterminate UIP pattern: predominantly basal and subpleural fibrotic lesions, interstitial reticulations, possible lesions with ground glass appearance. The distribution of fibrosis is not typical of the UIP pattern;
- Alternative diagnosis: presence of cysts, predominance of ground-glass lesions, numerous micronodules, centrilobular nodules, consolidations, prevalent peribronchovascular and perilymphatic distribution of lesions, involvement of the middle-upper lung fields, pleural plaques, esophageal dilatation.

The UIP radiological pattern has a very high sensitivity and specificity for the histopathological diagnosis of UIP (42). The new ATS guidelines (1) applied changes in the definition of the three radiological patterns:

- pattern UIP (confident > 90% for diagnosis of IPF): distribution subpleural and basal predominant, heterogeneous distribution, honeycombing, with or without traction bronchiectasis, irregular thickening of interlobular septa, may have pulmonary ossification;
- pattern UIP probable (confidence 70-89%): subpleural and basal predominant, heterogeneous reticulation and traction bronchiectasis, may have mild ground-glass. Absence of honeycombing;
- indeterminate for UIP (confidence 51-69%): diffuse distribution without subpleural predominance;

- alternative diagnosis (confidence < 50%): subpleural sparing as NSIP, perilymphatic distribution as sarcoidosis, fibrosis of upper lung lobes as HP, cysts, predominant ground-glass, centrilobular micronodules, consolidations, pleural plaques, dilated esophagus.

The new diagnostic criteria explain that IPF diagnosis not always need the biopsy confirmation (Fig2).

Pathological Anatomy

The histological criteria that define the UIP pattern are:

- the presence of "patchy" fibrosis (spatially heterogeneous lesions);
- temporally heterogeneous lesions;
- architectural distortion with honeycombing aspects.

The term "Patchy" describes areas of fibrotic lung alternating with spared areas. These changes typically affect the subpleural and paraseptal areas.

The fibrotic alterations also present in a heterogeneous way in a temporal sense with the alternation of inveterate fibrosis near to areas of younger and more active fibrosis, characterized by dense areas of collagen and subepithelial foci of fibroblasts and myofibroblasts. It is believed that fibroblastic foci are the areas where there is the greatest deposition of extracellular matrix which tends to transform into dense cicatricial fibrosis over time.

Honeycombing areas are composed of fibrotic cystic air spaces covered by bronchiolar epithelium and filled with mucin and represent the irreversible phase of lung remodeling.

The inflammation is usually moderate and consists of the presence of an interstitial infiltrate of lymphocytes and plasma cells associated with type 2 pneumocyte hyperplasia and bronchial epithelial cells (3).

The anatomical-pathological alterations allow us to describe four histological classifications:

- UIP pattern: evidence of marked fibrosis with distortion of the parenchymal architecture, presence of honeycombing with predominant subpleural and paraseptal distribution, presence of patchy fibrotic involvement of the lung

parenchyma, presence of fibroblastic foci, absence of all the characteristics that define an alternative diagnosis;

-Probable UIP pattern: there are some histological features of the UIP pattern but with an extension such as to preclude the definitive diagnosis and the absence of elements suggestive of an alternative diagnosis or the presence of only honeycombing;

-Indeterminate UIP pattern: fibrosis with or without architectural distortion with characteristics referable to a non-UIP pattern or to a secondary UIP pattern to another cause;

-Alternative diagnosis: Histological features related to other interstitial diseases (such as absence of fibroblast foci) in all biopsies, consistent with hypersensitivity pneumonitis, sarcoidosis, pulmonary histiocytosis.

Diagnosis

Starting from ATS guidelines of 2018 (3), a strong recommendation was made to use a multidisciplinary approach for the diagnosis of idiopathic pulmonary fibrosis, i.e. thanks to the collaboration of specialists such us pulmonologist, radiologist, pathologist and possibly rheumatologist.

The history and clinical situation are the first fundamental step in excluding all known causes of fibrosis; it is therefore important to investigate the exposure to organic or inorganic dust, the use of pneumotoxic drugs or to evaluate a possible connective tissue disorder (both through the clinic and with the dosage of autoantibodies). The next step is to evaluate the appearance on high resolution CT-chest (HRCT-chest) and, in selected cases, to resort to surgical lung biopsy (SLB) or transbronchial criobiopsy (TBLC).

In the case of radiological pattern UIP defined on chest HRCT and a compatible clinical situation, it is already possible to diagnose IPF, without recourse to lung biopsy.

The guidelines also provide a grid for combining HRCT and histological results for an accurate diagnosis of IPF (1). Clinically suspected of having IPF is defined as unexplained patterns of bilateral pulmonary fibrosis on

chest radiography or chest computed tomography, bibasal inspiratory crackles and age > 60ys.

The new diagnostic algorithm for IPF (1) says that patients with a radiological pattern of probable UIP can receive a diagnosis of IPF after a multidisciplinary discussion (MDD), without confirmation by lung biopsy in the appropriate clinical setting. Bronchoalveolar lavage (BAL) may be performed before MMD in some patients evaluated in experienced centers, to exclude secondaries from exposure. Therefore, in the event of a compatible clinical pattern and the presence of a defined UIP pattern, it is possible to diagnose IPF without resorting to more invasive investigations. On the other hand, in cases of indeterminate UIP pattern, we proceed with a multidisciplinary discussion of the clinical case to decide whether to subject the patient to further tests (BAL and/or lung biopsy) and in the light of these results, always agree on the diagnosis in a multidisciplinary context (Fig3).

Surgical lung biopsy and transbronchial lung criobiopsy

To obtain a biopsy sample of the lung parenchyma (43), the patient can undergo a surgical biopsy (SLB), usually performed by video-laparoscopy. The sampling areas, given the heterogeneous distribution of the disease, must be in at least two lung lobes, avoiding the tip of the lingula, the middle lobe and the honeycombing areas. Transbronchial biopsies allow sampling only small fragments on which it is not always possible to recognize the UIP pattern, for this reason it is not considered a useful method (44). In recent years, some centers have begun to use cryobiopsy: it is a method performed in rigid bronchoscopy through a probe that reaches a temperature of -89°C; it is possible to obtain lung fragments of considerable size and larger than in trans-bronchial biopsies (45). The new ATS guidelines says that transbronchial lung cryobiopsy (TBLC) may be preferred to surgical lung biopsy (SLB) in centers with appropriate expertise and in some patient populations, with a low risk of pneumothorax and bleeding. A subsequent SLB may be justified in some patients with no diagnostic findings on TBLC (1).

Bronchoalveolar lavage

Bronchoalveolar lavage is not a decisive method for the final diagnosis but can be useful in the diagnostic process to exclude other interstitial diseases (46,47).

IPF is not specifically associated with any cellular pattern, but a modest increase in neutrophils (>5%) and eosinophils (>2%) is common.

A lymphocyte count >25% is typical in granulomatous disease (sarcoidosis), a lymphocyte count >50% suggests hypersensitivity pneumonitis or NSIP. The presence of neutrophils >50% can direct the clinician to an inflammatory form; the presence of eosinophils >25% can instead diagnose eosinophilic pneumonia (47,48). The presence of neutrophils in BAL of IPF patients predicts early mortality. Neutrophils secrete various MMPs, playing a role in ECM deposition and maintenance (61).

Recent studies have been carried out about the S100 calcium binding protein (S100A12), as a prognostic biomarker of IPF. S100 protein is mainly expressed in monocytes and higher levels in BAL in IPF patients are associated to a higher mortality. Higher levels of S100A12 were found in the blood of patients with acute exacerbation of IPF. However, the role of the S100A12 in the lung tissue and in the BAL of IPF patients is unclear. We know that in the process of pulmonary fibrosis, monocytes are recruited into the lung in response to tissue injury and differentiate into long-lived macrophages producing TGF-β, metalloproteinasis (MMPs), eventually leading to fibroblast activation, myofibroblast differentiation and extracellular matrix remodeling (49).

Biomarkers

Biomarkers are defined as “characteristics that are objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes or pharmacologic responses to a therapeutic intervention” (83).

To date clinical, diagnostic, prognostic biomarkers are not well characterized for IPF. However, they could become useful tools helping facilitate diagnoses, monitoring disease progression and treatment efficacy.

IPF pathogenesis involves an unknown exogenous injury (cigarette smoke,

pollution, dust, virus, superoxide, reactive nitrogen species), or genetic change or autoimmune response or ageing related predisposal mediated alveolar epithelial cells (AEC) dysfunction (78, Fig4).

Some studies showed the role of S100A9 (calgranulin B) as a biomarker of IPF dosed on BALF (bronchoalveolar lavage fluid) to differentiate IPF from different interstitial pneumonias, using enzyme-linked immunosorbent assay-ELISA. The authors concluded that BALF S100A9 is a specific and sensitive biomarker in differentiating IPF from CVD-IP (connective vascular disease interstitial pneumoniae) and iNSIP. These findings suggest that serum S100A9 reflects systemic inflammatory conditions, whereas BALF S100A9 reveals local inflammation in the lung (79).

Interleukin-6 (IL-6) plays a key role in the pathogenesis of IP, stimulating myofibroblast proliferation. Myofibroblasts excessively secrete extracellular matrix and remodel the matrix through remodeling proteins (cathepsin S, periostin) (80).

Other studies demonstrate that the levels of CRP and fibrinogen are higher in IPF patients and that antithrombin-III correlated significantly with forced vital capacity (FVC), reflecting the severity of the disease (81-82).

Surfactant proteins, secreted by AEC type II, are encoded by SFTPA, SFTPB, SFTPC, SFTPD genes. Variants SP-C, SP-A2, SP-A1 have been associated to familiar pulmonary fibrosis. As surfactant protein levels can be measured in bronchoalveolar lavage fluid (BALF) and in blood, they could have a role in identifying risk individuals in families with pulmonary fibrosis. SP-A serum levels appear to be significantly higher in patients with IPF than in patients with other ILDs and are associated to a reduced survival in IPF (84,85).

Krebs von den Lungen-6 (KL6)/mucin 1 (MUC1) is a glycoprotein expressed on the extracellular surface of type II AECs and bronchiolar epithelial cells in the lung largely studied in ILDs due to its overexpression in affected lung and regenerating type II AECs (86, 87). KL-6 is increased in serum of several ILDs including IPF (88,89). In one study, KL-6 levels in BALF seems to be a specific diagnostic marker in IPF compared with other

ILDs (90),while Bennet et al. proved that higher levels of BALF KL-6 are related to a more severe and extended disease (91). However, since KL-6 reflects AECs damage, it is not specific enough to distinguish IPF from the other ILDs, but it could facilitate stratification of severity. Increased values of KL-6 in IPF is a predictor of AE risk (92,93). Response to pirfenidone therapy correlates with changes in serum KL-6. Bergantini et al. evaluated serial measurements of serum KL-6 in IPF patients treated with Nintedanib and demonstrated an indirect correlation with FVC percentages and KL-6 values. Moreover, after 1 year of treatment, patients on therapy showed stable FVC percentages and KL-6 levels compared with baseline values. (98,99).

Metalloproteases (MMP) are proteins (family of zinc-dependent matrixins) that participate in extracellular matrix degradation and in the fibrotic process. MMP-1 and MMP-7 are overexpressed in serum and BALF of IPF patients, but alone they are not sufficiently specific to distinguish IPF from other ILDs (94-96).

Periostin, another ECM protein involved in tissue development, has been shown part of the pathogenetic process in IPF. Periostin has prognostic values, in fact total periostin can predict both short-term declines of pulmonary function and overall survival in IPF patients. However, total periostin is not specific for IPF. On the contrary, the monomeric periostin form is more specific and can be used not only to predict pulmonary function decline but also to distinguish IPF patients from healthy controls (97).

Blood monocytes: peripheral blood monocyte count has recently emerged as a promising and easily measurable prognostic biomarker in IPF patients, with several studies showing that a monocyte count > 0.60 x $10^{\wedge 9}$ cell.L^{-1} is strongly associated with disease progression; a monocyte count > 0.95 x $10^{\wedge 9}$ cell.L^{-1} indicating a very high risk for poor outcomes (120-122).

The need of reliable biomarkers is becoming more and more fundamental. The validation of useful and accurate diagnostic markers could reduce uncertainty and the use of invasive procedure, could help to predict AE events and improve diagnostic accuracy. No biomarkers have demonstrated utility in the assessment of treatment response to antifibrotic therapy (123).

Treatment

The 2011 guidelines (3) analyzed the possible therapeutic choices for patients with IPF and the recommendations for each drug were classified as strong or weak based on the quality of the data and scientific evidence. At the time, however, no medical therapy presented a strong recommendation in favor of its use and therefore lung transplantation was the only intervention that received a positive recommendation.

In 2015, the 2011 guidelines update was published (100) where there was a strong recommendation against the use of prednisone+azathioprine+N-acetylcysteine (NAC), warfarin, macitentan and ambrisentan.

In 2009, the PANTHER-IPF study (101) was conducted with three study arms: NAC+prednisone+azathioprine; NAC monotherapy and placebo. In the triple therapy arm the study was discontinued early for safety reasons, the NAC alone arm completed the study but without showing substantial efficacy. However, a post-hoc analysis has shown interesting results in patients with the TOLLIP genotype (TT) (102). The phase III studies with warfarin (103) and the one with ambrisentan (104) were prematurely discontinued, in the first case due to an increase in the mortality of the treated arm, in the second due to lack of efficacy; studies for bosentan and macitenan showed negative results as early as phase II (105–107).

New in the 2015 update is the conditional recommendation in favor of the use of pirfenidone and nintedanib which are currently the only approved drugs for IPF.

Both drugs showed a slowdown in the progression of the disease and in the case of nintedanib also a reduction in exacerbations.

The prescription according to AIFA criteria of both drugs is represented by functional and diffusion characteristics. In particular, for both the FVC values must be greater than 50%; the DLCO must be greater than 35% predicted for pirfenidone and greater than 30% predicted for nintedanib. The choice of drug must therefore be weighted on the basis of the level of respiratory compromise, but also on the basis of patient compliance and associated comorbidities and therapies.

Lung transplantation, which has a strong recommendation in the guidelines, remains the only therapeutic procedure that modifies the natural history of the disease.

- *Non pharmacological treatments*

In case of hypoxemia at rest, **oxygen therapy** is indicated although there are no data of such long-term therapy in patients with IPF.

The guidelines identify a low level of recommendation, however, **pulmonary rehabilitation** can improve the quality of life, the six-minute walk test (6MWT) and the patient's symptoms (108), it is also essential for patients waiting for a lung-transplant for a better post-operative course (109).

In clinical practice, it is also recommended to carry out influenza and pneumococcal vaccination in patients with IPF.

Lung transplantation is the only intervention that can modify the natural history of the disease.

Patients with IPF and under the age of 65 need close monitoring in order to be able to evaluate this therapeutic choice, in case of worsening. The indication for inclusion in the list is present in the case of (110):

- FVC decline > 10% in 6 months of follow-up;
- DLCO decline > 15% in 6 months of follow-up;
- desaturation to values < 88% or distance traveled <250 m at 6MWT, or reduction >50 m in distance over 6 months of follow-up;
- pulmonary hypertension;
- hospitalizations for worsening respiratory symptoms, pneumothorax or acute exacerbation.

It is therefore essential to send patients with IPF to Transplant Centers as soon as possible; however, the optimal timing in which to propose this therapeutic choice to the patient remains very complex. The problems are represented by the fact that IPF has a substantially unpredictable course, however with a median survival to diagnosis between 2.5 and 3.5 years (111,112).

- *Pirfenidon*

Pirfenidone is a pyridine with anti-fibrotic, anti-inflammatory and anti-oxidant actions which is taken orally at a dosage of 2403 mg/day.

The two CAPACITY trials (113), comparing pirfenidone versus placebo therapy in patients with mild to moderate IPF, showed a significant reduction in the decline of forced vital capacity (FVC) at 72 weeks. The other end-points of the study, represented by the reduction of the decline of the 6-minute walk test and by the progression of the disease (expressed as a decrease in DLCO by 15% and as a decrease in FVC by 10%) were satisfied.

A third phase III study (ASCEND) (114) for the evaluation of the efficacy of pirfenidone showed positive results which overlapped with the previous ones. This study also evaluated mortality in the entire patient cohort of the CAPACITY and ASCEND studies showing a 48% reduction in one-year mortality from any cause.

Since June 2013, AIFA has approved the distribution of this drug by the National Health System at a dose of 2403 mg/day for patients with mild-moderate IPF.

The criteria for prescribing the drug in Italy are:

- aged between 40-80 years
- diagnosis of IPF (exclusion of other causes of disease; consistent UIP radiological pattern or probable UIP or histological diagnosis of IPF)
- FVC>50% predicted and DLCO>35% predicted.

Pirfenidone can only be prescribed by the centers identified in the regional resolutions and is subject to AIFA monitoring with a specific monthly plan.

At the beginning of the therapy, the dosage is that of one 267 mg tablet 3 times a day in the first week, then 2 tablets 3 times a day for another week and then 3 tablets 3 times a day to thus reach the dosage of 2403 mg/day. From 2019, the single 801 mg tablet was introduced to be taken 3 times a day with greater compliance on the part of the patient in taking the therapy and better gastric tolerance.

The intake of the drug is recommended after main meals to avoid gastrointestinal problems; patients also perform regular checks of liver function and to prevent possible photosensitivity reactions, it is

recommended to limit exposure to the sun. In fact, the most frequent side effects are represented by gastrointestinal effects (nausea, vomiting, dyspepsia, diarrhea), weight loss, increased transaminases and photosensitivity. In the ASCEND study, the use of pirfenidone, these adverse events were mild to moderate in intensity and led to discontinuation of the drug in 14% of patients on treatment.
The drug is not recommended in case of concomitant use of omeprazole, ciprofloxacin and fluvoxamine and in smoking patients.

- *Nintedanib*

Nintedanib is an inhibitor of tyrosine kinases, fibroblast growth factor receptor (FGFR), endothelial growth factor receptor (VEGFR), and platelet growth factor receptor (PDGFR). The phase II TOMORROW study (115) evaluated the efficacy of Nintedanib at four different doses (50 mg/day, 50, 100 or 150 mg/twice a day). The 300 mg/day dose demonstrated in patients with IPF a reduction in lung function decline in terms of FVC, a lower incidence of acute exacerbations and a reduction, although not significant, in the St. George's Respiratory Questionnaire score (SGRQ). Side effects, represented by gastrointestinal symptoms and increased transaminases, were always more frequent in the group with the highest dose. The INPULSIS 1 and 2 phase III studies (116) were then conducted to evaluate the efficacy and safety of the drug at a dosage of 150 mg twice a day, confirming the reduction of the decline in respiratory function. The study was performed on 1066 patients; patients enrolled in the study were > 40 years of age, FVC values ≥50% predicted and a DLCO between 30-79% predicted. The FVC annual decline benefit was -114 mL with nintedanib vs. -239 ml with placebo in INPULSIS-1 and -113 ml with nintedanib vs. -207 ml with placebo in INPULSIS-2. In INPULSIS-1 there was no difference between nintedanib and placebo in terms of time from the first exacerbation, while in INPULSIS-2 patients on therapy had an exacerbation later and also fewer events. The most frequent adverse events observed were: diarrhea, transaminases, nausea, vomiting, weight loss, lack of appetite. Bleeding events were also reported (10.3% vs 7.8% in placebo). Patients who completed the two

INPULSIS trials were eligible to be enrolled in the open-label INPULSIS-ON study regardless of the annual decline in FVC (117). In INPULSIS-ON the decline in annual FVC in both subgroups was similar to that found in the INPULSIS studies and suggested that nintedanib may have similar effects on disease progression in advanced as in mild disease (117).

Since November 2014, the drug has been approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the treatment of IPF.

Since then, the manufacturing company has made itself available to supply the drug, on a compassionate basis, for patients suffering from IPF without any restriction of functional or age-related parameters.

Since April 2016, the drug has been available in Italy and is dispensed by the national health system for patients with mild-moderate IPF, which is also subject to monthly monitoring by AIFA.

The criteria for prescribing the drug in Italy are:

- age greater than 40 years
- diagnosis of IPF (exclusion of other causes of disease; consistent UIP radiological pattern or probable UIP or histological diagnosis of IPF)
- FVC>50% predicted and a DLCO>30% predicted.

Nintedanib can only be prescribed by the centers identified in the regional resolutions where the therapeutic plan is renewed every month. The starting dosage is one 150 mg tablet twice a day which, in case of side effects, can be reduced to one 100 mg tablet twice a day. The most frequent side effects are gastrointestinal symptoms (diarrhea, dyspepsia) and increased transaminases. Given the side effects, patients perform liver function checks every month for the first three months and then every three months.

The risk of bleeding was reported in the INPULSIS studies, for this reason there is still no clear indication regarding the use of antiplatelet drugs (at therapeutic and non-prophylactic doses) and both old and new generation oral anticoagulants.

In 2022 through the results of the INBUILD trial, Nintedanib was approved as a treatment for **Progressive Fibrosis Phenotype (PPF).** According to the new 2022 ATS guidelines (1), in a patient with ILD of known or unknown

etiology other than IPF who has radiological evidence of pulmonary fibrosis, PPF is defined as at least two of the following three criteria occuring within the past year with no alternative explanation:

1) Worsening respiratory symptoms;

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

a. Absolute decline in FVC >5% predicted within 1 year of follow-up;

b. Absolute decline in DLCO (corrected for Hemoglobin) >10% predicted within 1 year of follow-up;

3) Radiological evidence of disease progression (one or more of the following):

a. Increased extent or severity of traction bronchiectasis and bronchiolectasis;

b. New ground-glass opacity with traction bronchiectasis;

c. New fine reticulation;

d. Increased extent or increased coarseness of reticular abnormality;

e. New or increased honeycombing;

f. Increased lobar volume loss.

Identifying novel targets for therapy in IPF requires a clear understanding of disease pathogenesis, genetic risk factors, and biomarkers of disease progression.

- *A novel approach to treatment of IPF- The stem cells*

  Mesenchymal Stem cells (MSCs) represent multipotent cells that are easily harvested from many tissues such as adipose tissue, peripheral blood, bone marrow and umbilical cord. Several lines of experimental evidence suggest strong anti-fibrotic, anti-inflammatory and immunomodulatory effects for MCS. Administration of MSCs was associated with downregulation of TGF-β signaling, reducing lung collagen content and bronchoalveolar lavage neutrophilia. Despite relative enthusiasm, there are still major concerns on the therapeutic role of stem cells in IPF mainly arising from experimental data suggesting a detrimental role for MSCs within a pro-fibrotic microenvironment. Some

studies demonstrated that intravenous delivery of placental derived MSCs had an acceptable safety profile in patients with moderately severe IPF, showing no statistically significant decline of FVC and DLCO (diffusing capacity of the lung for carbon monoxyde) during the first 6 months of follow-up. Many unanswered questions remain regarding the administration (intravenous or endobronchial), the origin of stem cells (bone marrow, adipose tissue or placental-derived), frequency and dose therapeutic regimens and phenotypic selection (early or advanced IPF) (118).

- *A phase II trial about Phosphodiesterasi-4B (PDE4B) inhibitor*

In this phase 2, double-blind, placebo-controlled trial, was investigated the efficacy and safety of **BI 1015550**, an oral preferential **inhibitor of the PDE4B subtype**, in patients with idiopathic pulmonary fibrosis. A total of 147 patients were randomly assigned to receive BI 1015550 or placebo. The most frequent adverse event was diarrhea. A total of 13 patients discontinued BI 1015550 treatment because of adverse events. The percentages of patients with serious adverse events were similar in the two trial groups.

This study demonstrate that BI 1015550 prevented a decrease in lung function (FVC) in patients with idiopathic pulmonary fibrosis (125).

Hypersensitivity Pneumonitis

Definition and Epidemiology

Hypersensitivity pneumonitis (HP) is an inflammatory and/or fibrotic interstitial lung disease (ILD) affecting the lung parenchyma and small airways in susceptible individuals. HP involves an inflammatory and immunological response (T-helper cell type 1) to repeated inhalation by sensitized individuals of various organic substances and chemical compounds of sizes capable of reaching the alveolar spaces (126-128).

A large group of organic antigens may be responsible for this disease (thermophilic actinomycetes in farmer's lung, pigeon and parrot breeder, exposure to isocyanates, fungi such as Aspergillus Fumigatus) however, as working practices have changed, some causes of HP have declined significantly, while new exposures, such as metalworking fluids, have been implicated as the causative agent of HP among mechanical workers. The development of extrinsic allergic alveolitis would derive from the combination of a humoral and a cell-mediated reaction which would act sequentially: in the initial phase of exposure to the antigen, the humoral reaction predominates with induction, in predisposed subjects, of acute alveolitis from immune complexes; later, when exposure to the antigen ends, the cell-mediated reaction follows with the characteristic formation of granulomas. Characteristic of the disease is the presence in the serum of precipitating antibodies (IgG serum precipitins) against the various antigens, which in any case can also be only an index of exposure and not of disease (126). The antigen exposure is not identified in up to 60% of patients with HP (cryptogenic HP) (129).The prevalence of HP varies with regional disparities in climate, occupational exposures and environmental exposures. Available studies estimate an incidence between 0.3-0.9 per 100.000 individuals (146).

Clinical presentation and Diagnosis

HP is a disease with heterogeneous clinical presentations and outcomes.

The historical clinical classification of 1989 was in three clinical stages based on the onset and symptoms:

• **Acute HP**: Characterized by episodic respiratory symptoms and systemic response (fever, general malaise) occurring within hours of exposure to antigen. Recovery is spontaneous but symptoms recur whenever the antigen is inhaled. The severity of the acute crisis depends on the amount of inhaled antigen, on the predisposition of the individual and on the exposure time;

• **Chronic HP (cHP):** Patients develop more insidious dyspnea and cough over months to years (repeated inhalations of the low-dose antigen) and a clear temporal relationship to the causative antigen may be absent, making identification impossible of the responsible antigens and the diagnosis of cHP (130,131).

Evidence of lymphocytosis in the bronchoalveolar lavage fluid (BAL) is considered valuable for the differential diagnosis (prevalence of CD8+ lymphocytes, with CD4+/CD8+ ratio reversed). Lymphocytes constitute the most represented cell type in the interstitial infiltrate and alveolar cells consist predominantly of foamy macrophages. However, every effort should be made to determine the etiology through medical history, as antigen avoidance has a positive impact on mortality (132).

The prognosis depends on the duration and intensity of the exposure, and even more on the stage of the disease in which the diagnosis is made, and is excellent when only a few acute episodes have occurred and the exposure can subsequently be discontinued. Specific serum IgG testing is one way to confirm a medical history of HP exposure and risk (133).

A classification based on the presence or absence of fibrotic features in histology or high-resolution computed tomography (HRCT) specimens has been proposed, since the presence and extent of pulmonary fibrosis is critical for survival (135).

Delphi's International Modified Survey aimed to identify the most useful clinical, radiological, laboratory and pathological diagnostic criteria for HP through a three-round survey from April to August 2017 involving 45 interstitial lung disease experts from 14 countries (136). The experts identified a positive history of environmental exposure known to cause HP with a temporal relationship between symptoms and exposure and HRCT evidence of mosaic attenuation, centrilobular nodules (CLN), and ground-

glass opacities (GGO). However, CLNs are not a pathognomonic sign of cHP, as they can be found in a variety of interstitial and airway pathological conditions, and histological correlation may also vary (137). They are defined as opacities ranging from a few millimeters to 1 centimeter, separated by a few millimeters from the pleural surfaces and may be dense or ground-glass in appearance. Centrilobular nodules are thought to represent intraluminal granulation tissue in the adjacent bronchioles and alveoli and are clearly visible on HRCT (138-140). Another radiological aspect detectable by HRCT is air trapping or retention of air in the lung distal to an obstruction, which in HP is usually at the level of the secondary pulmonary lobule and can be evaluated with CT scan end of exhalation. HRCT evidence of ground-glass is a sign produced by partial filling of air spaces, with or without partial collapse of alveoli, and may be an expression of a variety of conditions ranging from inflammatory and infectious to neoplastic disorders; interstitial thickening, and particularly thickening of the interlobular septa, due to fluid and cells and/or fibrosis, can also produce this radiological sign (138). The ground-glass appearance can therefore be found in a variety of widespread chronic infiltrative lung diseases such as some interstitial pneumonias, both idiopathic and of known etiology (141). The associated radiological features and lobular distribution of GGO may aid in the diagnosis; in the case of HP, for example, a centrilobular distribution of the ground glass is envisaged. The diagnostic algorithm for chronic hypersensitivity pneumonitis is schematized in Fig5(136). In advanced stages of fibrosis with honey-combing, on HRCT is essential to make the differential diagnosis with idiopathic pulmonary fibrosis (IPF).

The new ATS guidelines of HP published on 2020 (142), categorized HP into two clinical phenotypes:

- **Non-fibrotic HP**: patients without radiological and/or histopatological evidence of fibrosis;
- **Fibrotic HP**: patients with radiological and/or histopatological evidence of fibrosis.

Both the two phenotypes have common symptoms such as dyspnea, cough, mild inspiratory squeaks (143). Less frequently, there may be constitutional

symptoms such as weight loss, flu-like symptoms, chest tightness, wheezing (144). The nonfibrotic and fibrotic HP onset may be acute (developing over days to weeks, occasionally with pleural effusion) or may also be insidious (developing and worsening over months to years); episodes may be recurrent. Although an acute presentation with or without constitutional symptoms seems more consistent with nonfibrotic HP and the insidious presentation seems more consistent with the fibrotic HP, duration of symptoms has not been rigorously characterized with respect to fibrosis status (144,145).

The new radiological classification of the HRCT-pattern is (142):

- **tipical HP:** highly suggestive of HP
- **compatible with HP**: less frequently reported but compatible with HP
- **indeterminate for HP**: when the HRCT findings are neither suggestive nor compatible with features of HP.

Nonfibrotic radiological pattern is characterized by ground-glass opacity, small airway disease, centrilobular nodules, air trapping. These parenchymal patterns are usually bilateral and symmetric with a diffuse distribution.

Fibrotic radiological pattern is characterized by lung fibrosis and bronchiolar obstruction. Lung fibrosis in HP most frequently manifests as irregular fine reticulation with architectural lung distortion, with traction bronchiectasis in areas of ground-glass opacities. Honeycombing can be present and is often described as minimal (but extensive honeycombing in severe forms may also occur). Lung fibrosis is most severe in the mid and lower lung zones, with relative basal sparing. Typical radiological pattern is the *"headcheese sign"* characterized by three density patterns: ground-glass, lobules of decreased attenuation and normal appearing lung (123). This pattern emphasizes the diagnostic value of lobules with decreased attenuation and vascularity on inspiratory HRCT-images, especially when concomitant with air trapping at expiration, both suggesting the presence of severe bronchiolar obstruction.

The *mosaic attenuation* is another important radiological sign because its presence in each of three or more lobes bilaterally was found to have the highest specificity for fibrotic HP and helped differentiate this disorder from IPF (Fig6,156).

A confident histopathological diagnosis of nonfibrotic HP requires the presence of bronchiolocentric cellular interstitial pneumonia and granulomatous inflammation (154, 156-158).

The new guidelines underlined the crucial role of the multidisciplinary discussion (pulmonologists, radiologists, methodologists, pathologists) to diagnosis HP. Serum IgG testing against potential antigens associated with HP distinguished patients with HP from exposed control subjects with a sensitivity and specificity of 90% and 91%, respectively. Precipitin testing performed best for patients with metal worker's lung and farmer's lung.

BALF analyses help to distinguish fibrotic HP from IPF and nonfibrotic HP from sarcoidosis when there is a lymphocytes count >40%.

The diagnostic algorithm of HP according with the new ATS guidelines is illustrated in Fig7.

Prevalence of HP is highest among older individuals (65 years and older), with the average patient receiving a diagnosis in their fifth or sixth decade (146). HP can also be diagnosed among younger adults and children (147).

Pulmonary function tests are usually done at diagnosis and during follow-up to evaluate for functional impairment. A restrictive pattern is the most frequent alteration found. A reduction in pulmonary diffusing capacity of carbon monoxide (DLCO) is usually an earlier finding than a reduction in lung volume (134).

Patients with fibrotic HP are more likely to be older, have an unidentified inciting agent and have lower FVC, lower DLCO and lower lymphocytes in their BAL fluid than patients with nonfibrotic HP (148).

The natural history of HP ranges from improvement to progressive decline and death due to respiratory failure (149). Patients with nonfibrotic HP who avoid ongoing exposure to the inciting agent may have a favorable prognosis with the possibility of stabilization or full recovery (149, 150). Patients with fibrotic HP, particularly those with UIP-like pattern, have reduced survival (149, 151-155).

No specific diagnostic or prognostic biomarkers are available to identify HP in the early stages and/or to assess treatment response or prognosis.

Prognosis and Treatment

The prognosis basically depends on the duration and intensity of the exposure, and even more on the stage of the disease in which the diagnosis is made. This depends on the intensity of the symptoms and the subjective tolerance of the patients. The prognosis is excellent when only a few acute episodes have occurred and exposure can subsequently be discontinued. A history of repeated acute episodes carries a much less favorable prognosis. The presence of pulmonary fibrosis is evidently a sign of an irreversible alteration. According to the data in the literature, for the majority of patients with extrinsic allergic alveolitis, survival is around 10 years from diagnosis. In the chronic forms, with evident signs of pulmonary fibrosis, survival is similar to that of fibrosis of another nature, and is linked to the functional alterations of the lung typical of this anatomical situation.

The prognosis is worse if the patient is atopic: while this condition, in fact, does not have a favorable effect on the development of the disease, it involves, through the bronchial obstruction to which it in itself predisposes, a worse functional evolution of the disease itself. Cigarette smoke, which paradoxically seems to carry out a protective action against sensitization (decreasing, through the reduction induced on the bronchial caliber, the penetration towards the pulmonary periphery of the antigens and immunologically through the stimulus induced on the macrophages, which therefore would be more active also in the purification action towards the inhaled antigens) once the disease is established, on the other hand, it causes a worse evolution, due to the chronic bronchitis and emphysematous component. The disappearance of serum precipitins in subjects removed from exposure represents a favorable element in the prognostic evaluation. Indispensable for the improvement of the disease is the removal from the allergen. This fact, however, implies a change or a reorganization of the profession which is not always possible.

Corticosteroids, administered systemically, represent the only effective therapy and should be used when environmental prophylaxis is ineffective. The treatment of the acute phase involves the administration of prednisone at doses of 40-60 mg/day, which can be progressively reduced after two

weeks. Other immunosuppressive drugs to treat HP are Azathioprine or Micofenolate Mofetil (which inhibits B and T lymphocytes proliferation) or Rituximab (which depletes $CD20^+$ B lymphocytes and inhibits T-cell co-stimulation).

Through the INBUILD study from 2022 Nintedanib was approved for Progressive Pulmonary Fibrosis included HP (1).

AIFA criteria for the prescription of the drug are:

- Age ≥18 years (no upper limit);
- >10% extent of fibrosis on HRCT;
- FVC ≥ 45% predicted value;
- DLCO ≥ 30% predicted value;
- Met at least one of the following criteria for ILD progression within 24 months before:
  - Relative FVC decline of ≥10% predicted;
  -Relative FVC decline of 5%-<10% predicted and worsening respiratory symptoms, or increased extent of fibrosis on HRCT;
  - Worsening respiratory symptoms and an increased extent of fibrosis on HRCT.

Exclusion criteria:

- Physician-diagnosed IPF;
- Relevant airways obstruction (ie, pre-bronchodilator FEV_1/FVC <0.7);
- Significant arterial hypertension;
- Significant pulmonary hypertension;
- impaired liver function (ALT, AST, or bilirubin 1.5x);
- impaired renal function (creatinine clearance < 30 mL/min);
- Known risk or predisposition to bleeding;
- Patients receiving a full dose of anticoagulation treatment with risk for bleeding;
- Recent history of myocardial infarction or stroke.

- *Non-pharmacological treatments*

In HP patients significant physical deconditioning can develop due to avoidance of activity and generalized sarcopenia due to chronic inflammation and metabolic derangement (160). Furthermore, corticosteroid use can lead to proximal myopathy. Many patients will benefit from pulmonary rehabilitation (161). Supplemental oxygen can improve endurance and is advised for all ILD patients with resting hypoxemia, and for those with exercise-induced hypoxemia (162).

A select group of HP patients with progressive disease may be suitable for lung transplantation. Transplanted HP patients appear to have better outcomes than those with IPF, with lower rates of acute rejection in the HP group in one retrospective series. Notably, HP may recur in the allograft in a small proportion of patients if the exposure is not removed (163).

In conclusion, it should be underlined that, since HP causes irreversible lung damage, it is necessary to formulate the diagnosis as early as possible and to set up a consequent therapy, even aggressive, when removal from the antigen alone is unable to control the disease (159).

Histiocytosis

Definition and classification

The term histiocytosis defines a group of rare pathologies that share the proliferation and accumulation of dendritic cells in various organs (164). Most of the histiocytes derive from the CD34+ stem cell which, conditioned by the cytokines of the cellular microenvironment (165).

The first classification of histiocytosis dates back to 1987, published by the Working Group of the Histiocyte Society (166,167) and which included 3 categories of the disease differentiated on the basis of clinical and immunohistochemical characteristics:

• Langerhans cell histiocytosis (ICL): derived from dendritic cells;

• non-Langerhans cell histiocytosis: deriving from the macrophage line;

• malignant histiocytosis.

The distinguishing feature of LCH is the presence of Langerhans cells (CL), CD1a+/CD207 Langerin+/S100+ histiocytes. Non-Langerhans cell histiocytosis, on the other hand, comprised a heterogeneous group of disorders (juvenile xanthogranuloma, Rosai-Dorfman disease, or breast histiocytosis with massive lymphadenopathy and Erdheim-Chester disease).

A new classification of histiocytosis has been proposed based on pathogenetic mechanisms, the clinical presentation and the molecular characteristics (168). These disorders were divided into 5 groups:

• L (Langerhans histiocytosis),

• C (cutaneous and mucocutaneous non-Langerhans histiocytosis),

• M (malignant histiocytosis),

•R(Rosai-Dorfman disease and non-cutaneous non-Langerhans histiocytosis),

• H (haemophagocytic lymphohistiocytosis) (169).

Langerhans Cells Histiocytosis

Definition and etiopathogenesis

Langerhans cell histiocytosis (LCH) is a rare disease and the most frequent chronic histiocytic disorder, with an estimated annual incidence of 5.4 cases per million in children (170) and 1-2 cases per million in adults (171). In childhood, males are more than twice as affected as females, but with increasing age, the predominance of males decreases almost to the point of being reversed. No genetic component has been demonstrated to date (172). The spectrum of clinical manifestations ranges from a single lesion that tends to regress spontaneously (eosinophilic granuloma), to multiple osteolytic lesions of the skull associated with exophthalmos and diabetes insipidus (DI), defined as Hand-Schüller-Christian disease, up to a very rare disseminated multi-organ form with an acute and potentially fatal course, called Letterer-Siwe disease. The term "Histiocytosis X" used by Lichtenstein in 1953 to define the three different disorders was due to the lack of knowledge on the etiopathogenesis. In 1973, Nezelof demonstrated that granulomas are characterized by the presence of intracytoplasmic granules of Birbeck (173). In 1987, the Histiocyte Society (HS) replaced the term histiocytosis X with the term Langerhans Cell Histiocytoses (LCH) to define these disorders. LCH is a proliferative disorder characterized by the accumulation of atypical histiocytes (CD1a+/CD207 langerina+/S100+), alongside normal histiocytes, lymphocytes and eosinophils, which form granulomas, characteristic infiltrates of the disease.

The etiopathogenesis remains unknown, even if various hypotheses have been formulated, from the neoplastic nature of the disease to an alteration of the immune system (174). Langerhans cells have the function of presenting the antigen, belong to the category of dendritic cells. The presence of specific surface markers, such as CD1a and CD207, also known as langherin (which acts by internalising the antigen in connection with Birbeck's granules), allow the identification of abnormal histiocytes whose finding is diagnostic for LCH. Immunohistochemical characterization with these antigens allows the differential diagnosis with indeterminate cell histiocytosis (CD207-), with Rosai-Dorfman disease (CD1a- or CD207-). LCH is distinguished from

malignant histiocytosis by the presence of histiocytes with nuclear atypia and a high mitotic index.

The variability in clinical manifestations, ranging from a single lesion to fatal disseminated forms with multiorgan involvement, has fueled doubts on considering LCH an inflammatory disease or a cancer. The function of the activated Langerhans cell is to collect and process the antigen, it migrates to the regional lymph nodes where it initiates an adaptive immune response, presenting the transformed antigen to the T lymphocytes. Compared to normal CLs, LCL histiocytes have a higher proliferative capacity and can infiltrate multiple organs (may be due to a dysregulated expression of chemokine receptors (175). In support of the inflammatory nature of the LCH, there is also clinical evidence, in particular the possibility of spontaneous regression of the lesions and the good response to therapy (176). The increased production of some cytokines (IFN-γ, TNF-α, IL-6) by CL and associated lymphocytes could explain some manifestations clinical signs of the disease (the increase in TNF-α could be responsible for fever, osteolytic phenomena, haematological and hepatic alterations) (177).

In LCH lesions, osteoclast-like multinucleated giant cells play an important role in the chronic tissue destruction observed in lesions of LCH (178-179); there is also an increased expression of metalloproteases (MMP2-MMP9) promoting invasion and metastasis (180-182). However, the fact that cell clonality is not indicative of malignancy and the persistent inability to identify specific genetic abnormalities have prevented the classification of LCH as a neoplastic disorder (183). About genetic background, there is a correlation with the mutation of BRAF gene (recurrent somatic point mutation, BRAF-V600E, in approximately 60% of LCH lesions (184). The BRAF-V600E mutation has been identified in about 8% of all human cancers, it is rarely associated with non-histiocytic malignant hemopathies, with the exception of hairy cell leukemia, of which it represents the diagnostic marker (185). BRAF V600E is specific to CLs of the LCH and is absent in normal proliferating CLs and other types of histiocytosis (186). Furthermore, in another study it was shown that patients with BRAF-V600E mutation show greater resistance to chemotherapy and a higher 5-year reactivation rate (187). BRAF plays an important role in the signaling cascade,

which starts with the activation of the tyrosine kinase receptor and proceeds with the phosphorylation of several kinases (Ras, Raf, MEK) (188).
LCH, therefore, could be considered as an uncontrolled proliferative disorder arising from a somatic mutation with inflammatory cytokines activation (189).

Clinical presentation

The clinical signs of LCH are highly variable, conditioned by the type of tissue involved and the extent of the disease. The course of the disease is generally benign although in children (age <2 years) it can be more aggressive. The disease can be:

• **localized:** mostly at bone-skin level

• **diffuse:** involving multiple organs and/or systems (bone, pituitary, hematopoietic system, liver, spleen, lymph nodes, eye, intestine, heart, nervous system, lungs) with a more severe prognosis.

In some cases, the diagnosis may be incidental. In the disseminated forms, typical of the pediatric age, general symptoms such as fever, asthenia and reduced height-weight growth are frequent. In a minority of cases the disease can explode, very serious, already in the first months of life with a leukemic-like clinical presentation: diffuse purpuric and/or necrotic skin lesions, fever, hepatosplenomegaly, adenomegaly, cytopenia. This form, formerly called Letterer-Siwe disease, carries a 20% mortality rate, even with the most modern treatments.

Bone involvement

Bone involvement is present in about 80-90% of patients with LCH and the clinical manifestations depend on the affected site (190). The most common presentation is swelling accompanied by pain. The bone segments most frequently involved are reported to be the skull (49%), pelvis (23%), femur (17%), ribs, clavicle and scapula (8%), humerus, facial mass (mandible, maxilla and orbit) and vertebrae (7%) (191). The lesions have the characteristics of an osteolysis with sharp margins in the absence of periosteal reaction.

Skin involvement

The skin is affected in about one third of patients with LCH. In 10% of cases the skin disease is isolated and can undergo, especially in children, spontaneous

regression over the course of weeks or months. The isolated form is more common in children under one year of age, while the multiorgan forms are found with equal frequency in children and adults. The areas most affected are the folds, the head, the trunk. The lesions can take on the most diverse appearances: they usually appear as purpuric papules ranging in color from brown to red, vesicles, pustules, ulcers and/or crusts. In some cases, the skin lesions appear as erythematous plaques covered with thin scales located on the scalp, behind the ears, in the inguinal or axillary folds or in the perineal area. In rare cases, growing lesions are observed, particularly at the perineal level, which can be confused with condylomas (192).

Involvement of the oral cavity

The localizations affecting the oral cavity, more frequent in adults, have a variable incidence from 10 to 35% in the different series. The lesions can affect the mandibular bone and, less frequently, the maxilla and/or the oral mucosa and/or the teeth. Bone involvement is a single osteolytic lesion with or without alveolar bone compromise. Involvement of the dental socket can lead to hypermobility of the tooth(s). Localized hypertrophy and/or ulceration of the gingival mucosa can also be observed as an isolated manifestation or associated with socket and/or bone lesions (192).

Lung involvement

Pulmonary Langerhans cell histiocytosis (PLCH) is relatively rare and usually occurs in cigarette smokers (young adults aged 20 to 40 years, with no gender predilection). The pathological hallmark of PLCH is the accumulation of Langerhans cells and other inflammatory cells in the small airways, resulting in the formation of nodular inflammatory lesions (bronchiolocentric granulomas, 1 to 10 mm in diameter). While the vast majority of patients are smokers, the mechanisms by which smoking induces this disease are not known. A combination of more results in the activation of Langerhans cells in the small airways. Cigarette smoking induces the production of growth factor TNFα by epithelial cells and macrophages, resulting in the activation of Langerhans cells (193). It also induces the production of the growth factor TGFβ by epithelial cells (194), which has been shown to be overexpressed in PLCH lung biopsies. TGFβ is an essential factor in the development of Langerhans cells and is an

important cytokine involved in the process leading to tissue remodeling for fibrosis (195).

While cellular inflammation is prominent in early disease, the more advanced stages are characterized by cystic lung destruction and pulmonary vascular remodeling. Respiratory function can therefore be compromised in various ways: from a reduction of gaseous exchanges at the alveolar level, clinically asymptomatic, up to a restrictive alteration of variable entity and respiratory failure. High-resolution CT scan chest imaging may show characteristic nodular and cystic abnormalities.

The presence of > 5% CD1a cells in bronchoalveolar lavage fluid is highly indicative of disease. Biopsy shows proliferation of Langerhans cells with occasional clusters of eosinophils (giving rise to the obsolete definition of eosinophilic granuloma) centered in cellular and fibrotic nodules that may take on a stellate configuration. Immunohistochemical stains are positive for CD1a. Lung biopsy is required for a definitive diagnosis, although it may not be required in cases where the imaging findings are highly characteristic. The most severe lesions are typically found in the upper lung fields and perihilar areas, with sparing of the lung bases. All smokers should be educated on the importance of smoking cessation, which can lead to disease regression and obviate the need for systemic immunosuppressive therapy. The prognosis for most patients is relatively good, in particular the functional trend is stable (median survival 12 years). Complications such as pneumothorax (spontaneous in 10 to 25% of patients, due to the formation of emphysematous bullae and subsequent rupture) and secondary pulmonary hypertension can shorten life expectancy. The total cessation of smoking leads to a regression of the lesions or a stabilization of the same and of the symptoms (dyspnea, stingy cough, haemoptysis, weight loss, pleuritic chest pain). In 15% of patients the pathology is asymptomatic and is found incidentally on a chest x-ray performed for other reasons (196). Patients with progressive disease may require a lung transplant, usually curative if associated with smoking cessation.

Lymph node involvement

Lymph node involvement can be isolated (the only compromised system), generally laterocervical or it can be accompanied by bone and/or skin lesions; rarely, it can be part of disseminated disease, especially in children (197).

Bone marrow impairment

The hematopoietic system is rarely involved in adults, while in children it is part of disseminated and more serious disease, and is almost always associated with hepatosplenomegaly. Bone marrow involvement can manifest as anemia, leukopenia, thrombocytopenia, and associated symptoms such as fever or bleeding. The extent of the cytopenia may not be related to the degree of bone marrow infiltration; in this case, it is often due to hypersplenism resulting from splenic compromise of the disease (198).

Involvement of liver and spleen

Hepatomegaly is very common in multisystem histiocytosis, but organ dysfunction is not always present. The enlargement of the liver can be due to various factors, such as a localization of the disease in the liver parenchyma or a hypertrophy and hyperplasia of the Kuppfer cells, and/or, more rarely, a compression by the lymph nodes of the hepatic hilum . Obstruction of the bile ducts with signs of even severe cholestasis may also be found. In advanced cases, cholangitis can result in fibrosis and cirrhosis, the pathogenesis of which is unclear; at this stage, hepatic evolution may be independent of the state of systemic disease activity. Splenomegaly is rare at onset and is characteristic of disseminated forms, especially in childhood (199).

Nervous system involvement

In some cases, LCH may begin with polyuria and polydipsia (drinking 6-8 liters of water per day) which represent the clinical manifestation of diabetes insipidus (DI), due to pituitary compromise. DI can be the only symptom of the disease, even preceding the diagnosis of LCH by many years, or it can occur at a variable distance from the diagnosis. In this case, the risk of developing diabetes insipidus is higher in patients with involvement of the bones of the face or the skull base. Other cerebral districts involved are: the pons, the cerebral hemispheres, the basal ganglia, the chiasma and the optic nerves; diffuse meningeal involvement is seen sporadically. Neurologic symptoms depend on

the site and size of the lesion and include: ataxia, dysarthria, nystagmus, cranial nerve deficits, hyperreflexia, impaired intellectual function and behavior, hemi- or paraparesis. These symptoms, however, may also be due to the extension of lesions present in a contiguous bone or in the meninges. Extra-axial lesions exert a mass effect that can cause focal symptoms, intracranial hypertension, and hydrocephalus (200).

Diagnosis

Differential diagnosis is fundamental in the diagnostic process as LCH can be confused with various other pathologies due to the extreme variability of the clinical manifestations (201). The definitive diagnosis is based on histological and immunohistochemical examination which allows the morphological identification of the CLs that are positive for the S-100 protein and for the CD1a antigen. Where the biopsy is too risky for the patient due to the site of the lesion, in this case the diagnosis is radiological, being the typical LCH lesion. Currently, a new specific marker for langherina is used for the diagnostic definition, CD207, which has replaced the search for Birbeck's granules since its expression fully correlates with their presence. There are, however, organs, such as the liver, in which the CLs do not contain Birbeck granules and CD1a and/or langherina (CD207) may be negative (202). Once the histological diagnosis has been made, it is necessary to carry out further tests to identify any asymptomatic localizations of the disease. The history should be oriented towards identifying the type and duration of symptoms, such as: pain, swelling, rash, otorrhea, irritability, fever, loss of appetite, diarrhea, weight loss, failure to thrive, polydipsia, polyuria, dyspnea , exposure to smoke, lifestyle habits and neurological alterations. In adults, the assessment of any comorbidities is important. The physical examination is aimed at a thorough evaluation of the skin and mucous membranes, the oral cavity (gingival and palatal lesions), the abdomen (hepatosplenomegaly), the nervous system (papilledema, cranial nerve abnormalities, cerebellar signs) and of the lungs. The objective examination is fundamental to exclude hepato-splenomegaly to be confirmed with radiological investigation (abdominal ultrasound). In adult patients, it is useful to perform targeted investigations for the main target organs affected by the disease, such as the lung, pituitary gland and oral cavity, even in the absence

of symptoms. Pulmonary function tests with diffusion carbon dioxide (DLCO) can identify early radiologically undetectable pulmonary compromise; nuclear magnetic renosance (MR) of the brain with gadolinium can give indications of the pituitary gland and orthopanoramic radiography with dental evaluation can highlight lesions not otherwise identifiable. As regards the evaluation of any bone lesions, in vertebral involvement it is useful to deepen the spine with MR to exclude the presence of newly formed tissue that could affect the spinal cord. From the data available so far, PET has no indications either in the staging or in the clinical management of LCH, since despite being a highly sensitive investigation it is not very specific (203). Bone marrow biopsy is indicated only in the presence of anemia and/or thrombocytopenia and/or leukopenia, with no obvious cause. In case of isolated pulmonary involvement, if the broncholavage is not diagnostic for LCH (<5% CD1a+ cells in non-smokers) it is mandatory to perform a lung biopsy, for a correct differential diagnosis with other pathologies, even if the biopsy is hampered by the multifocality of the lung lesions. Alternative procedures such as cryobiopsy could be used but with the risk of pneumothorax (201,204).

Clinical classification

The disease is defined as single system when only one organ is affected; in turn, based on the number of lesions, it is defined as uni-focal (single lesion), multifocal (several lesions involving the same organ or system). The disease is defined as multisystem when two or more organs are involved. The clinical classification is used at diagnosis to plan the therapeutic strategy.

Treatment

The disease has a variable and unpredictable course. The localized forms (skin, bone) have a good prognosis and can also regress spontaneously or with local therapies. Diffuse forms, on the other hand, require chemotherapy which is not always effective, especially in multi-system disease with organ dysfunction, frequent in children aged <2 years. The first cytotoxic drugs that demonstrated efficacy in the treatment of LCH, in mono-chemotherapy or in combination, were: chlorambucil, vincristine (VCR), cyclophosphamide (CTX), methotrexate (MTX), 6-mercaptopurine (6-MP) and vinblastine (VBL).

Approximately 50% of patients are refractory to induction therapy or develop disease reactivations within 5 years (205). Clofarabine, a second-generation nucleoside analogue, has shown significant single-agent activity against low- and high-risk cladribine- or cytarabine-refractory LCLs (206). For patients with high-risk refractory LCH, the combination of cladribine and cytarabine has proven to be the most effective rescue regimen, which, however, is burdened by high toxicity at high doses (207). In the isolated pulmonary form, honey-combing type, difficult to treat, the use of low-dose of prednisone (0.5-1 mg/kg/day for several months) associated with respiratory physiotherapy gives good results both in terms of outcome and quality of life (208). Regardless of the type and/or combination of drug used, the response to treatment in adults is lower than that observed in children, especially in some particular forms. Cladribine has been shown to be effective in multifocal forms with organ dysfunction and in those with neurological involvement (209). Patients with central nervous system involvement have been successfully treated with the combination of cladribine and cytarabine, probably because both of these drugs are able to pass the blood-brain barrier (210).

New therapeutic perspectives concern the use of imatinib mesylate, a potent competitive inhibitor of PDGFRB-associated tyrosine kinases (platelet derived growth factor receptor beta), which has been shown to be able to inhibit the differentiation of CD34+ progenitors into dendritic cells (211). Recent advances concern the use of BRAF inhibitors. The first drug used was a BRAF-V600E inhibitor, Vemurafenib. Used in 4 patients with ICL and concomitant Erdheim Chester disease, it gave a sustained response (10-16 months) even if burdened by complications, especially cutaneous, in the short and long term (212).

Lymphangioleiomyomatosis

Definition and etiopathogenesis

Lymphangioleiomyomatosis (LAM) is a rare disease that predominantly affects women of childbearing age (20-40 years). Although it has also been reported in men, it is extremely rare in men. It is characterized by cystic destruction of the lungs, lymphatic manifestations including lymphangioleiomyomas, and abdominal tumors known as angiomyolipomas, usually affecting the kidneys (213). The disease can be sporadic or associated with tuberous sclerosis complex (TSC), an autosomal dominant syndrome characterized by cerebral calcifications, mental retardation and hamartomatous lesions in several organs (214, 215). LAM is caused by mutations in the tuberous sclerosis gene TSC1 and, in most cases, the TSC2 gene, resulting in loss of function of their protein products hamartin and tuberin, respectively (216-218). The main role of the hamartine/tuberin heterotrimer is the inhibition of the mechanistic target of rapamycin (mTOR), an intracellular kinase that serves as a central regulator of cell growth and proliferation (219-221). In both sporadic and tuberous sclerosis-associated LAM, inactivating mutations of the TSC1 and TSC2 genes result in constitutive activation of mTOR.

LAM lesions are characterized by the presence of LAM cells, which include two types of subpopulations: small spindle-shaped cells expressing specific smooth muscle proteins (α-actin, vimentin and desmin) and epithelioid-like cells expressing cell markers of melanoma and immature melanocytes (gp100 and MelanA/Mart1) (222,223).

Indeed, LAM has been included in the family of "perivascular epithelioid cell tumors" (pecomas), a heterogeneous group of mesenchymal tumors composed histologically of perivascular epithelioid cells in which myogenic and melanocytic markers typically coexist (224). Furthermore, numerous genetic and cellular findings support the neoplastic nature of LAM cells.

TSC mutations that occur in LAM result from inappropriate signaling through the mTOR cascade, which controls protein transcription and is activated in many forms of human cancer (225). Identical TSC somatic mutations were detected in angiomyolipomas and lung lesions from the same patient, suggesting that LAM cells might originate from a common source (216). Furthermore, the recurrence of

LAM in lung transplant recipients and the presence of LAM cells in blood and other body fluids is consistent with the metastatic behavior of these cells (226-229). More recently, CD44v6, a glycoprotein that binds hyaluronic acid and is associated with metastatic tumors, has been shown to be expressed on the cell surface of LAM (230). The CD44v6 protein could allow LAM cells to adhere to the extracellular matrix, thus facilitating metastasis. Although LAM cells show low-grade proliferation and no evidence of cell atypia, progressive lung parenchyma infiltration, tissue destruction, angiogenesis, lymphangiogenesis, and extracellular matrix degradation are additional features that LAM cells have in common with neoplastic cells (231-234).

Clinical presentation

The disease may present acutely with pneumothorax (spontaneous PNX), manifesting with chest pain and dyspnea. Pneumothorax can be difficult to manage because it often recurs, is bilateral, and is less responsive to standard treatments. A recurrence of pneumothorax requires pleural abrasion, talcum powder, chemical pleurodesis, or pleurectomy. Patients with LAM should be informed that there is an increased incidence of pneumothorax and disease progression if they become pregnant (in which case the patient should be closely followed up). In the event of severe functional respiratory impairment, the patient should be discouraged from becoming pregnant. Acutely, the disease can also begin with hemoptysis.

Stingy cough and exertional dyspnea are instead the most frequent symptoms of insidious onset, together with asthenia and weight loss. The disease can also manifest itself with lumbar pain due to the presence of angiomyolipomas (from 1 mm to 20 cm in diameter, at risk of bleeding if > 4 cm, to be treated with embolization), which however can also be present in asymptomatic patients. Studies have found that renal angiomyolipomas are present in 100% of TSC-LAM patients and 50% of patients with sporadic LAM (235).

There are two clinical phenotypes of LAM:

- *with worse prognosis*: onset with dyspnea, weight loss, respiratory failure, reversible obstructive disease, high serum VEGF-D levels. These patients have a shorter survival and a rapid functional decline;

- *with better prognosis*: onset with pneumothorax, age >40 years, elevated FEV1 and DLCO values at diagnosis. These patients have a longer survival and a slower functional decline (236).

Radiological diagnosis

The radiological diagnosis of LAM pulmonary involvement is made by high-resolution chest CT (HRCT) which detects a pattern of multiple cysts, spread throughout the lung area and of various sizes (10 mm-10 cm). The characteristic HRCT-chest X-ray pattern for LAM is the presence of multiple (more than 10) well-defined thin-walled cysts without other significant pulmonary involvement; the HRCT-chest compatible pattern for LAM consists in the presence of a few cysts (more than two but less than 10) with thin walls (237). Biopsy (surgical) is indicated only when high-resolution CT findings are non-diagnostic. Findings of abnormal proliferation of smooth muscle cells (LAM cells) associated with cystic changes on histological examination confirm the disease. The 2010 ERS guidelines described three possible levels of diagnosis of LAM:

- *Definite LAM*: HRCT-chest pattern characteristic for LAM with at least one other disease manifestation including: angiomyolipoma, chylous thoracic/abdominal effusion, lymphangioleiomyoma, lymphadenopathy (positive for LAM cells on biopsy), tuberous sclerosis;
- *Probable LAM*: characteristic HRCT-chest pattern for LAM with compatible clinical history or compatible HRCT-chest pattern for LAM with presence of angiomyolipoma or chylous effusion;
- *Possible LAM*: characteristic or compatible HRCT-chest pattern only for LAM (237).

In the presence of an HRCT-chest pattern compatible with LAM, the abdomen must always be studied with Magnetic Resonance (MRI) or alternatively CT-abdomen with contrast medium. Conversely, in the event of an abdominopelvic lesion compatible with lymphangioleiomyoma or angiomyolipoma, chest HRCT must be performed to exclude/confirm the possibility of LAM.

The follow-up of LAM patients involves the execution of a series of blood chemistry and diagnostic tests (respiratory function tests, chest-HRCT, abdominal echography, walking test, arterial blood gas analysis, heart echography) to be

performed at a time of 3-6-12 months from diagnosis to mainly evaluate the clinical course, the radiological progression of the disease and the functional deterioration.

Biomarkers

- *VEGF-D*

Vascular endothelial growth factor D (VEGF-D) is a lymphangiogenic growth factor involved in the formation of lymph vessels and the spread of cancer cells to lymph nodes. A decade ago, serum VEGF-D levels of patients with sporadic LAM were found to be higher than those found in healthy controls (238). Subsequently, serum VEGF-D levels in patients with LAM were also higher than those in healthy volunteers and in patients with other cystic or chylous lung diseases, including PLCH and emphysema; all of which suggested that such a parameter could be a diagnostic biomarker. Furthermore, VEGF-D levels were higher in women with TSC and LAM than in women with TSC showing no signs of cystic changes on CT scans (239). A subsequent study in a larger population of LAM patients confirmed that serum VEGF-D levels were higher in LAM patients than in healthy subjects (240). However, when patient samples were pooled, based on the extent of extrapulmonary lymphatic involvement, the significant difference was maintained only for LAM patients with lymphatic involvement, and higher VEGF-D levels were associated with a score of higher severity on CT scan analysis and reduced pulmonary diffusing capacity (DLCO). This suggested that serum VEGF-D levels could be a measure of lymphatic involvement in patients with LAM (240). A prospective study of 48 patients with cystic lung disease of unknown etiology confirmed the validity of serum VEGF-D concentration as a diagnostic test. According to the authors' findings, serum **VEGF-D levels above 800 pg/mL could** be diagnostic for LAM with a sensitivity and specificity of 73 and 100%, respectively (241). A subsequent study of 75 patients with cystic lung disease reported that serum VEGF-D levels could be diagnostic for LAM with a sensitivity and specificity of 87% and 90%, respectively, with 468 pg/mL as the diagnostic threshold (242).

Based on these findings, the recent American Thoracic Society / Japanese Respiratory Society (ATS / JRS) 2016 guidelines for the diagnosis and

management of LAM recommend VEGF-D testing for a noninvasive diagnostic confirmation of LAM in cases with radiological HRCT-compatible chest, when the other confirmatory features previously reported by the European Respiratory Society (ERS) 2010 guidelines are missing (e.g. TSC, angiomyolipomas, pleural effusions, ascites, chylosis. The recommended diagnostic threshold is 800 pg/mL (243-244).

The low false-positive rate of the test indicates that serum VEGF-D concentrations above 800 pg/mL may preclude the need for a lung biopsy in patients with a chest-HRCT pattern typical for LAM. However, serum tests for VEGF-D have a high false negative rate, which means that a negative test does not rule out LAM. In these cases, confirmation with lung biopsy is required. Serum VEGF-D may also be useful as a marker of disease severity and response to therapy. These results derive from the analysis of a study on the safety and efficacy of sirolimus in LAM patients: Multicenter International Lymphangioleiomyomatosis Efficacy of Sirolimus (MILES), demonstrated that serum VEGF-D levels were reduced in the group of patients taking sirolimus , but remained stable in the placebo group during the treatment period (245). Lung function improved with sirolimus in the group with high baseline VEGF-D values, and declined more rapidly in the placebo group (246).

Therefore, the serum assay of the biomarker VEGF-D can be used both to confirm the diagnosis of LAM in patients with a characteristic HRCT-chest pattern but who do not have other typical lesions, and to evaluate the response to therapy especially in patients with lymphatic manifestations (lymphangioleiomyoma, enlarged lymph nodes, pleural or abdominal chylous effusion). However, it must be specified that serum levels < 800 pg/ml do not exclude the diagnosis of LAM, just as serum levels > 800 pg/ml in the absence of a characteristic HRCT-chest pattern and a compatible clinical presentation cannot alone confirm the diagnosis of LAM. Certainly the combination of the criteria of the 2010 ERS guidelines and the use of serum VEFG-D values has significantly reduced the need for invasive diagnosis with biopsy (243).

- *Extracellular matrix metalloproteinases (MMPs)*

Matrix metalloproteinases (MMPs) are proteases with a central role in the turnover and degradation of the extracellular matrix. In the lung, they are involved in tissue remodeling and lymphangiogenesis (247). MMPs are implicated in the pathogenesis of LAM: MMPs are present in LAM lesions (248-250). Furthermore, serum MMP-9 levels are higher in patients with LAM than in normal subjects (251). They may facilitate cell migration and contribute to the formation of pulmonary cysts (248). Monitoring of serum and urine MMPs could be a useful, though nonspecific, assessment of disease severity and response to treatment in patients with LAM (252,253). In a patient with severe LAM, urinary MMP-9 and MMP-2 levels became undetectable after 3 months of treatment with doxycycline, an MMP inhibitor (254).

Functional tests in LAM

The most common respiratory symptom of LAM is dyspnea (over 70% of patients), although pulmonary function tests (PFRs) may be normal in up to 30% of patients (255, 256).

Airflow obstruction and decreased pulmonary diffusing capacity (DLCO) are the most frequent functional abnormalities (255-258). Both ventilatory deficits correlate with disease severity as assessed by chest CT, histology, and exercise capacity (224, 259-260). The 25-30% of patients with LAM show reversible airflow obstruction (257,259). The decline in forced expiratory volume in 1 s (FEV1) in untreated patients is variable and mostly unpredictable; the mean annual decline in FEV1 ranged from 60 to 134 mL per year across retrospective studies and clinical trials (223, 261-263). Patients with LAM frequently experience impaired exercise tolerance. Inhaled salbutamol caused a slight improvement in FEV1, but did not produce a significant reduction in dyspnea during maximal exercise compared with placebo (264). Disease progression should be evaluated with pulmonary function tests every 3 to 6 months in the first year after diagnosis and then at 12-month intervals, depending on the severity of the disease.

Treatment

Both sirolimus and everolimus, two inhibitors of mTORC1, target the signaling pathway activated by mTOR, thereby inhibiting the growth and proliferation of LAM cells (265).

- *Sirolimus*

The efficacy of sirolimus in reducing angiomyolipoma volume and increasing functional parameters in patients with LAM was first demonstrated ten years ago in a pilot study, *The Cincinnati Angiomyolipoma Sirolimus Trial* (CAST) (266).

The MILES study, a randomized placebo-controlled study of the safety and efficacy of sirolimus in patients with moderate functional impairment, showed stabilization of FEV1 over 12 months of treatment, with frequent, but mostly mild to moderate adverse events (i.e. mucositis, gastrointestinal events, hypercholesterolemia, acneiform rash, and swelling of the lower limbs) (263). Improvements in FVC, quality of life, and reductions in serum VEGF-D levels were also noted. Discontinuation of therapy caused a rapid decline in lung function, similar to that in the placebo group, suggesting the need for continued treatment. Sirolimus also ameliorated the chylous manifestations of the disease (267). Another current concern regarding mTOR inhibitor therapy is whether low doses of the drug could be used to maintain therapeutic effects and reduce toxicity. A retrospective study in a small cohort of patients before and after therapy suggested that sirolimus may be effective in maintaining pulmonary function and controlling chylous effusions even at trough serum levels below 5 ng/mL (268). However, the incidence of adverse events was similar to that reported in previous studies, with the exception of hypercholesterolaemia.

Recent ATS/JRS guidelines recommend sirolimus treatment for patients with impaired lung function, defined as FEV1 less than 70% predicted or patients with progressively declining lung function. Furthermore, the drug is recommended in LAM patients with chylous effusions before considering invasive procedures (243).

- *Everolimus*

Two randomized controlled trials (EXIST-1 and EXIST-2) showed that everolimus, a second generation mTOR1 inhibitor, was effective in reducing the size of giant cell astrocytomas in patients with TSC and the size of renal angiomyolipomas in patients with with TSC or LAM (269,270). A non-randomised study conducted in 24 patients with sporadic LAM or TSC-LAM demonstrated that the safety profile of everolimus was similar to that of sirolimus in the MILES study. There were four drug-related serious adverse events, including pneumocystis, heart failure, and pneumonia, that were not observed in the MILES study. Serum VEGF-D levels decreased throughout the duration of treatment. Treatment with everolimus resulted in stabilization of FVC and improvement in FEV1, compared to baseline (271).

- *Other therapies*

To date, hormonal treatments including progesterone, GnRH antagonists, anti-estrogen therapy (tamoxifen) are not recommended, because available study results are controversial or inconclusive and no controlled clinical trials have been conducted (272, 273).

Doxycycline is a tetracyclic antibiotic which inhibits the production and activity of several MMPs: its potential role in the treatment of LAM has been suggested in patients with severe pulmonary impairment, in whom treatment with this drug improved lung function with increased of FEV1 (254). Patients, however, may be unresponsive or intolerant to mTOR inhibitor treatment, or even recur after discontinuation of therapy. These results suggest that mTOR inhibitors are not curative for LAM. Recent advances in understanding the pathogenesis of LAM suggest a new therapeutic potential. A recent clinical study evaluated the tolerability and safety of sirolimus therapy in combination with hydroxychloroquine (200 and 400 mg per day) for a treatment duration of 24 months (274). The main side effects noted were mucositis, headache, diarrhea. An improvement in FEV1 and FVC was found at 24 weeks.

Furthermore, a tuberin deficiency due to TSC2 mutations results in increased activity and RhoA GTPase which promotes cell survival. Because of their inhibitory effects on RhoA GTPase, statins have been considered as a possible

therapy in LAM (275). A retrospective study of patients who were treated with a combination of sirolimus and simvastatin or with sirolimus or simvastatin alone showed that the combination therapy had no beneficial effect and did not increase the incidence of adverse events, in addition to those foreseen by the use of each single drug (276).

Sarcoidosis

Definition and etiopathogenesis

Sarcoidosis is a systemic inflammatory disease characterized by the presence of granulomas in any organ, although the lung is the most common site. Sarcoidosis has different clinical phenotypes (resulting from the combination of genetic variants and environmental factors). The course of the disease may vary (many patients recover without treatment, while others develop chronic inflammation and fibrosis) (277, 278). Sarcoidosis often substantially affects quality of life, decreases work capacity, and increases mortality. The incidence is highly variable and depends on gender, age and ethnicity. Seasonal and geographic variations in incidence indicate an influence of unknown agents, possibly including microorganisms, environmental factors and/or inorganic materials, which trigger inflammation in genetically predisposed individuals. Genetic factors clearly play an important role in the etiology of sarcoidosis, as evidenced by the considerably higher disease risk in first-degree relatives of sarcoidosis patients. HLA alleles and variants of other genes, such as TNF, may be associated with disease course and predict prognosis (279).

Sarcoidosis is characterized by the accumulation of activated T-helper cells in the lungs and the formation of non-necrotizing epithelioid cell granulomas, suggesting that a specific antigen or antigens trigger an immune reaction. In a subset of patients, T cells accumulated in the lungs express identical T cell receptors (TCRs), suggesting that these patients were exposed to an identical antigen (microorganisms or their non-degradable remains, mycobacterial antigens [ESAT6, KatG] or eg Mycobacterium tuberculosis or Propionibacterium acnes) which could act as a trigger (279). The autoimmune hypothesis is supported by the detection of vimentin-directed TCR receptors and anti-vimentin antibodies in patients with sarcoidosis. Vimentin is an important constituent of the cytoskeleton, predominantly expressed by mesenchymal cells and produced by activated macrophages in sarcoid granuloma. Anti-vimentin antibodies have been detected in the BAL of subjects with sarcoidosis (HLA-DR3+). Vimentin is known to be involved in autoimmune diseases such as systemic lupus

erythematosus, in which the levels of anti-vimentin antibodies correlate with disease severity (280).

Epidemiology

The incidence and prevalence of sarcoidosis and its clinical presentation vary greatly by geography and across genders, ethnicities, and age groups (281). Sarcoidosis incidence is highest in Scandinavian countries (11–24 cases per 100,000 person-years) (282-284) and among African Americans (18–71 cases per 100,000 person-years) (285-287) and lower in Asian countries (1 case per 100,000 individuals per year) (288, 289). Within countries, the distribution of sarcoidosis varies by geographic region (285, 287, 290, 291), and some studies have shown that the prevalence is higher in less densely populated areas (282, 292). The mean age of onset is between 40 and 55 years, with a younger peak age at diagnosis in men (30-50 years) than in women (50-60 years). The clinical manifestations of sarcoidosis vary according to the characteristics of the patient. African Americans have more severe disease at diagnosis and are more likely to have advanced pulmonary or multiorgan involvement (281) than individuals of other ethnicities. Löfgren syndrome has the highest reported incidence in white individuals and is rarely diagnosed in African Americans or Asian individuals. In Sweden, Löfgren syndrome comprises approximately one third of all sarcoidosis cases and patients usually carry the HLADRB1*03 (HLA-DR3) allele and have a good prognosis, with remission in 70–80% of these patients (293).

Risk Factors

- **Genetic factors**: genetic susceptibility is an important component of the risk of developing the disease, as demonstrated by genome association studies (294, 295) with the HLA class II allele (296,297). Having a family member with the disease is associated with a 2–4-fold increased risk of developing sarcoidosis (297, 298), and the risk increases with the number of affected relatives (298). The heritability of sarcoidosis is estimated at 39–70% (299, 300).

- **Environmental factors and lifestyle**: the ACCESS study conducted in the United States investigated occupational exposures retrospectively using questionnaires (301), identifying exposure to various environmental agents in patients with sarcoidosis such as molds, insecticides, agricultural environment, exposure to silica dust in foundries, firefighters (302,303). Several studies have demonstrated that inhalation of inorganic material can induce sarcoidosis. Smoking has been consistently associated with decreased risk of sarcoidosis, probably due to the immunomodulatory effects of nicotine (301). Several studies have found cases of sarcoidosis induced by immunotherapy in individuals treated with immunostimulant drugs for malignant or chronic conditions (anti-IFNα, anti-IFNγ as in psoriasis, anti-PD1 and BRAF inhibitors in melanomas). To date there is no consensus opinion on whether it is true sarcoidosis or sarcoidosis-like reactions in these individuals. These antibodies are able to restore the proliferative capacity of CD4+ T lymphocytes, leading to the formation of granulomas. Anti-TNFα biologics (etanercept, infliximab, adalimubab) are rarely associated with the onset of sarcoidosis (or sarcoidosis-like reactions) (304-309). Cases of sarcoidosis have also been reported in recipients of bone marrow transplants from donors with sarcoidosis (310).

Comorbidities and mortality

Epidemiological studies show that sarcoidosis is not a benign disease for many patients but instead sufferers have a high disease burden (311) and show increased mortality. Overall sarcoidosis mortality is 9–14 cases per 1,000 person-years, and 5-year survival is 93–95% (312-315). Mortality risk increased 60% in Sweden (314), 70% in Korea (313), doubled in the United Kingdom (312), and increased 2.4-fold in black American women (315). Mortality is higher in those with more severe disease at diagnosis.

Sarcoidosis is associated with an increased risk of infections, further increasing in patients receiving immunosuppressive therapy (316), congestive heart failure (317, 318), cerebrovascular events (318), venous

thromboembolism (319), autoimmune disorders (particularly thyroid autoimmune disease, Sjogren's syndrome and systemic lupus erythematosus) (320). In a systematic review and meta-analysis of 16 studies, sarcoidosis was associated with an increased risk of hematologic cancers, skin cancers, upper digestive tract cancer, kidney cancer, liver cancer, and colorectal cancer (321). About 30% of patients develop pulmonary fibrosis with increased mortality.

Granuloma's formation

The histopathological feature of sarcoidosis is the presence of non-necrotizing epithelioid cell granulomas with varying degrees of lymphocytic inflammation (322). Such granulomas are the result of exposure to unknown antigens in genetically predisposed individuals, in whom there is immune dysregulation with an exaggerated T-helper1 (Th1) lymphocyte response (279). In the 1960s and 1970s clinical studies demonstrated peripheral lymphocytopenia in patients with sarcoidosis and subsequently with the experience of bronchoscopy with alveolar broncholavage (BAL) an intense lymphocyte activation was detected in the affected disease sites (in the case of BAL the lung), with a prevalence of CD4+ T-helper lymphocytes. They differentiate into Th1 and Th17 effector cells with production of proinflammatory cytokines (IFNγ, IL17, TGFβ) (323-325).

The role of Th17 cells in sarcoidosis is not well understood, but they appear to be important in determining the clinical phenotype (they are highly expressed in Lofgren syndrome) (326-327). The immune response is crucial in the pathogenesis of sarcoidosis. Presumably there is a trigger (unknown antigen) which stimulates the response of macrophages and dendritic cells; activated macrophages transform into epithelioid cells that can cluster into multinucleated giant cells. Serum amyloid A (SAA) protein also contributes to the aggregation of these cells into granulomas, which amplifies the immune response of Th1 lymphocytes (328, 329).

Resolution of the granuloma occurs when the (unknown) peptide antigen is presented by HLA-DR3 molecules present on dendritic cells or macrophages, with recognition by the CD4+ T-cell receptor (TCR). The result is an immune response that involves a cytokine cascade that leads to

the elimination of the antigen and the resolution of the granuloma (**type 1 immune response**). Instead, the progression of the granuloma occurs when there is no recognition of the antigen perhaps due to the exposure of other molecules other than HLA-DR3. As a result, the granuloma continues to expand and the disease persists (**type 2 immune response**) (330) (Fig8).

Clinical presentation

The clinical classification of sarcoidosis can be made on the basis of onset, natural history and organ involvement. The onset of the disease can be acute or gradual, but sometimes asymptomatic sarcoidosis can be diagnosed incidentally during tests done for other reasons. The acute onset is typical of the Scandinavian populations with fever, erythema nodosum (inflammation of the subcutaneous adipose tissue), widespread arthralgias. In some cases, the pathology can also present with cardiac arrest or neurological manifestations. In 50% of patients, the disease resolves spontaneously within 2 years, in other cases within 5 years (remission is rare after 5 years) (331). The most severe clinical manifestations of the disease are pulmonary, cardiac and neurological.

The disease can also manifest by syndromes and be diagnosed without the need for histological examination (331). Lofgren's syndrome occurs in patients with a combination of genetic, immunological and environmental factors (onset mainly in the spring months). This syndrome is a well-characteristic phenotype of sarcoidosis (332-334).

Heerfordt syndrome is instead a rarer form that arises with uveitis, enlargement of the parotids, paresis of a cranial nerve (especially the VII) and is mainly a sporadic form (335).

Pulmonary sarcoidosis

In the thoracic area, the organs most involved are the lungs and mediastinal lymph nodes (80-90%) of cases. There is a **radiological staging of pulmonary sarcoidosis** described for the first time by **John Scadding** in 1950), subsequently also revealing a prognostic value (Table1). Pulmonary sarcoidosis can evolve into pulmonary fibrosis progressively and with poor prognosis. Different radiological patterns have been found on CT scans in

the fibrotic evolution of sarcoidosis, such as traction bronchiectasis, cysts, honeycombing (336).

Cardiac sarcoidosis

Clinically detected in 2-7% of patients with sarcoidosis, it presents occultly with myocardial involvement in 20% of cases. Cardiac sarcoidosis can also occur in the absence of pulmonary or systemic involvement. Early diagnosis and immediate therapeutic intervention can save the lives of these patients. Clinically it can present with ventricular arrhythmias, high-grade heart blocks, progressive heart failure, due to infiltration of the myocardium by granulomas (337-339). To date, the best diagnostic test for recognizing cardiac sarcoidosis is gadolinium-enhanced cardiac MRI (340).

Neurological sarcoidosis

It occurs in 4-10% of sarcoidosis cases. It predominantly affects the cranial nerves, meninges, brain, spinal cord, hypothalamus, neurohypophysis, and peripheral nerves (341). Hypothalamic and pituitary involvement can lead to hyperprolactinaemia, decreased circulating testosterone and follicle stimulating hormone (FSH), diabetes insipidus (342).

In 2002, small fiber neuropathy was recognized as a nongranulomatous parasarcoidosis syndrome (343). Clinically it manifests as pain, hyperesthesia or hypoesthesia or dysautonomia. It is a syndrome that is rarely recognized and difficult to treat.

Other organs involved in sarcoidosis

The location of sarcoidosis in organs other than those mentioned above depends on the individual's ethnicity and occupational exposure.

For example, ocular involvement (uveitis) is more frequent in black and Asian patients (10-30% of cases) (331). Skin involvement occurs in 15% of patients, mostly in black individuals.

Of interest is how granulomas tend to form on tattoos and scars (353). Although facial pernio lupus does not put patients' lives at risk, it causes aesthetic and psychological problems; however, sometimes it is associated with severe sarcoidosis of the paranasal sinus, putting the patient's life at risk. The systemic localizations of sarcoidosis are illustrated in Table2.

Diagnosis

The diagnosis of sarcoidosis has a clinical correlation with the histopathologic finding of non-necrotizing granulomas (344). Possible differential diagnoses should be excluded and a diagnostic conclusion should be reached after discussion with a multidisciplinary team (345).

Unfortunately, a diagnosis of sarcoidosis is made at the first visit in only 15% of cases (346).

As a first approach, laboratory tests including serum calcium, VitD dosage, angiotensin converting enzyme (ACE) and soluble interleukin 2 receptor (rIL2, indicator of lymphocyte activation) can be performed, but with little diagnostic value. More than anything else, they are useful biomarkers for monitoring the progress of the disease and the response to therapy (347).

Although chest X-rays can detect early signs suggestive of pulmonary sarcoidosis, it is high-resolution pulmonary computed tomography (HRCT) of the chest that most definitely shows sarcoidosis pulmonary involvement. In patients with Lofgren's syndrome, the diagnosis is made only with chest radiography and monitoring the progress of the disease. In more complex cases of pulmonary sarcoidosis, HRCT allows to evaluate the presence of pulmonary fibrosis, or complications secondary to immunosuppressive therapy (aspergilloma) or non-infectious complications such as pulmonary hypertension (348). Magnetic resonance with gadolinium (MRI) cardiac and encephalic allow to evaluate the possible involvement of the pathology in these sites, just as with an echocardiography it is possible to verify alterations at the level of the left ventricle (alterations of the kinetics) or of the right (increase of diameters secondary to pulmonary hypertension), indicative of a poor prognosis (338). Fluorodeoxyglucose (18F-FDG) positron emission tomography (PET-CT) nuclear radiology may also help detect occult inflammatory lesions in the lungs and heart, indicative of disease activity (349).

Bronchoscopy is currently a fundamental diagnostic test, with the possible finding of endobronchial granulomas on which to perform a biopsy (350). Alveolar broncholavage (BAL) helps to exclude alternative diagnoses, being

strongly diagnostic for sarcoidosis when there is significant lymphocytosis with an increase in the CD4+/CD8+ ratio > 3.5 (351).

Ultrasound-guided transbronchial lymph node aspiration (EBUS-TBNA) is very useful for excluding other possible pathologies responsible for mediastinal lymphadenopathy (e.g. lymphoma or infections) (352).

Pulmonary function tests have no diagnostic role in this pathology, but are used to evaluate the degree of pulmonary involvement and response to therapy. The diagnostic algorithm of sarcoidosis is summarizes in Fig9.

Treatment

The clinical course of sarcoidosis is highly variable, from spontaneous resolution to severe forms with pulmonary and cardiac involvement leading to lung transplantation (354). The introduction of a therapy must be evaluated on the basis of the clinic, the therapeutic benefits and the side effects.

There are patients who only need corticosteroids, others oxygen therapy, others implantable defibrillators due to ventricular arrhythmias, others desmopressin therapy for diabetes insipidus.

The main anti-inflammatory therapy administered in sarcoidosis is that with glucocorticoids, which must be personalized in the light of the damage it causes if taken chronically (arterial hypertension, iatrogenic diabetes mellitus, neuropathy, myopathy, adrenal insufficiency) (355). In patients with evident functional deterioration, steroid therapy leads to stabilization or improvement (356-358). Studies show that significant lymphocytosis in the BAL is associated with forms of sarcoidosis that undergo spontaneous remission, while neutrophilia would be indicative of progressive forms that require close monitoring (359, 360). Glucocorticoids are the first line of treatment in the symptomatic patient. Inhaled steroids cannot be used as a substitute for oral steroid therapy (331). Initial treatment often involves prednisolone 0.5-0.75 mg/kg/day for 4 weeks, then tapered to 10 mg/day for another 4 weeks, to be adjusted according to the patient's response. Usually this therapy is continued for 12 months, evaluating its suspension on the basis of functional improvement and disappearance of symptoms. There may

be disease relapse 24 months after diagnosis in the refractory forms. In any case, a low maintenance dose for 6-12 months is frequently associated with the onset of weight gain, insomnia, glaucoma, cataract, diabetes, myopathy, personality change, osteoporosis (361). In some patients after the suspension of cortisone therapy taken chronically, a recurrence of the disease can occur. In these cases, corticosteroid therapy should be reintroduced at a dosage of 0.5 mg/kg/day for 8-12 weeks, followed by a maintenance dose of 5-10 mg/day, with the addition of an immunosuppressant drug. If this therapy is well tolerated, it should be continued for at least two years (356, 362, 363).

Hypercalcemia occurs in 5 to 10% of patients with sarcoidosis; it could be prevented by the administration of bisphosphonates (364, 365).

In case of no response to steroid therapy, immunosuppressants such as azathioprine, methotrexate, mycophenolate mofetil can be administered.

If these therapies fail, cyclophosphamide may be considered. These drugs should be administered together with a minimal dose of oral steroid therapy (366).

Several studies show TNFalpha as a potential therapeutic target (367); the use of monoclonal antibodies inhibitors of this cytokine (infliximab, adalimumab), prevails in extrapulmonary forms of sarcoidosis refractory to therapy (368).

Lung transplantation should be considered as a therapeutic intervention in end-stage pulmonary sarcoidosis or pulmonary hypertension (369).

The treatment algorithm of sarcoidosis is illustrated in Fig10.

The new ERS guidelines 2020 explain that for patients with pulmonary sarcoidosis, initiation of systemic treatment is reserved for patients who communicate symptomatic disease impacting quality of life and/or whether the patient's disease can lead to progressive lung function decline or significant morbidity or mortality. For patients with extra-pulmonary involvement, the decision to treat is similarly dependent on the presence of clinically significant disease activity in the affected organ (presumed to impair quality of life (QoL) and/or threaten organ function) and is left to the clinicians judgment (370).

Presence of clinically significant cardiac, neurologic, ocular or renal involvement is often associated with significant morbidity and mortality and treatment is usually indicated (371). The ERS guidelines published a consensus statement from a large group of sarcoidosis experts advocate an approach to therapy that balances the use of reduced doses of corticosteroids with the early stepwise addition of steroid sparing anti-inflammatory non-biologic and biologic agents (371).

The proposed approach to corticosteroid use is to limit continuation to a 3-6 months period to allow for demonstration of therapeutic response. During that time period, attempts are made to taper to the minimal effective dose with a goal maintenance dose of < 10 mg/day of prednisone/prednisone equivalent (372). If the patient's disease remains uncontrolled on minimal steroid doses or significant steroid side effects develop, therapy is then stepped up to steroid-sparing non-biologic immunosuppressive therapy. The guidelines make for early/concomitant initiation of steroid-sparing non-biologic agents (so-called "second-line agents") for patients with severe or multi-organ disease where prolonged therapy is anticipated or where there is a high risk of steroid-induced toxicity. For patients with symptomatic pulmonary sarcoidosis believed to be at higher risk of future mortality or permanent disability from sarcoidosis, the guidelines recommend addition of methotrexate (MTX). This drug is considered the preferred "second-line agent" with the most data supporting its use in sarcoidosis (373,374). Other commonly used "second-line agents" include: Azathioprine, Leflunomide and Mycophenolate Mofetil, however the evidence behind these medication recommendations is very weak. For patients with symptomatic pulmonary sarcoidosis with higher risk of future mortality or permanent disability from sarcoidosis who have been treated with glucocorticoids and MTX and have continued disease, the guidelines suggests the addition of infliximab to improve and preserve lung function and QoL (371). Infliximab is a tumor necrosis factor inhibitor (TNFi) that has been shown to be effective in severe and refractory forms of pulmonary and extrapulmonary sarcoidosis (375, 376). TNFi Infliximab and Adalimumab are regarded as "third line agents"

to be added in patients whose sarcoidosis is uncontrolled on second-line therapy (Fig11).

Systemic Sclerosis associated to interstitial lung disease

Definition and epidemiology

Systemic sclerosis (SSc) is an autoimmune connective tissue disease, which is characterised by immune dysregulation and progressive fibrosis that typically affects the skin, with variable internal organ involvement. It is a rare condition that affects mostly young and middle-aged women. It was initially described in mid-18th century Italy by Carlo Curzio as a condition that turned skin to wood (hard-skin) (377).

The diagnostic criteria for SSc were revised in 2013 by the European League Against Rheumatism (EULAR) and the American College of Rheumatology, to include important immunological, fibrotic and vascular features of the disease (Table3) (378).

SSc has traditionally been divided into three subsets on the basis of the extent of skin involvement:

- **limited cutaneous**: skin changes limited to distal to the elbows and knees but can involve the face and neck;
- **diffuse cutaneous**: skin involvement extends proximally to the elbows and knees;
-**sine scleroderma**: absence of skin thickening but internal organ involvement and serological abnormalities.

Although there are different risks of internal organ involvement in each subset, interstitial lung disease (ILD) can occur in all three causing morbidity and mortality. In the European Scleroderma Trials and Research database (EUSTAR), pulmonary fibrosis accounted for 35% of SSc mortality (379).

All patients with a diagnosis of SSc should be screened for ILD.

Risk factors for develop ILD in Systemic Sclerosis

Interstitial abnormalities are evident in chest-HRCT in up to 80% of patients, but only 30-40% will develop clinically significant ILD, with a 10-year mortality of up to 40%. Although, the prevalence of ILD increases during the course of the disease, its onset is often within 5 years of the first non-Raynaud phenomenon symptom and almost never more than 15 years after

diagnosis of SSc (380). Early development of SSc-ILD (less than 3 years after diagnosis) is associated to an aggressive clinical course (381).
The demographic factors associated with the presence of SSc-ILD include male sex, African American race and diffuse skin disease (382).
A genetic component has been well established, with most risk attributed to polymorphisms in the HLA region and in genes implicated in innate immunity, B-lymphocyte and T-lymphocyte activation. Patients with positive test for anti-topoisomerase antibodies have increased risk of develop ILD, that is rare in patients with anti-centromere antibodies (Fig12) (383-384).

Pathobiology, clinical presentation and radiological pattern

SSc-ILD is a result of the interplay between fibrosis, autoimmunity, inflammation and vascular injuries. The initial event has been proposed to be an injury to the alveolar epithelium or vasculature, followed by aberrant activation of the immune system, which promotes fibroblast recruitment and activation and extracellular matrix overproduction (385).
Clinical manifestations of SSc include:
- **sclerodactyly**: thickening of the skin on the fingers with associated flexion contractures;
- **raynaud phenomenon**: vasospasm of the fingers resulting in cyanotic discoloration;
- **telangiectasias**: dilated blood vessels near the surface of the skin, on the face and palms;
- **abnormal nailfold capillaroscopy:** active pattern revealing dilated capillaries as well as areas of drop-out;
- **digital ulcers.**

A considerable proportion of patients with SSc-ILD are asymptomatic, especially in the initial stages of disease. When reported, dyspnoea (initially on exertion and eventually at rest), non-productive cough, fatigue are the most common symptoms. Physical examination typically reveals velcro-like crackles on auscultation, in addition to the cutaneous findings associated with SSc. Pulmonary function tests often reveals restriction with reduced FVC and DLCO (386).

The most common imaging pattern on HRCT is NSIP (non-specific interstitial pneumonia) in more than 80% of patients. This pattern is characterized by peripheral ground-glass opacities mostly at the lower lobes with subpleural sparing, traction bronchiectasis and bronchiolectasis. A UIP pattern is present less than 10% of patients with SSc-ILD (387, 388).

Other common findings include dilated eosophagus, chronic aspiration, pulmonary artery enlargement, right ventricular dilatation which indicates pulmonary hypertension (PH). PH is reported in up to 20% of patients with SSc-ILD. Gastrointestinal involvement could also lead to a poor nutritional status, resulting in sarcopenia, neuromuscular impairment and respiratory failure (399).

SSc-ILD prognosis

Although SSc-ILD was traditionally considered slowly progressive with an expected median survival of 15 years. Male sex, active smoking, older age at presentation are associated with increased risk of disease progression and early mortality (389,390). The presence of a radiological UIP pattern, arthritis, digital ulcers, pulmonary hypertension, progressive skin fibrosis, renal disease, myocardial fibrosis are associated with an aggressive clinical course (389, 391). In contrast, the absence of ILD on an initial HRCT is a favourable sign.

Treatment

The first line therapies for SSc are immunosuppressive drugs cyclophosphamide (intravenous form has lower toxicity than the oral form), corticosteroids, azathioprine.

Important is to treat aggressive reflux (with proton pump inhibitors or surgery), supplemental oxygen, pulmonary rehabilitation.

Second line agents are biological therapies such as rituximab and mycophenolate mofetil, if failed first line immunosuppressive therapy (392-394).

Nintedanib is a tyrosine kinase inhibitor that has been shown to affect crucial fibrotic mediators, including the platelet-derived growth factor receptor, fibroblast growth factor receptor, vascular endothelial growth factor receptor

and the kinase. It inhibits human fibroblast proliferation, myofibroblast differentiation and collagen release (395, 396). In the 2019 SENSCIS trial demonstrated the efficacy of Nintedanib in 580 patients with SSc-ILD positive to anti-topoisomerasi antibodies test. The study demonstrated that the antifibrotic drug in these patients reduced the FVC decline by 44% than in the placebo group (397). In 2019 the INBUILD trial demonstrated the efficacy of Nintedanib in patients affected by SSc-ILD with progressive fibrosis, reducing the annual FVC decline rate by almost 60% (398). Patients with extensive disease (> 20% on HRCT) or clinically significant disease progression should be treated with antifibrotic drug in monotherapy or with a combination therapy with immunosuppressive drugs. Cyclophosphamide or mycophenolate can be considered first line in patients with a more inflammatory phenotype. Rituximab could be considered for refractory disease (401).

Other possible treatment are autologous stem cell transplantation and lung transplantation (with an average survival of about 5 years) (400).

Corticosteroids maintenance dose could be use after lung transplantation to prevent renal crisis. Iloprost can be used to treat digital ischaemia.

If there is no interstitial lung disease the patient with SSc-ILD could be monitored with pulmonary function tests every 3-6 months for 3-5 years, than annually.

NSIP (non specific interstitial pneumonia)

Non specific interstitial pneumonia (NSIP) is an interstitial lung disease that may be idiopathic or secondary to connective tissue disease (polymiositis/dermatomyositis, Sjogren, Systemic sclerosis), toxins (chemotherapy) or numerous other causes. Idiopathic NSIP (iNSIP) is a rare diagnosis and requires exclusion of these other possible causes (also IgG4-related disease or bone marrow-transplant). Only in 2013 iNSIP was determined to be an official idiopathic interstitial pneumonia (402-404). Prevalence of iNSIP is estimated to ben 1 to 9/100000 patients. Some studies demonstrated 10% of familial cases of NSIP (405-406).

Clinical presentation of NSIP is characterized by cough, dyspnoea, fatigue, weight loss (402, 407, 408). Physical exam common features are inspiratory crackles, digital clubbing (408-410). Approximately one half (42-69%) of patients have never smoked (409, 411).

Pulmonary function tests reveal a restrictive ventilatory defect with a decreased DLCO (407,409). Bronchoalveolar lavage fluid often shows a lymphocytic population (412).

An HRCT-scan is necessary for the radiological confirmation of ILD, but the diagnosis of NSIP need an hystological confirmation (by surgical biopsy or criobiopsy in more lung lobes). Radiological characteristics are:

- reticular abnormalities;
- traction bronchiectasis;
- lobar volume loss;
- ground-glass attenuation;
- sub-pleural sparing (not pathognomonic) and peribronchial thickening (413).

Honeycombing is rare and when present in NSIP is associated to a worse prognosis (414). Important is the difference that UIP pattern can be diagnosed in the correct setting by radiology alone, whereas NSIP pattern requires a biopsy for diagnosis (407, 415). Honeycombing as an isolated findings does not make a diagnosis of UIP.

After histologic diagnosis of NSIP is established, it is necessary to re-evaluate and rule out other secondary causes of NSIP (occult connective

tissue diseases-CTD, inhalation exposure), ensuring the case is in fact idiopathic. Crucial is the role of a multidisciplinary discussion with pathologist, radiologist, pulmonologist that assists in reaching a consensus diagnosis and improves diagnostic accuracy.

The prognosis for iNSIP is overall favourable in comparison with IPF (410, 412), although there is an approximate 20% mortality rate in 5 years (414, 416). The prognosis for iNSIP is similar to that of secondary NSIP from CTD (417). Fibrosing NSIP patients have worse outcomes than those with cellular NSIP, but they have better survival rates than IPF (418).

A fall in FVC of > 10% within 6-12 months of follow-up has independently been associated with mortality (414). Patients who have been declines in FVC > 10% or DLCO > 15% unexplained by other causes (e.g. infection, pulmonary hypertension) should be considered for escalation of treatment and/or lung transplantation.

In cases of mild or asymptomatic disease, serial monitoring of symptoms and pulmonary functions may be employed. Treatment should be implemented if progression of disease is noted (419).

Corticosteroids are the predominant and often initial agent of choice. Cytotoxic agents (azathioprine, cyclophosphamide, cyclosporine, mycophenolate mofetil) are used to augment or supplant corticosteroid treatment. Rituximab demonstrated good results in patients with NSIP and undefined connective tissue disease (420).

There are no clear guidelines regarding glucocorticoid dosage or duration of therapy. Some studies reported an initial dose of prednisolone (0.5 mg/kg or 1 mg/kg) for 1 months, then tapering in 4-7 months (414, 421,422). Optimal treatment length is not defined nor is known if indefinite treatment is beneficial. No consensus exists as to whether a cytotoxic agent should be started at diagnosis versus upon disease progression or corticosteroid dependence (423). Cyclophosphamide may be considered in patients who fail other immunosuppressive regimens (424).

Improvement or stability of spirometry, lung volumes, DLCO, radiographic findings as well as diminished symptoms of cough and dyspnoea reveal a

favourable treatment response (414,421). Spirometry and DLCO should be monitored to determined treatment response and disease progression.
In case of disease progression without a treatment response is it necessary an evaluation for lung transplantation.

Rheumatoid arthritis (RA)

Rheumatoid arthritis (RA) is a chronic autoimmune disease affecting the joints, characterized by a symmetric inflammation of joints leading to cartilage destruction and bone erosion. It affected few or many joints and could have also extrarticular symptoms. The prevalence of this disease ranges from 0.4% to 1.3%, affecting mainly women (age 60 years). It is one of the most prevalent chronic inflammatory disease, higher in urban than rural areas. There is an inflammation of the joint capsule by T cells, macrophages, B cells, neutrophils, fibroblasts, osteoclasts.

The etiology of RA is unknown, but genetic (HLADRB1 alleles) and environmental factors contribute to its development. There are many risk factors linked to RA: smoking, obesity, exposition to UV-light, drugs, periodontal disease, viral and bacterial infections (in genetic predisposed individuals the trigger antigen activate antigen presenting cells which stimulate Th1-Th17 and B immune response with tissue injury). Activated T cells migrating to the synovium locally interact with resident macrophages, synoviocytes, osteoclasts and dendritic cells. There is the secretion of many cytokines (IL-2, IL-6, IFN- γ, TNF-α) that activate B cells, leading to the perpetuation of inflammatory process in the synovium. TNF-α induce the differentiation of macrophages into osteoclasts, causing cartilage degradation and bone resorption.

Typical symptoms are: fatigue, flu-like feeling, tender joints, morning stiffness. However, insufficiently treated RA could lead to systemic manifestations such us lung nodules, pleural effusion, interstitial lung disease, anemia, leukopenia, eosinophilia, thrombocytopenia, bone erosion, cartilage destruction.

The two main autoantibodies found in RA are rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPAs); the presence of RF and ACPAs defined RA seropositive and is correlated to a worse prognosis with aggravated symptoms and increased mortality. The risk of onset of disease in patients positive for RF and ACPAs is 40% (even 10 years after the discovery of seropositive).

Early diagnosis can arrest disease in many patients, preventing or slowing disease progression, irreparable joint damage and disability.

Reactive C protein (CRP), a pentraxin protein release by liver by IL-6 stymulus, is a useful marker in RA diagnosis, because its blood levels correlate with disease activity (CRP activates neutrophils, promotes proliferation of macrophages inducing the release of pro-inflammatory cytokines via production of MMPs).

The 2010 ACR-EULAR (American College of Rheumatology- European League against Rheumatism), has defined classification criteria with relative score for making diagnosis of RA: joint involvement (large or small and numbers), serology (RF, ACPA negative or low-high positive), acute phase reactant (CRP, ERS-eythrocyte sedimentation rate), symptoms duration (< or > 6 weeks). RA diagnosis is made if the score is greater than six and other causes for synovitis can be excluded.

Treatment should be initiated promptly. Non-pharmacological treatments include a combination of physical therapy, patient counseling in lifestyle factors, and surgical procedures to remove and/or replace the affected joint and bone areas. Non-steroidal anti-inflammatory drugs (NSAIDs) are usually used only for symptomatic treatment to reduce pain and stiffness in the affected patients, but have no influence on disease progression (aspirin, diclofenac, ibuprofen). In contrast to this, non-specific immune system suppression via the application of glucocorticoids (prednisolone) has rapid effects, but side effects due to long-term use. Finally, disease-modifying antirheumatic drugs (DMARDs) are used to target inflammation, preventing joint damage and progression. These drugs are divided into 2 groups: synthetic (methotrexate, hydrochloroquine, sulfadiazine); biological (anti IL-6R, anti-TNF-α, B-cell depletion, inhibitor of T cells) such as tocilizumab, infliximab/adalimumab, rituximab, abatacept, respectively to the mechanism of action mentioned above. Other drugs are inhibitors of JAKs, such as tofacitinib/baricitinib (they inhibit the activation of Janus activated Kinase-JAK preventing the immune cell activation and inflammatory response). These drugs could have side effects based on the duration of treatment: increased frequency of infections (Herpes Zoster, mild

upper respiratory tract infections), cytopenia, liver toxicity, gastrointestinal side-effects, elevation of blood cholesterol.

In case of failure to respond to a single drug, some studies have shown the efficacy of a triple therapy (methotrexate+hydrochloroquine+sulfadiazine). Subsequent, the application of JAK inhibitors or biological DMARDs. Once patient has reached stable remission over at least 6 months, sequential tapering or dose reduction of the treatment can be considered in order to reduce side effect and costs (481).

Interstitial lung disease associated to rheumatoid arthritis (RA-ILD) is the most serious extra-articular manifestation (58%) with significant mortality; is more common in men. Several studies have reported a similarly low survival rate or RA-ILD to IPF. A study of the Western population reported the *MUC5B* promoter variant rs35705950 (very rare genetic polymorphism in the Asian population) as a risk factor for developing RA-ILD, especially in those patients with a UIP radiological pattern . Other risk factors to develop RA-ILD are: smoking, that leads to the generation of the citrullinated proteins in alveolar cells, resulting in autoimmunity to the citrullinated proteins in the genetically susceptible individuals; RF and ACPAs positive.

Lung involvement in RA can be the first manifestation of RA and this is typical of most aggressive forms, developing in progressive fibrosing phenotype. Patient under methotrexate therapy have an higher prevalence of lung fibrosis and nodules or emphysema and nodules at HRCT-chest.

The more frequent radiological patterns of fibrosis in RA-ILD are UIP and NSIP. There is a tendency of rheumatologists and pulmonologists to interrupt MTX treatment when lung damage occurs; it si correct in case of lung nodules, but there is still not enough evidence about the association of MTX exposure to RA-ILD development.

Patients on biological DMARDs monotherapy, instead, have higher prevalence of combined bronchiectasis and nodules at HRCT-chest.

The major causes of death in RA-ILD are: acute exacerbation, progressive fibrosis, lung cancer, respiratory infections with pneumonia. Antifibrotic therapy (Nintedanib) have shown to reduce the decline of FVC and the rate of acute exacerbations (482, 483).

Interstitial Pneumoniae With Autoimmune Features (IPAF)

In 2015, the European Respiratory Society (ERS) and American Thoracic Society (ATS) "Task Force on undifferentiated Forms of connective tissue disease-associated interstitial lung disease" proposed classification criteria for the category of Interstitial Pneumonia with Autoimmune Features (IPAF). These classification criteria were based on a combination of features from three domains:

- clinical: consisting of extra-thoracic features (Raynaud's phenomenon, arthralgias, multiple joint swelling, weight loss, morning stiffness, dysphagia, recurrent unexplained fever, oral ulceration, proximal muscle weakness, mechanics hands);
- serologic: specific autoantibodies (ANA, RF, anti-Scl70, SS-A, SS-B, anti-Jo1, ACPAs, anti-PM, anti-dsDNA);
- morphologic: with imaging patterns (NSIP, LIP, OP, DAD, DIP if no smoking history) and histopathological findings (lymphoid aggregates with germinal centers, plasmocytic infiltration); for diagnosis we need one feature from at least two domains.

IPAF patients tend to have a history of smoking similar to IPF.

The prevalence of IPAF is between 7-34% of all ILDs.

The most frequent clinical and serological markers of autoimmune features are Raynaud' phenomenon and positive antinuclear antibodies, respectively.

NSIP is the predominant radiological and histopathologic pattern (41%), but they can also present a UIP pattern. The histological UIP pattern observed in IPAF has been reported as non-typical, with diffuse lymphoplasmacytic infiltration and interstitial lymphoid aggregates (some authors called these patient UIPAF).

Prognosis of IPAF is generally intermediate between that of IPF and connective tissue disease-associated ILD (CTD-ILD), variable according to the predominant histologic and radiological patterns; non UIP-IPAF pattern have a very similar prognosis to those with CTD-ILD, while UIP-IPAF have higher disease progression rate. Smoking history and male gender are associated to an increased mortality. Some studies had shown that UIP-IPAF with positive Myositis specific antibodies (MSAs) have a better prognosis

and a good response to immunosuppressive treatment. Not all IPF-UIPs are equal and demographic/clinical characteristics will impact on prognosis.

We know that an histological NSIP pattern would support immunosuppressive treatment, while histological confirmation of a UIP pattern lead to a cautios approach to immunosuppressive therapy, keeping the patient monitored for possible antifibrotic therapy in case of progression.

Therefore, morphological features of IPAF in a biopsy characterised by a UIP pattern, lead to monitor the patient over time to detect features that can suggest a definite connective tissue disease.

Short telomere length in IPAF was associated with faster functional decline and needing of lung transplantation, similar to IPF.

Data regarding IPAF treatment are limited to case series and further research is needed to define the optimal treatment strategy in IPAF patients.

As in other ILDs, treatment of gastro-esophageal reflux, long-term oxygen, pulmonary rehabilitation, prevention of infections are indicated.

Some studies suggest that non-UIP pattern IPAF benefit from immunomodulatory drugs (intravenous cyclophosphamide, azathioprine, mycophenolate mofetil, corticosteroids), calcineurin inhibitors (ciclosporine, tacrolimus) and occasionally rituximab. Treatment decisions in IPAF patients must be based on evaluation of benefit in a multidisciplinary setting with input from pulmonologists, radiologists, pathologists, rheumatologists and immunologists.

The choice of first line therapy is based on: age, gender, extrapulmonary manifestations, histopathological pattern, radiological pattern, functional impairment, comorbidities, patient's expectations. These factors allow to distinguish between a predominantly fibrosing or inflammatory phenotype.

IPAF is a research entity and not a diagnosis (484,485,486).

Diffuse pulmonary ossification (DPO)

Diffuse pulmonary ossification (DPO) is a rare entity characterized by ectopic bone formation within lung parenchyma, in the interstitial or alveolar spaces. DPO could be idiopathic or related to cardiovascular or respiratory diseases; diagnosis is often made by histological examination (surgical biopsy).

This disease is mainly found in men between 70-80 years, in advanced stages of chronic obstructive pulmonary disease or interstitial lung disease; however some cases have been reported in young women and men. A possibile family predisposition was described.

DPO is characterized by two forms:

- nodular: associated to chronic pulmonary congestion (mitral stenosis);
- dendriform: in advanced ILD (IPF).

The main risk factors to develop DPO are age, male sex, history of smoking, IPF. The pathogenesis is uncertain, some studies support that inflammation is a precursor of DPO (inflammation→anoxia→ TGF-β → fibroblastic proliferation → transformation into osteoblasts → metaplastic bone formation in the lung interstitium).

Patients affected by DPO may be asymptomatic or with mild symptoms; when DPO is secondary to IPF the lung function shows a restrictive pattern with low DLCO.

Pulmonary ossification is not usually visible in chest X-rays. It consists in mature bone in peripheral areas of lower lobes with reticulonodular aspect.

In chest-CT DPO is identified as multiple tiny calcifications in the bibasilar lung periphery superimposed on subpleural lung fibrosis. On the other hand, CT findings of multiple lung calcifications could help to make differential diagnosis beetwen UIP and NSIP patterns.

There is no consensual treatment for DPO and low-calcium diets or systemic corticosteroids therapy didn't proven a clear benefit.

According to the case reports published in literature, the evolution of DPO is slow and when associated to IPF the prognosis is related to the pulmonary fibrosis (487).

Pleuroparenchymal fibro-elastosis (PPFE)

Pleuroparenchymal fibro-elastosis (PPFE) is a clinico-radiologic-pathologic interstitial lung disease, characterized by fibrosis that has upper lobe and subpleural predominance, involving the visceral pleural and the subpleural lung parenchyma. PPFE was recognized in 2013 as a rare idiopathic interstitial pneumonia in the ATS/ERS classification of ILDs. The true incidence and prevalence of PPFE are not known. It has been suggested that acute or subacute lung injury including diffuse alveolar damage with interstitial inflammation has a central role in the pathological cascade that leads to PPFE. The development of PPFE is not linked to cigarette smoking or to immunodeficiency states.

PPFE is divided into two types: idiopathic (iPPFE) and non-idiopathic PPFE, which could be secondary to many potential initiating factors such as:

- bone marrow and hematopoietic stem cell transplant (restrictive chronic allograft dysfunction);
- lung transplant;
- chemotherapy treatment (cyclophosphamide) and radiation treatment;
- autoimmune or connective tissue disease (scleroderma, rheumatoid arthritis);
- recurrent infections (Aspergillus);
- lung fibrosis (UIP, NSIP, HP);
- occupational exposure to asbestosis;
- familial history of pulmonary fibrosis (57% of cases of PPFE);
- short telomere lengths and genetic mutations of TERT and TERC.

Most patients presenting PPFE have 40-70 years; however, some cases have also been reported in children. Younger patients were predominantly female, in the nontransplant setting and characterized by telomere gene mutations. Male preponderance of PPFE has rarely been reported.

The duration of symptoms before the radiological manifestation of PPFE varies from 6 to 24 months. Typical symptoms are: progressive breathlessness, cough, chest discomfort, pleuritic pain (in the absence of pneumothorax), weight loss (PPFE patients have lower BMI than other

fibrotic patients). Auscultatory findings may be normal, however if PPFE is combined with a pulmonary fibrosis (UIP, NSIP, HP) there could be inspiratory crackles. Fingers clubbing is uncommon. PPFE patients develop platythorax, which resulted from marked upper lobe volume contraction and reduction of anteroposterior thoracic depth (that could lead to secondary pulmonary hypertension and a rigid chest wall possible controindication for lung transplantation). In some individuals could be seen the suprasternal notch (clinically and radiologically, with concomitant retraction of the trachea).

The main differential diagnosis for PPFE are: sarcoidosis, nontubercolosis mycobacterial infection, post-lung injury remodeling, pneumoconiosis, malignancy pleural cap.

Criteria for diagnosing PPFE (radiologically and histopathologically) were first proposed in 2012 by Reddy and colleagues, that proposed the classification in three categories, on the basis of histological and radiological descriptions:

- definite: pleural thickening with subpleural fibrosis in the upper lobes;
- consistent: upper lobe pleural thickening with subpleural fibrosis, with features of coexistent disease elsewhere;
- inconsistent: absence of features of definite and consistent categories.

Diagnosis of PPFE needs a multidisciplinary consideration of clinical and radiological aspects. Surgical biopsy is often avoid for the high risk of iatrogenic pneumothorax, pneumomediastinum (air leak syndrome) or bronchopleural fistula.

The first radiological description emerged in the 1970s. Later, in 2004, Frankel and colleagues proposed the term PPFE, describing the characteristics of bilateral upper zone pleural thickening and lobar volume loss. A histopathological diagnosis of PPFE requires demonstration of intralveolar fibrosis and elastosis (dense collagenous fibrosis in the alveolar spaces and elastin deposition in the alveolar walls), with visceral pleural

fibrosis (that may be absent in biopsies because of its patchy distribution). These features dominate in the upper lobes; foci fibroblastic may be present. The functional profile of PPFE is characterized by a restrictive ventilatory defect denoted by a rapidly decline of FVC (300 ml/year), reduced TLC, increased RV (reasons remain unclear) and reduced DLCO but with elevated alveolar carbon monoxide uptake, as a result of extrapulmonary restriction. Functional criteria of RV/TLC % pred ≥ 115% and or BMI ≤ 20 kg/m^2 plus RV/TLC % pred ≥ 80%, may successfully discriminate patients with idiopathic PPFE from those with chronic ILDs (491).

Patients affected by PPFE have a predilection for hypoxemic respiratory failure; at advanced stage they could develop hypoventilation with increased arterial carbon monoxide pressure.

PPFE has a median survival of 11 years; however there are progressive disease phenotype of idiopathic PPFE (iPPFE) with a median survival of 5 years (similar to PPFE combined with UIP fibrosis). Patients with PPFE combined with IPF have been reported to have a lower BMI than patients with IPF and a worse prognosis (a BMI < 18.5 kg/m^2 is associated to a significant risk of mortality after lung transplantation) (488).

However, PPFE has been shown to be an independent determinant of higher mortality in chronic HP or in patients with short telomere lengths. Recurrent pneumothorax worsen the prognosis.

No treatment has been demonstrated to be effective in PPFE. Low dose of prednisolone may be useful, although unproven effects. Immunosuppressive agents (azathioprine, methotrexate) should be avoided due to the high risk of infections. Useful therapies are: prophylactic antibiotics, antifungal therapy, oxygen-therapy, nutritional input, psychological support, pulmonary rehabilitation. Successfully transplanted cases have been reported, one from a living donor and one as a bilateral lung transplantation (489).

A small retrospective study suggests that nintedanib may slow disease progression, reducing the decline of FVC percentage; however, these results were not achieved in the idiopathic PPFE, in which Nintedanib efficacy may be limited (490).

Serum Amyloid A (SAA)

Definition

Serum amyloid A (SAA) is an acute phase protein produced primarily in the liver in response to the release of proinflammatory cytokines from activated monocytes. It is a precursor to amyloid, which was discovered a quarter of a century ago. SAA shares antigenicity with amyloid A, the major constituent of the fibrillar component in amyloidosis (425,426). SAA has been measured in body fluids of patients with various lung diseases including lung cancer and obstructive/restrictive lung diseases such as interstitial lung diseases, sarcoidosis and COPD. The potential clinical value of SAA as a biomarker of different lung diseases is the main topic of this work.

Protein structure and metabolism of SAA

Four genes on chromosome 11 have been identified in the human genome responsible for the synthesis of SAA1, SAA2, SAA3 and SAA4 (427). The SAA1 and SAA2 genes encode the proteins SAA1 and SAA2, which constitute the acute phase protein SAA (A-SAA), of which SAA1 accounts for approximately 70% (428,429). A-SAA is a single polypeptide of 104 amino acid residues; although its exact tertiary structure is unknown, the critical role of the 10 amino-terminal residues is recognized in the formation of amyloid fibrils that bind high-density lipoprotein (HDL) (427,430). The SAA1 and SAA2 proteins act as acute phase reactants in plasma and are also plasma precursors of amyloid A fibrils (429). Most plasma A-SAA is synthesized by the liver, although extrahepatic synthesis of macrophages, endothelial cells, smooth muscle cells, adipocytes, atherosclerotic lesion sites (428) and tumor cells (431) has been demonstrated. Upon release of A-SAA from cells, the protein binds to the HDL and enters the bloodstream (432).It is then rapidly sequestered by phagocytes and broken down by lysosomal proteinases (433). Degradation of A-SAA in the liver is catalyzed by enzymes such as serine proteases, elastase, collagenase , cathepsins (434).

Role of SAA in inflammation and in connective tissue diseases with lung involvement

In inflammatory diseases, plasma concentrations of SAA have been shown to be thousands of times higher than the baseline value. Serum SAA levels rise three to six hours after an inflammatory stimulus, peaking in the blood in 2 to 3 days and then returning to baseline levels. The cytokines TNF-α, IL-1 and IL-6 can induce the release of SAA within 2-3 hours. SAA and C-reactive protein (CRP) are known as the first class of acute phase proteins because they are the most sensitive plasma markers of acute inflammation (435). Depending on the severity of the inflammatory state, SAA concentrations can be in the order of thousands of mg/l; similar levels are achieved in bacterial and fungal infections, invasive malignant diseases, tissue damage (such as acute myocardial infarction), and autoimmune diseases (such as rheumatoid arthritis, CTD, and vasculitis). SAA is a better indicator of inflammatory activity in patients with autoimmune gastrointestinal disorders than CRP; one reason is that CRP returns to baseline faster than SAA (426). Inflammation in systemic sclerosis is an important but not completely characterizing feature of the early stages of the disease. High concentrations of SAA are associated with progressive systemic sclerosis characterized by severe skin thickening and poor five-year survival (436). Elevated levels of SAA were found in 25% of patients with SSc, the highest levels being reported in those with early stage disease and diffuse skin involvement. Serum SAA levels were correlated with lung involvement in SSc patients and prognosis: they were inversely correlated with DLCO rates, suggesting that SAA may be a biomarker of interstitial lung involvement in SSc patients (437,438) . The gene expression of SAA in patients with rheumatoid arthritis (RA) has been related to the transcription of extracellular matrix metalloproteinases. Increased concentrations of SAA have been found in synovial tissue in patients with rheumatoid arthritis (439, 440).

SAA is a marker of the acute phase of rheumatic diseases responsible for the stimulation of angiogenesis, leukocyte recruitment and extracellular matrix degradation via an Nf-kb-dependent signal transduction pathway (441).

SAA as biomarker of acute exacerbation of chronic obstructive pulmonary diseases

Chronic obstructive pulmonary disease (COPD) is a disease characterized by persistent airflow limitation associated with chronic inflammatory response of the airways and lungs to harmful particles or gases. COPD is divided into phenotypes based on clinical and functional characteristics associated with different prognoses (442). A major subgroup is characterized by acute exacerbations and worsening of symptoms, including increased dyspnea, cough, sputum production, and purulence. The pathology and impact of COPD results from an abnormal inflammatory process that induces tissue damage with ineffective repair in response to toxic inhalants (particularly cigarette smoke). It has been shown that SAA and CRP are mainly produced during acute exacerbations of COPD after the release of proinflammatory mediators such as IL-6, IL-1β, and TNF-α (443, 444). SAA has been proposed as a blood biomarker of acute COPD exacerbation: its changes correlate with COPD severity scores, lung function data, risk of respiratory failure, and hospitalizations (443). Using a proteomic approach, SAA was identified in the serum and BAL of COPD patients, demonstrating overexpression in patients with glucocorticoid-refractory exacerbations. SAA appears to be a serological marker correlated with acute exacerbations and resistant to corticosteroids (444).

SAA in obstructive sleep apnoea

In a study conducted in 2003, plasma levels of SAA were increased in subjects with obstructive sleep apnea syndrome (OSAS) (445). Clinically, OSAS is currently defined on the basis of sleepiness and one or two of the following symptoms: severe snoring, nocturnal respiratory arrest, repeated nocturnal awakenings, non-recuperative sleep, daytime fatigue, and impaired concentration. The polysomnographic criterion consists of the detection of more than five episodes of apnea-hypopnea plus micro-awakenings related to respiratory efforts per hour of sleep (446). Patients with OSAS are at high risk of cardiovascular and cerebrovascular events. It

is not understood how OSAS might affect serum concentrations of SAA. One possible explanation is that sleep apnea-related hypoxia/reoxygenation stimulates proinflammatory cells and mediators, including SAA and CRP (445). It has been shown that serum concentrations of SAA are higher in patients with severe OSAS than in those with mild or moderate OSAS. After 3 months of CPAP ventilation therapy, SAA levels decreased significantly in patients with OSAS along with parameters of cardiovascular risk and inflammation (447). SAA has been suggested as a promising biomarker of treatment response in patients with OSAS to be confirmed with further studies.

Role of SAA in sarcoidosis

SAA has an important pathogenetic role in granulomatous inflammation and has been extensively studied in these disorders. It induces the production of some cytokines and regulates T-helper1 (Th-1) lymphocytes in the immune response through interaction with the toll-like receptor 2 (TLR-2). Increased serum levels of SAA have been reported in patients with active sarcoidosis (defined by progressive impairment of radiological and functional characteristics) (448). Our group of Siena performed a study in which higher levels of SAA were detected in the BAL of sarcoidosis patients compared to healthy controls and other interstitial lung disease (ILD) patients with a proteomic approach; SAA levels on BAL fluid were also inversely correlated with FEV1 percentages. Furthermore, SAA was elevated in patients with serious pathologies requiring steroid therapy (448). Mijoshi et al. in 2001 showed that unlike ACE, the production of SAA is not inhibited by immunosuppressive drugs and its use has been recommended during the follow-up of patients with sarcoidosis, supporting the hypothesis that SAA may be a better biomarker of treatment response than ACE (449). Chen et al.in 2009 demonstrated that SAA can induce nuclear factor NF-κB, stimulating the expression of TLR-2. SAA is expressed by macrophages and giant cells of sarcoid granulomas; it is found within granulomas and is expressed by CD68+ macrophages and giant cells, but is related to CD3 lymphocytes linked to the expression of the local Th-1 response (450).

Because it is an innate regulator of granulomatous inflammation that occurs in sarcoidosis through TLR-2, it offers new therapeutic targets.

SAA appears to be a structural component of the granulomatous inflammation of sarcoidosis, as demonstrated by Moller et al. It has been hypothesized that SAA produced in response to an initial mycobacterial infection may promote specific pathogenic pathways, inducing epithelioid granulomatous inflammation (451). The protein is also a mediator of lipid metabolism in sarcoidosis. After synthesis, SAA is released from cells and binds HDL isoform 3 (HDL3), becoming the major apo-lipoprotein A1 (Apo-A1), critical in reverse cholesterol transport and prevention of atherogenesis. The first description of alterations in lipid metabolism in patients with sarcoidosis in the medical literature appeared in 1989, when reduced serum levels of HDL cholesterol were detected in patients with sarcoidosis, without any correlation with disease activity. In 1998, Salazar demonstrated that patients with active sarcoidosis had lower HDL cholesterol levels than patients with inactive disease, concluding that a reduced serum HDL cholesterol concentration found in untreated patients with active sarcoidosis was associated with elevated serum levels of SAA (452). Our group contributed to the definition of SAA as a regulator of lipid metabolism with a role in the pathogenesis of sarcoidosis (448, 453).

SAA in bronchial asthma

Asthma is a heterogeneous condition, its prevalence is 5-10% in adults and is characterized by different clinical phenotypes, however there is no standardized classification system (454). Diagnostic and prognostic serum biomarkers of asthma with adequate sensitivity and specificity are needed to counteract misguided therapies and misdiagnoses. SAA is strongly induced during inflammatory responses: elevated levels of SAA, IL-1β and IL-17 are recorded in subjects with severe allergic asthma, although the relationship between these mediators is unclear. The Th17 response in allergic airway disease is associated with neutrophilia, tissue destruction, and steroid insensitivity. Recently, SAA-induced IL-1β, IL-6, and IL-23 have been implicated in the initiation and expansion of IL-17-producing T cells

involved in respiratory allergies (455-457). SAA is a pro-inflammatory mediator capable of promoting antigen-specific pulmonary Th17 responses through the activity of the cytokine IL-1.

Role of SAA in lung cancer

SAA is known as an acute phase protein involved in carcinogenesis. Elevated CRP levels have been reported to have prognostic significance in lung cancer patients, and a single study demonstrated increased CRP-SAA levels in lung cancer patients (458). Elevated CRP-SAA levels were significantly associated with severe clinical features of lung cancer and with lower survival rates. Sung HJ et al. identified both SAA1 and SAA2 expression in sera from lung cancer patients but not in healthy controls. Lung cancer cells have higher expression of SAA1-SAA2 than normal lung cells, and contact culture of lung cancer cells with macrophages was seen to result in increased IL-1β and IL-6 , which in turn stimulate lung cancer cells to induce SAA1-SAA2. While chronic inflammation is considered to be a promoting condition of cancer development, the infiltration of immune cells and their secreted cytokines are also components of the tumor microenvironment. The role of SAA in tumor pathogenesis is also related to extracellular matrix adhesion proteins. In vitro experiments showed that SAA is induced in lung cancer cells inducing MMP-9 expression by macrophages. MMP-9 is involved in cancer invasion and metastasis. In conclusion, higher concentrations of SAA (SAA1-2) are associated with lung cancer (especially adenocarcinoma) and the protein can therefore be suggested as a therapeutic target to prevent lung cancer metastasis (459-462). Thus, quantification of serum SAA levels in patients with non-small cell lung cancer may be a prognostic biomarker to monitor during follow-up (463,464).

Role of SAA in other lung diseases

Few data are available on SAA in other lung disorders. This interesting molecule, involved in alterations of lipid metabolism, was related to impaired respiratory function (465). The expression of SAA in infectious

lung diseases such as tuberculosis has been explored in some manuscripts, particularly in miliary tuberculosis SAA has been shown to predict response to antibiotic treatments (466).

Radiation pneumonitis is a serious interstitial lung disease. Patients with high-concentration SAA at baseline showed an increased risk of lung involvement after lung irradiation, and SAA can be considered as an auxiliary bioindicator for predicting the severity of radiation pneumonitis (467).

In patients with cystic fibrosis, SAA and other blood biomarkers (such as C-reactive protein and calprotectin) have been studied in order to evaluate its potential role in detecting response to inflammatory treatments (468).

Unfortunately, no data are available on other interstitial lung diseases including smoking-related ILD, nonspecific interstitial pneumonia; few data have been reported on SAA expression in patients with bronchiectasis and chronic obstructive pulmonary diseases such as emphysema (469).

SAA and SARS-COV-2

SAA serum levels were analyzed in some studies on patients affected by acute COVID-19. SARS-COV-2 infection increase the SAA amyloid formation; overexpression of SAA does not always lead to systemic amyloidosis. SARS-COV-2 proteins can increase the risk of SAA fibrils formation by these mechanisms:

- binding of the SK-9 viral protein reduces the stability of the biologically active SAA hexamer in which SAA transports lipids during inflammation, shifting the equilibrium towards monomers;
- an enzymatic cleavage transform monomers in smaller fragments that are found in SAA fibrils.

The presence of the viral protein SK-9 increase the risk of amyloid fibrils formations and deposition in the blood vessels, causing inflammation and thrombosis. It leads to the multisystem inflammatory syndrome observed in patients with severe COVID-19 with cardiovascular, gastrointestinal, dermatologic and neurological symptoms (470).

Patients with severe COVID-19 have diffuse alveolar damage, which produce multiple inflammatory factors (IL-1, IL-6) that can rapidly induce a 1000-fold increase of SAA serum levels (198,32 mg/l). This biomarker were significantly higher in patients with critical COVID-19 (471, 472).

After his release, SAA binds HDL cholesterol and this complex induce the neutrophils activation aggravating the degree of inflammation and leading to the worsening of the patient.

The project

Aims and objectives

Some studies in the literature have shown that lipid metabolism can influence the pathogenesis of inflammatory and fibrotic lung disorders. Serum amyloid A (SAA) is an acute phase protein (apolipoprotein) mainly produced by the liver in response to proinflammatory cytokines from activated monocytes.

Its physiological role is still unclear, although roles in acute-phase response control and/or propagation, lipid metabolism and transport, cell communication and many inflammatory/immune responses, such as granuloma formation and carcinogenesis, have been suggested. SAA was investigated in different biological fluids for its potential as a biomarker of many inflammatory lung diseases such as sarcoidosis, obstructive lung diseases (COPD, asthma), obstructive sleep apnoea (OSAS) and lung cancer. Few data are available on SAA levels in patients with IPF, a chronic progressive lung disease associated with a poor survival and a radiological and histological pattern of usual interstitial pneumonia (UIP). The clinical course of IPF is unpredictable; some patients have a stable form, also favored by the use of new antifibrotic drugs, while other patients have rapidly progressive variants with phases of acute acceleration and very rapid clinical progression. To date, we don't have a marker of severity and prognosis capable of establishing the evolution early and being able to send patients early to lung transplantation. The clinical management of IPF has improved since the introduction of two new antifibrotic drugs. A current goal of research is to diagnose IPF early in order to start pharmacological therapy as soon as possible. Since IPF is currently diagnosis by exclusion of other fibrotic lung diseases, it would be useful to have a specific biomarker. In this project we improve the results of our previous studies published on 2019 and 2020, were we demonstrated that IPF patients had higher SAA serum levels as compared to healthy controls and other ILDs (473, 474).

We evaluated SAA levels in IPF cohort and patients with other ILDs, to definite the potential value of this protein as a biomarker of fibrosis and its specificity in IPF. Our aim is to demonstrate the role of the lipid metabolism in the fibrotic process, especially the role of the apolipoprotein SAA as a

biomarker of IPF that can predict clinical course, prognosis and survival of IPF patients.

Materials and methods

Study population

We recruited **185 patients** and we compared SAA serum concentrations in: **40 clinically stable IPF** (32 males, 12 former smokers; mean age 73,32 ± 6,25 years) and **8 IPF on acute exacerbation - AE-IPF** (6 males, 8 former smokers; mean age 69,38 ±7,90 years), compared to other lung diseases with a fibrotic and cystic radiological patterns, but also with acute exacerbated patients affected by pulmonary emphysema;

- **30 patients with Sarcoidosis** (6 males, 2 former smokers; mean age 59,27 ± 11,35 years);
- **30 patients with hypersensitivity pneumonitis** (**HP)** with a fibrotic pattern (18 males, 16 former smokers; mean age 66,72 ± 8,44 years);
- **17 patients with cystic lung diseases: 11 patients with Pulmonary Langerhans cell Histiocytosis (PLCH) and 6 patients with Lymphangioleyomiomatosis (LAM)** (considering the two groups together there were 3 males, 7 former smokers; mean age 50,94 ± 12,08 years);
- **6 patients with non-specific interstitial lung disease (NSIP)** (0 males, 2 former smokers; mean age 66 ± 12,09 years);
- **9 patients with ILDs with a radiological UIP pattern non IPF** (7 males, 7 former smokers; mean age 78 ± 5,61 years);
- **16 patients with Systemic sclerosis** with interstitial lung disease **(SSc-ILD)** (0 males, 4 former smokers; mean age 65,25±10,35 years);
- **6 patients with acute exacerbation of COPD in pulmonary emphysema (**4 males, 4 former smokers, 1 current smoker; mean age 69,8 ± 6,3 years);
- **6 other-ILDs** (3 patients affected by diffuse pulmonary ossification and 3 patients with pleuroparenchimal fibroelastosis). Considering the two groups together there were 3 males, 3 former smokers with mean age 74,4± 8,01 years.

SAA serum levels were also analyzed in **17 healthy controls (HC)** (7 males, 8 former smokers; mean age 43,35 ± 13,03 years). They were monitored for 12 months and did not develop any disease (Table4).

All these patients were being follow-up at the Regional Reference Center for Sarcoidosis and other Interstitial Lung Disease in Siena and the Respiratory Department of the Saint Anna Hospital at University of Ferrara, Italy.

The diagnosis of IPF was made according to the ATS/ERS/JRS/ALAT guidelines (1) in a multidisciplinary setting.

At the time of SAA sampling, patients and controls had been fasting for at least 6 hours. After being collected, the blood samples were centrifuged to obtain the serum and stored at -80 degrees, before being analyzed.

Clinical, functional, radiological and immunological data were collected from all patients. Laboratory parameters included assessments of complete blood count, erythrocyte sedimentation rate (ESR), C-reactive protein, fibrinogen, D-dimer, lactate dehydrogenase (LDH), creatinine, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides. Pulmonary function tests (PFTs) included forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), forced expiratory volume in 1 second/vital capacity (Tiffenau index), intrathoracic gas volume, total lung capacity (TLC), residual volume, diffusing capacity of the lung for carbon monoxide (DLCO) and transfer coefficient of the lung for carbon monoxide (absolute values expressed in milliliters and as a percentage of predicted). All patients underwent clinical and functional follow-up with PFTs at 6-months intervals in accordance with our protocol. Patients with relevant cardiovascular comorbidities, on statin therapy or with ongoing cancer were excluded, potentially responsible for the increased SAA levels. All these patients underwent bronchoscopy for diagnostic reasons and bacterial, viral and tuberculosis infections were excluded.

Comorbidities were also recorded for the entire population, with particular attention to: hypertension, hypercholesterolemia, ischemic heart disease, multi-district atherosclerosis (ATS), hepatic steatosis, mild OSAS, diabetes

mellitus type II, atrial fibrillation, visceral obesity, bronchial asthma, gastro-esophageal reflux (GER).

The incidence of these comorbidities in each group of patients is illustrated in Table5.

In the cystic diseases group there were also 4 patients (23%) who were smokers at the time of serum sampling.

Patients suffering from Sarcoidosis were on oral steroid therapy (22 patients, 73%) and immunosuppressive therapy with Methotrexate (2 patients, 6%), Azathioprine (5 patients, 16%).

Sarcoidosis manifested itself with:

- acute onset in 10 patients (33%), with non-productive cough, fever, dyspnoea on exertion, diffuse arthralgias, erythema nodosum;
- subacute onset in 14 patients (46%), with low-grade fever, asthenia, night sweats.

The radiological staging of sarcoidosis placed 1 patient (3%) at Stage I, 15 patients (50%) at Stage II, 7 patients (23%) at Stage III, 3 patients (10%) at Stage IV I (Table6).

Extrathoracic involvement of sarcoidosis resulted in:

- subcutaneous nodules (2 pts, 6%);
- retropancreatic lymphadenopathy (2 pts, 6%);
- uveitis (3 patients, 10%);
- meningeal granuloma (2 patients, 6%), (Table7).

Patients affected by HP had a positivity for anti-Candida Albicans serum precipitins (8 pts, 26%), anti-Aspergillus Fumigatus (7 pts, 23%); anti-Thermoactinomyces Vulgaris (2 pts, 6%); anti- Micropoluspora Faeni (1 pt, 3%). Of these patients 24 patients (81%) were taking oral steroid therapy, 5 patients (16%) were on immunosuppressive therapy with azathioprine and 1 patient (3%) were on antifibrotic therapy (Nintedanib).

The group of patients with cystic lung diseases included 6 LAM and 11 PLCH. Patients with LAM presented with hemoptysis and dyspnea on exertion. The presence of renal angiomyolipoma was found in 2 patients (11%). At the time of serum sampling, 2 LAM patients (33%) were on Sirolimus therapy. In the group of PLCH 6 patients (54%) had exclusive

pulmonary involvement, 1 patient (9%) also had bone involvement (femur), 1 patient (9%) had neurological involvement (pituitary), 3 patients (27 %) also had centrilobular emphysema. The onset of PLCH was manifested by exertional dyspnea, but in some patients the pathology was occasionally found in a chest X-ray performed as a preoperative evaluation for other types of surgical interventions.

Patients on antifibrotic treatment are listed in Table8. In the group of other-ILDs the 2 patients under antifibrotic treatment (Nintedanib) were affected by progressive pleuroparenchimal fibroelastosis.

The study was approved by the ethics committee (approval number 180712) of University of Siena and by the ethics committee (Lung-Biomarkers protocol, CE: 303/2023/Oss/AOUFE) of University of Ferrara.

All patients and controls were Caucasians and signed written informed consent to participation in the study, which was approved by the ethics committee mentioned before.

The data were entered in a specific database with survival data.

SAA assay

The SAA assay was performed using a commercially available enzyme-linked immunosorbent assay kit (Human Invitrogen KHA0011) in accordance with the manufacturer's instructions. The microplate wells were coated with a highly purified monoclonal antibody specific for Human SAA. During the first incubation, standards with known SAA contents, controls and samples were pipetted into the coated wells, followed by the addition of a second biotinylated monoclonal antibody. After washing, the enzyme Streptavidin-Peroxidase was added; the latter, by binding to the biotinylated antibody, completed the characteristic sandwich of the ELISA method. Subsequently to a second incubation and a second washing (necessary to remove the unbound enzyme) a solution was added, so as to be welcomed by the bound enzyme to produce color. The intensity of the resulting coloration was considered directly proportional to the concentration of Human SAA present in the analyzed samples.

Statistical analysis

Results were expressed as means and standard deviations. Since the data were not normally distributed, Kruskal-Wallis one-way analysis of variance and Dunn test were used for multiple comparisons. The Mann-Whitney test was used for pairwise comparison of variables. Areas under receiver operating characteristic (ROC) curves were assessed and Youden's index was used to obtain cut-off values with the best sensitivity and specificity.

The Spearman test was used to look for correlations between variables. A p-value less than 0.05 was considered statistically significant. Statistical analysis and graphic representation of the data were performed by GraphPad Prism 9.0 software.

Results

Significant differences in gender and smoking habits were observed between patient groups. Considering patients affected by interstitial lung diseases, in the groups IPF, AE-IPF, UIP-non IPF, emphysema and other-ILDs patients were predominantly males and older than the other groups of patients (over 65 years of age).

At time of serum SAA sampling:

- in the **IPF group** most of patients had a radiologic *UIP definite* pattern (25 pts, 63%); the remaining 15 patients (37%), had a *UIP probable* radiological pattern;
- in the **AE-IPF group**, 6 patients (75%) had a radiological *UIP definite* pattern, 2 patients (25%) had a *UIP probable* radiological pattern. All these patients were hospitalized and on high-dose systemic steroid therapy (1 mg/kg);
- in the **Sarco group** 3 patients (10%) had a radiological StageIV characterized by upper lobes fibrosis with traction bronchiectasis;
- in the **HP group** the radiological pattern was mostly characterized by apical fibrosis with traction bronchiectasis and honeycombing in the upper lung lobes; in 2 patients (6%) there was a *UIP definite* radiological pattern and in 3 patients (10%) there was diffuse ground-glass with concomitant emphysema. In this group 5 patients

(16%) were on immunosuppressive treatment (azathioprine), 1 patient (3%) was on antifibrotic treatment (Nintedanib), 24 patients (81%) were on oral steroid therapy;

- in the **NSIP group** 4 patients (66%) had a radiological fibrosing pattern prevalent at the bases with traction bronchiectasis; the other 2 patients (34%) had a chest HRCT pattern of ground-glass in the lower lobes, apical fibrotic findings and apical paraseptal pulmonary emphysema. In this group 2 patients (33%) were on immunosuppressive drugs (rituximab and micofenolate mofetil), 4 patients (67%) were under oral steroid treatment;
- in the **UIP non IPF group** 7 patients were affected by rheumatoid arthritis (RA), instead, the other 2 patients had autoimmune features but do not fulfill the diagnostic criteria for a defined connective tissue disease (IPAF). The group with rheumatoid arthritis had a radiological *UIP definite* pattern in 5 patients (71%); 2 patients (28%) had a *probable UIP* pattern. In this group 3 patients with rheumatoid arthritis (42%) were on antifibrotic treatment (Nintedanib) for progressive pulmonary fibrosis; only 1 patient (14%) was on immunosuppressive treatment (methotrexate); the IPAF group has serological domain with ANA (1:320) positive and clinical domain with Raynaud phenomenon;
- in the **SSc-ILD group** 5 patients (31%) had a radiological *UIP definite* pattern, while 11 patients (69%) had a NSIP pattern. In this group 2 patients (12%) were on antifibrotic treatment (Nintedanib), 10 patients (62%) were on micofenolate mofetil therapy, 1 patient (6%) was under tocilizumab therapy, 3 patients (20%) were on steroid therapy;
- **in the other-ILDs group** 3 patients affected by diffuse pulmonary ossification (DPO) had a radiological pattern of bilateral bone density lung lesions in lower lobes, with ground-glass opacities and traction bronchiectasis; these patients were not on steroid therapy. The 3 patients affected by pleuroparenchymal fibroelastosis (PPFE) had a radiological pattern of upper lobes fibrosis with subpleural

predominance, with concomitant interstitial thickening at lower lung lobes and traction bronchiectasis (UIP-probable pattern) in an underlying scleroderma pattern. Two of these patients were under antifibrotic (Nintedanib) treatment;

- **in the COPD-emphysema group included** 3 patients GOLD2D, 1 patient GOLD1B, 2 patients GOLD3D. These patients had a radiological pattern of centrolobular and paraseptal emphysema, predominantly in the upper lung lobes with clinical acute exacerbation of the obstructive lung disease with acute respiratory failure, requiring hospitalization, steroid, antibiotic and oxygen therapy.

The functional worsening of the study population is described in Table9.

The decline of FVC and DLCO percentage in each group of fibrotic patients and sarcoidosis group is illustrated in Fig13. The graphical representation shows how, especially in the IPF group, there was no serious functional worsening over the years, probably thanks to the antifibrotic therapy that all patients in our study were taking since diagnosis.

Our results show that IPF patients have significantly higher SAA serum levels than the other ILDs (Table10).

A comparison analysis with non-parametric ANOVA test shows a clear and statistical significant difference between SAA serum levels in the IPF group vs healthy controls ($p<0.0002$), which indicates that SAA is a disease marker. Furthermore, the analysis shows that IPF patients have higher SAA serum levels than the other groups with a statistically significant difference, which would indicate how SAA is a disease marker that allows discriminating the IPF patient in a group of fibrotic patients (Fig14-Fig15).

A ROC curve analysis showed that IPF patients have statistical significance higher SAA serum levels than SSc-ILD with a progressive fibrosis, identifying a cut-off value (45,21 mcg/ml, $p<0,0001$, AUC 97,6 with specificity 100%). This data indicates that a patient with a progressive fibrosis with serum SAA levels above that cut-off is more likely an idiopathic pulmonary fibrosis than another progressive fibrosis with a pattern UIP or another radiological pattern.

Instead, the ROC-curve analysis between healthy controls SAA levels vs the other patients show a statistical significance difference with high specificity (AUC > 80%, p < 0,001) which indicates that a subject with serum SAA levels < 5 mcg/ml is an healthy subject. This data strengthens our hypothesis of SAA as a disease marker but also a discriminating biomarker of IPF. Furthermore, if a patient' serum SAA levels have values around 6 mcg/ml, this data indicates that an acute inflammation state is already underway (see for example the cut-off value for the sarcoidosis group 5.9 mcg/ml). Instead, if SAA values are very high with a cut-off > 50 mcg/ml, we can suspect a progressive fibrosis lung disease, probably idiopathic (IPF).

Furthermore, the analysis detected very low cut-off value (5.7 mcg/ml) of SAA serum levels in the other-ILDs compared to the other groups with progressive fibrosis, confirming the data explained before of SAA being a marker of progressive fibrosis and probably specific for IPF (which is a progressive fibrosis by definition) (Table11).

The difference in serum SAA concentration in the IPF and AE-IPF groups was statistically significant (p<0.002). By performing a ROC curve of SAA in AE-IPF vs stable IPF we detected a cut-off 48,84 mcg/ml highly specific for acute exacerbation (AUC: 90.4%; p<0,0024; Se: 87,5%; Sp: 92,31%). These results show how serum SAA levels are higher in IPF patients in the stable phase compared to those with acute exacerbation, suggesting a role for SAA as a biomarker of fibrosis in IPF rather than as an indicator of inflammation (Fig16). These data need to be further investigated in a larger cohort of patients.

Considering the “age factor”, a statistical analysis with ANOVA test confirms the well-known data from the literature that patients suffering from IPF are older than other fibrosis and then patients suffering from interstitial lung diseases in which an inflammatory state prevails. It also shows that patients with a radiological UIP pattern non IPF are older than other ILDs (Fig17). This data could be related to what reveals the statistical analysis with Spearman correlation, which shows a direct correlation (rho 0.28, p<0.0004) between “Age” and “SAA”. This indicates that in older patients (as IPF), we have higher serum SAA levels. It would be interesting in this

context to understand whether the age-related alteration of lipid metabolism is somehow involved in the process of fibrogenesis.

We didn't found statistically significant correlation between SAA and smoking.

A statistically significant inverse correlation was found between peripheral neutrophilia (in percentage) and DLCO in percentage at T0 (rho -0.25, p 0.04), which indicates that high peripheral neutrophilia is associated with a functional worsening of gas exchange. So higher neutrophil blood counts could be involved in the fibrotic process leading to a worsening of DLCO for the fibrotic subversion of the alveolar-capillary interface. In our study we can hypothesize that this data is to be referred in particular to IPF patients, strengthened by the statistically significant and inverse correlation between DLCO in percentage at T0 and age (rho -0,25, p<0,009), demonstrating that the older patients in our study population (i.e. IPF) already present at T0 (time of diagnosis) a more compromised functional state, with a high peripheral blood neutrophilia, which has a negative predictive value. This result demonstrates the role of innate immunity in the pathogenesis of IPF.

We know that pulmonary fibrosis is the result of a persistent alveolitis with abnormal deposition of connective tissue. The alveolitis consists of infiltration by inflammatory cells including eosinophils, which express cytokines that can promote inflammation and tissue injury. In our study we found that in the IPF group there was a lower level of eosinophil blood counts in patients on Pirfenidon treatment, with a statistical significance difference against HP group (p <0,005). This data is in line with literature were some authors demonstrated higher eosinophils levels in the BALF of IPF patients, associated with a poor prognosis and increased resistance to therapy. Furthermore, IPF patients on Nintedanib treatment had higher eosinophil blood counts (0,45x10^3/ µL); this data could be interpreted as an effect of the antifibrotic itself at the endothelial level.

We also found that for the same UIP radiological pattern, IPF patients have higher absolute levels of monocytes in peripheral blood (p<0.006) than UIP non-IPF patients and we know from literature that this data is associated to a worse prognosis (Table12, Fig18).

In the group of patients suffering from cystic diseases, such as LAM and PLCH, statistically significant correlations were detected between serum SAA levels and peripheral eosinophils (r=-0.7, p=9E-03). The inverse correlation indicates how the increase in serum levels of SAA corresponds to a decrease in blood eosinophil counts, supporting the role of SAA in the formation of parenchymal cysts (through the stimulation of MMPs), contributing to the progression of these interstitial diseases towards a chronic evolutionary disease, leaving the initial inflammatory one behind.

Discussion

SAA is a pleiotropic protein involved in the regulation of inflammatory processes and lipid metabolism. SAA levels are elevated in patients with chronic inflammatory lung diseases such as sarcoidosis and chronic obstructive pulmonary disease (COPD), but also in lung cancer, connective tissue diseases and interstitial lung diseases. In 2019 we evaluated the serum levels of SAA in IPF patients, publishing for the first time a study that demonstrated a statistically significant difference in SAA concentrations between healthy controls and IPF patients (stable phase), and a direct correlation between SAA values and functional decline in FVC. High serum SAA values correlated with worse survival, suggesting a prognostic role of this biomarker.

The current study aims to validate our previous results on a larger population of IPF patients in order to evaluate the specificity of SAA in IPF compared to other ILDs and better define its role as a possible prognostic biomarker of the disease.

It is not clear how an acute phase protein involved in inflammatory processes can be overexpressed at a peripheral level in a fibrotic disease, where inflammation has a marginal pathogenetic role; while its increase in interstitial lung diseases in which the inflammatory process prevails, such as sarcoidosis, was already known and well documented. Probably, SAA is produced following profibrotic and hypoxic stimuli. For example, SAA levels have been found to be elevated in other lung diseases associated with hypoxic stimuli, such as COPD, asthma and obstructive sleep apnea syndromes (OSAS). The low partial pressure of oxygen would induce the activation of many mediators including acute phase proteins. The correlation between hypoxemia and SAA expression in IPF patients is intriguing and could partially explain our results; hypoxia, which favors the onset of cardiovascular comorbidities in IPF patients, would also have a negative prognostic role due to the involvement of SAA as a mediator of lipid metabolism (with reduction of HDL cholesterol) in these patients.

Our findings allow to hypothesize SAA as a possible specific biomarker for IPF patients.

Our results clearly demonstrated higher serum SAA levels in IPF compared to other pulmonary fibrosis (61,64 ± 8,52 mcg/ml), including sarcoidosis in which SAA had been proposed as a prognosis marker potentially involved in the pathogenesis.

The results of our study showed higher SAA values in IPF patients in stable phase compared to those with acute exacerbation (AE-IPF), although with the limitation of the small sample of AE-IPF patients (due to the fact that our patients often present exacerbated phases of the disease which lead them to be hospitalized in hospitals other than our center, making it difficult to obtain serum samples for our laboratory). This data suggest the role of SAA as a biomarker of fibrosis in IPF rather than as an indicator of inflammation (since in the exacerbated phase were inflammation prevails over fibrosis).

It almost seems that this data indicates that AE-IPF is not determined by a true inflammatory state, at least not systemic, in fact detecting a decrease in serum SAA levels in the acute phase compared to the stable phase of IPF (unlike as demonstrated in the literature where in systemic inflammation from chronic intestinal diseases or in septic states there is an increase in serum SAA resulting from the inflammatory state). Probably, this protein could be considered a marker of fibrosis/ progressive fibrosis. Perhaps, the increase of SAA serum levels in IPF vs other ILDs, in a statistically significant manner, would indicate that upstream of the entire pathway of its release there is not exactly an inflammatory stimulus but rather a profibrotic stimulus.

SAA levels, as known in the literature, are not modified by ongoing steroid or immunosuppressive therapy. In our study many patients with pulmonary fibrosis with a prevalent inflammatory pattern or with acute exacerbation of the disease were taking high-dose steroids or immunosuppressive drugs at the time of SAA sampling.

Our results show a clear statistically significant difference between SAA serum levels in the IPF group vs healthy controls ($p<0.0002$), which indicates that SAA is a disease marker.

Furthermore, IPF patients have higher SAA serum levels than the other ILD groups with a statistically significant difference, which would indicate how

SAA is a disease marker that allows discriminating the IPF from others fibrosis.

Our data show that IPF patients have statistical significance higher SAA serum levels than SSc-ILD with a progressive fibrosis, indicating that a patient with a progressive fibrosis with serum SAA levels above that cut-off (45,21 mcg/ml) could have IPF, regardless of the radiological pattern. According to our results, with low serum SAA concentrations < 6 mcg/ml we can discriminate healthy subjects from inflammatory pathological status. Instead, if the SAA values are very high with a cut-off > 50 mcg/ml, we can suspect a progressive fibrosis lung disease, probable IPF.

In line with literature, our IPF patients were older than the other groups.

Our statistically significant positive correlation between “age” and “SAA” confirm the correlation of IPF with age. This indicates that in older patients (as IPF), we have higher serum SAA levels. It would be interesting in this context to understand whether the age-related alteration of lipid metabolism is somehow involved in the process of fibrogenesis.

Our results confirm what is being studied in the literature about neutrophils involvement in the fibrotic process. In our patients the high peripheral neutrophilia is associated to a functional worsening of gas exchange (DLCO) This could be explained by the fact that the SAA-HDL complex chemoattracts inflammatory cells and it can interfere with the lipoxin signaling pathway, which can increase the survival time of neutrophils and aggravate the degree of inflammation. The neutrophil elastase is involved in extracellular matrix turnover, as well as the proliferation of lung fibroblasts and myofibroblast differentiation. Some studies demonstrated that blood neutrophilia is associated with a decline in FVC and mortality in IPF; it is also associated to a progression to IPF in patient with a radiological UIP indeterminate pattern (476). In our population older patients (as IPF) already present at T0 (time at diagnosis) a more compromised functional state, with a high neutrophilia with a negative predictive value. This result demonstrates the role of innate immunity in the pathogenesis of IPF. Innate immunity can be a target for IPF treatment, including via neutrophils.

We know that pulmonary fibrosis is the result of a persistent alveolitis with abnormal deposition of connective tissue. The alveolitis consists of infiltration by inflammatory cells including eosinophils, which release cytokines and stimulate the proliferation, migration and activation of mesenchymal cells increasing matrix synthesis (477,478). In our results IPF patients have lower eosinophils blood count and this data is explained by what is described in the literature relating to the higher BALF eosinophilia in IPF patients, associated to a poor prognosis. The low level of eosinophil blood counts is probably a consequence of alveolar tissue eosinophilia, the place where the fibrogenetic process occurs with the involvement of eosinophils. Eosinophil blood counts results to be lower in IPF patients compared to other ILDs. Furthermore, the peripheral eosinophilia increases during antifibrotic therapy (Nintedanib) in the IPF group and this data could be interpreted as an effect of the antifibrotic itself at the endothelial level. It could be hypothesized that this drug causes a recall of eosinophils from the tissue compartment to the vascular one, with consequent functional stability of the disease. It is known from the literature that some authors have analyzed the BALF in IPF patients, detecting eosinophilic alveolitis (>10%), which suggests that these inflammatory cells may be involved in the process of fibrogenesis and therefore it would be interesting to study how SAA may be related to it.

IPF patients have, instead, higher absolute levels of monocytes in peripheral blood than UIP non-IPF patients. We know that in the process of pulmonary fibrosis, monocytes are recruited into the lung in response to tissue injury and differentiate into long-lived macrophages producing TGF-β, metalloproteinasis (MMPs), eventually leading to fibroblast activation, myofibroblast differentiation and extracellular matrix (ECM) remodeling. Peripheral blood monocyte counts has recently emerged as a promising and easily measurable prognostic biomarker in IPF patients, with several studies showing that a monocyte count > 0.60 x 10^9 cell/μL^{-1} is strongly associated with disease progression; a monocyte count > 0.95 x 10^9 cell/μL^{-1} indicating a very high risk for poor outcomes (120-122).

Regarding cystic diseases, such as LAM and PLCH, statistically significant and inverse correlation was detected between SAA levels and peripheral eosinophilia. This correlation indicates how increasing serum levels of SAA corresponds to a decrease in eosinophilia, supporting the role of SAA in the formation of parenchymal cysts (through MMP stimulation), contributing to the progression of these interstitial diseases towards a chronic form evolutionary, leaving the initial inflammatory one behind. In fact, literature data report how SAA induces the overproduction of matrix metalloproteases (MMP-5, MMP-7, MMP-1, MMP-9).

In IPF patients, the marked expression of SAA could be a consequence of fibrogenesis, given that this protein is not only produced at the liver level but also by fibroblasts and in turn, with the induction of metalloproteases, could favor the expansion of the fibrotic process. Metalloproteases are also known to be involved in the metastatic process of lung tumors, in tissue remodeling and in lymphangiogenesis. They are in fact implicated in the pathogenesis of LAM: MMPs are present in LAM lesions. Furthermore, literature data reports that serum MMP-9 levels are higher in patients with LAM than in normal subjects. They can facilitate cell migration and contribute to the formation of pulmonary cysts, with clinical and functional worsening of the disease.

Our data about SAA in ILDs support the hypothesis that lipid metabolism is involved in the fibrotic process. We know that phospholipids and sphingolipids are the structural components of the membranes and regulate intra and intercellular signaling. These lipids can release some mediators that regulate cellular functions, migration, proliferation and apoptosis. Aberrations in phospholipids and sphingolipids metabolism have been identified as potential contributors to the pathophysiology in IPF. Some studies demonstrated that lipotoxicity due to accumulation of saturated fatty acids may have a role in the development of lung fibrosis in IPF by inducing reactive oxygen species (ROS) and apoptosis of alveolar epithelia cells (AECs). Nitrated fatty acids (NFAs) are physiological activators of the nuclear hormone receptor peroxisome-activated receptor-γ (PPARγ). Agonists of this receptor exhibit antifibrotic properties in vitro. NFAs

upregulated PPARγ and blocked TGF-β induced fibroblast differentiation in vitro. This data suggest therapeutic potential of NFAs in pulmonary fibrosis. (479).

SAA is an apolipoprotein that can replace apolipoprotein A1 (apoA1) as the major apolipoprotein of HDL. Some studies demonstrated that apoA1 BALF levels were lower in IPF patients compared to healthy controls. This apolipoprotein is produced by the liver and has many anti-inflammatory mechanisms, including inhibition of TNF, IL-6, IL-8 release. ApoA1 is the major apolipoprotein of HDL, the anti-inflammatory effect of HDL may be caused by the action of ApoA1. We know that SAA binds HDL (isoform3) cholesterol, reducing HDL levels in blood stream and the Apo1 protection from acute lung damage (480).

Limitations of the study

- IPF patients are all on antifibrotic treatment;
- AE-IPF group includes few patients (but we must also consider the fact that acute exacerbations of the disease are seen less thanks to antifibrotic therapy);
- healthy-controls are younger than all others patients.

Conclusions

Our findings about SAA support the hypothesis of such protein as a potential specific biomarker for IPF that can predict clinical course and prognosis of patients. SAA could discriminate an inflammatory ILD from a pulmonary progressive fibrosis mainly for IPF (which is a progressive fibrosis by definition). Monitoring serum levels could be useful to identify patients with rapidly progressive IPF disease phenotype or at risk of acute exacerbation, in order to direct such patients to antifibrotic therapies and lung transplantation earlier.Moreover, it strengthens the hypothesis about the role of lipid metabolism in the fibrotic process. SAA may play a crucial role in the regulation of lipid metabolism and production of MMPs in IPF. May be SAA could become a potential target for IPF treatment, including via apolipoproteins.

References

1. Ganesh Raghu, Martine Remy-Jardin, Luca Richeldi, Carey C-Thomson, Yoshikazu Inoue et al. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline. Idiopathic Pulmonary Fibrosis (an Update) and Progressive Pulmonary Fibrosis in Adults. February 2022.
2. Travis WD, Costabel U, Hansell DM, King TE, Lynch DA, Nicholson AG, et al. An official American Thoracic Society/European Respiratory Society statement: Update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. Am J Respir Crit Care Med; 15 settembre 2013;188(6):733–48.
3. Ganesh Raghu, Martine Remy-Jardin, Jeffrey L. Myers, Luca Richeldi et al. Diagnosis of Idiopathic Pulmonary Fibrosis. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline. Approved by ATS, JRS and ALAT on May 2018, and the ERS on June 2018.
4. Raghu G, Weycker D, Edelsberg J, Bradford WZ, Oster G. Incidence and prevalence of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med; ottobre 2006;174(7):810–6.
5. Olson AL, Swigris JJ, Lezotte DC, Norris JM, Wilson CG, Brown KK. Mortality from pulmonary fibrosis increased in the United States from 1992 to 2003. Am J Respir Crit Care Med; agosto 2007; 176(3):277–84.
6. Raghu G, Chen S-Y, Yeh W-S, Maroni B, Li Q, Lee Y-C, et al. Idiopathic pulmonary fibrosis in US Medicare beneficiaries aged 65 years and older: incidence, prevalence, and survival, 2001-11. Lancet Respir Med; luglio 2014; 2(7):566–72.
7. Ley B, Collard HR, King TE. Clinical course and prediction of survival in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med; febbraio 2011; 183(4):431–40.
8. King TE, Pardo A, Selman M. Idiopathic pulmonary fibrosis. Lancet Lond Engl; dicembre 2011; 378(9807):1949–61.
9. Hopkins RB, Burke N, Fell C, Dion G, Kolb M. Epidemiology and survival of idiopathic pulmonary fibrosis from national data in Canada. Eur Respir J; luglio 2016; 48(1):187–95.

10. Agabiti N, Porretta MA, Bauleo L, Coppola A, Sergiacomi G, Fusco A, et al. Idiopathic Pulmonary Fibrosis (IPF) incidence and prevalence in Italy. Sarcoidosis Vasc Diffuse Lung Dis Off J WASOG; ottobre 2014; 31(3):191–7.
11. Selman M, King TE, Pardo A, American Thoracic Society, European Respiratory Society, American College of Chest Physicians. Idiopathic pulmonary fibrosis: prevailing and evolving hypotheses about its pathogenesis and implications for therapy. Ann Intern Med; gennaio 2001; 134(2):136–51.
12. Chilosi M, Doglioni C, Murer B, Poletti V. Epithelial stem cell exhaustion in the pathogenesis of idiopathic pulmonary fibrosis. Sarcoidosis Vasc Diffuse Lung Dis Off J WASOG; luglio 2010; 27(1):7– 18.
13. Desai TJ, Brownfield DG, Krasnow MA. Alveolar progenitor and stem cells in lung development, renewal and cancer. Nature; marzo 2014; 507(7491):190–4.
14. Rock JR, Barkauskas CE, Cronce MJ, Xue Y, Harris JR, Liang J, et al. Multiple stromal populations contribute to pulmonary fibrosis without evidence for epithelial to mesenchymal transition. Proc Natl Acad Sci U S A; dicembre 2011; 108(52):E1475-1483.
15. Liang J, Zhang Y, Xie T, Liu N, Chen H, Geng Y, et al. Hyaluronan and TLR4 promote surfactant-protein-C-positive alveolar progenitor cell renewal and prevent severe pulmonary fibrosis in mice. Nat Med; novembre 2016; 22(11):1285–93.
16. Chilosi M, Carloni A, Rossi A, Poletti V. Premature lung aging and cellular senescence in the pathogenesis of idiopathic pulmonary fibrosis and COPD/emphysema. Transl Res J Lab Clin Med; settembre 2013; 162(3):156–73.
17. Günther A, Korfei M, Mahavadi P, von der Beck D, Ruppert C, Markart P. Unravelling the progressive pathophysiology of idiopathic pulmonary fibrosis. Eur Respir Rev Off J Eur Respir Soc; giugno 2012; 21(124):152–60.
18. Armanios MY, Chen JJ-L, Cogan JD, Alder JK, Ingersoll RG, Markin C, et al. Telomerase mutations in families with idiopathic pulmonary fibrosis. N Engl J Med; marzo 2007; 356(13):1317–26.
19. Kropski JA, Lawson WE, Young LR, Blackwell TS. Genetic studies provide clues on the pathogenesis of idiopathic pulmonary fibrosis. Dis Model Mech; gennaio 2013; 6(1):9–17.
20. Noth I, Zhang Y, Ma S-F, Flores C, Barber M, Huang Y, et al. Genetic variants associated with idiopathic pulmonary fibrosis susceptibility and mortality: a genome-wide association study. Lancet Respir Med; giugno 2013;1(4):309–17.
21. Fingerlin TE, Murphy E, Zhang W, Peljto AL, Brown KK, Steele MP, et al. Genome-wide association study identifies multiple susceptibility loci for pulmonary fibrosis. Nat Genet. Giugno 2013;45(6):613–20.
22. Peljto AL, Zhang Y, Fingerlin TE, Ma S-F, Garcia JGN, Richards TJ, et al. Association Between the MUC5B Promoter Polymorphism and Survival in Patients With Idiopathic Pulmonary Fibrosis. JAMA. 5 giugno 2013;309(21):2232–9.
23. Steele MP, Speer MC, Loyd JE, Brown KK, Herron A, Slifer SH, et al. Clinical and pathologic features of familial interstitial pneumonia. Am J

Respir Crit Care Med. 1 novembre 2005;172(9):1146–52.
24. Richeldi L, Collard HR, Jones MG. Idiopathic pulmonary fibrosis. Lancet Lond Engl. 30 marzo 2017.
25. Meyer KC. Pulmonary fibrosis, part I: epidemiology, pathogenesis, and diagnosis. Expert Rev Respir Med. maggio 2017;11(5):343–59.
26. Schulze K. Management of idiopathic pulmonary fibrosis and pulmonary hypertension. Sarcoidosis Vasc Diffuse Lung Dis Off J WASOG. 1 settembre 2013;30 Suppl 1:33–6.
27. King CS, Nathan SD. Idiopathic pulmonary fibrosis: effects and optimal management of comorbidities. Lancet Respir Med. gennaio 2017;5(1):72–84.
28. Raghu G, Amatto VC, Behr J, Stowasser S. Comorbidities in idiopathic pulmonary fibrosis patients: a systematic literature review. Eur Respir J. ottobre 2015;46(4):1113–30.
29. Kim W-Y, Mok Y, Kim GW, Baek S-J, Yun YD, Jee SH, et al. Association between idiopathic pulmonary fibrosis and coronary artery disease: a case-control study and cohort analysis. Sarcoidosis Vasc Diffuse Lung Dis Off J WASOG. 5 gennaio 2015;31(4):289–96.
30. Hubbard RB, Smith C, Le Jeune I, Gribbin J, Fogarty AW. The association between idiopathic pulmonary fibrosis and vascular disease: a population-based study. Am J Respir Crit Care Med. 15 dicembre 2008;178(12):1257–61.
31. Sprunger DB, Olson AL, Huie TJ, Fernandez-Perez ER, Fischer A, Solomon JJ, et al. Pulmonary fibrosis is associated with an elevated risk of thromboembolic disease. Eur Respir J. gennaio 2012;39(1):125–32.
32. Navaratnam V, Fogarty AW, McKeever T, Thompson N, Jenkins G, Johnson SR, et al. Presence of a prothrombotic state in people with idiopathic pulmonary fibrosis: a population-based case-control study. Thorax. marzo 2014;69(3):207–15.
33. Lee JS, Ryu JH, Elicker BM, Lydell CP, Jones KD, Wolters PJ, et al. Gastroesophageal reflux therapy is associated with longer survival in patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 15 dicembre 2011;184(12):1390–4.
34. Ryerson CJ, Berkeley J, Carrieri-Kohlman VL, Pantilat SZ, Landefeld CS, Collard HR. Depression and functional status are strongly associated with dyspnea in interstitial lung disease. Chest. marzo 2011;139(3):609–16.
35. Milioli G, Bosi M, Poletti V, Tomassetti S, Grassi A, Riccardi S, et al. Sleep and respiratory sleep disorders in idiopathic pulmonary fibrosis. Sleep Med Rev. aprile 2016;26:57–63.
36. Kishaba T, Nagano H, Nei Y, Yamashiro S. Body mass index- percent forced vital capacity-respiratory hospitalization: new staging for idiopathic pulmonary fibrosis patients. J Thorac Dis. dicembre 2016;8(12):3596–604.
37. Kim JH, Lee JH, Ryu YJ, Chang JH. Clinical predictors of survival in idiopathic pulmonary fibrosis. Tuberc Respir Dis. settembre 2012;73(3):162–8.
38. Gribbin J, Hubbard R, Smith C. Role of diabetes mellitus and gastro-oesophageal reflux in the aetiology of idiopathic pulmonary fibrosis. Respir Med. giugno 2009;103(6):927–31.
39. Suzuki A, Kondoh Y. The clinical impact of major comorbidities on idiopathic pulmonary fibrosis. Respir Investig. marzo 2017;55(2):94– 103.

40. Collard HR, Moore BB, Flaherty KR, Brown KK, Kaner RJ, King TE, et al. Acute exacerbations of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 1 ottobre 2007;176(7):636–43.
41. Collard HR, Ryerson CJ, Corte TJ, Jenkins G, Kondoh Y, Lederer DJ, et al. Acute Exacerbation of Idiopathic Pulmonary Fibrosis. An International Working Group Report. Am J Respir Crit Care Med. Agosto 2016;194(3):265–75.
42. Hunninghake GW, Lynch DA, Galvin JR, Gross BH, Müller N, Schwartz DA, et al. Radiologic findings are strongly associated with a pathologic diagnosis of usual interstitial pneumonia. Chest. ottobre 2003;124(4):1215–23.
43. Poletti V, Ravaglia C, Gurioli C, Piciucchi S, Dubini A, Cavazza A, et al. Invasive diagnostic techniques in idiopathic interstitial pneumonias. Respirol Carlton Vic. gennaio 2016;21(1):44–50.
44. Tomassetti S, Cavazza A, Colby TV, Ryu JH, Nanni O, Scarpi E, et al. Transbronchial biopsy is useful in predicting UIP pattern. Respir Res. 29 ottobre 2012;13:96.
45. Tomassetti S, Wells AU, Costabel U, Cavazza A, Colby TV, Rossi G, et al. Bronchoscopic Lung Cryobiopsy Increases Diagnostic Confidence in the Multidisciplinary Diagnosis of Idiopathic Pulmonary Fibrosis. Am J Respir Crit Care Med. 1 aprile 2016;193(7):745–52.
46. Ohshimo S, Bonella F, Cui A, Beume M, Kohno N, Guzman J, et al. Significance of bronchoalveolar lavage pulmonary fibrosis. Am J Respir Crit Care Med. 1 giugno 2009;179(11):1043–7.
47. Meyer KC, Raghu G, Baughman RP, Brown KK, Costabel U, du Bois RM, et al. An official American Thoracic Society clinical practice guideline: the clinical utility of bronchoalveolar lavage cellular analysis in interstitial lung disease. Am J Respir Crit Care Med. Maggio 2012;185(9):1004–14.
48. Meyer KC. The clinical utility of bronchoalveolar lavage in interstitial lung disease - is it really useful? Expert Rev Respir Med. aprile 2014;8(2):133–5.
49. Yupeng Li, Yaowu He, Shibin Chen, Qi Wang, Yi Yang et al. S100A12 as biomarker of Disease Severity and Prognosis in Patients with Idiopathic Pulmonary Fibrosis. Frontiers in Immunology. February 2022; 13:810338.
50. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, et al. 2011. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am. J. Respir. Crit. Care Med. 183:788–824.
51. Broekelmann TJ, Limper AH, Colby TV, McDonald JA. 1991. Transforming growth factor beta 1 is present at sites of extracellular matrix gene expression in human pulmonary fibrosis. PNAS 88:6642–46.
52. Khalil N, O'Connor RN, Unruh HW,Warren PW, Flanders KC, et al. Increased production and immunohistochemical localization of transforming growth factor-α in idiopathic pulmonary fibrosis. Am. J. Respir. Cell Mol.
53. Biol. 1991; 5:155–62.
54. MaherTM, Oballa E, Simpson JK, Porte J,Habgood A, et al. An epithelial biomarker signature for idiopathic pulmonary fibrosis: an analysis from the multicentre PROFILE cohort study. Lancet Respir. Med. 2017; 5:946–55.
55. Romero Y, Bueno M, Ramirez R, Alvarez D, Sembrat JC, et al. 2016. mTORC1 activation decreases autophagy in aging and idiopathic pulmonary fibrosis and contributes to apoptosis resistance in IPF fibroblasts. Aging Cell

15:1103–12.
56. Gracey DR, Divertie MB, Brown AL Jr. 1968. Alveolar-capillary membrane in idiopathic interstitial pulmonary fibrosis: electron microscopic study of 14 cases. Am. Rev. Respir. Dis. 98:16–21.
57. Magro CM,WaldmanWJ, Knight DA, Allen JN, Nadasdy T, et al. Idiopathic pulmonary fibrosis related to endothelial injury and antiendothelial cell antibodies. Hum. Immunol.2006; 67:284–97.
58. RaghuG,Behr J,Brown KK, Egan JJ, Kawut SM, et al.Treatment of idiopathic pulmonary fibrosis with ambrisentan: a parallel, randomized trial. Ann. Intern.Med. 2013; 158:641–49.
59. Distler JHW, Györfi AH, Ramanujam M, Whitfield ML, Königshoff M, Lafyatis R. 2019. Shared anddistinct mechanisms of fibrosis. Nat. Rev. Rheumatol. 15:705–30.
60. Nuovo GJ, Hagood JS,Magro CM,ChinN, Kapil R, et al.The distribution of immunomodulatory cells in the lungs of patients with idiopathic pulmonary fibrosis. Mod. Pathol. 2012; 25:416–33.
61. Hashimoto S, Gon Y, Takeshita I, Maruoka S, Horie T. IL-4 and IL-13 induce myofibroblastic phenotype of human lung fibroblasts through c-JunNH2-terminal kinase–dependent pathway. J. Allergy Clin. Immunol. 2001; 107:1001–8.
62. Kinder BW, Brown KK, Schwarz MI, Ix JH, Kervitsky A, King TE Jr.Baseline BAL neutrophilia predicts early mortality in idiopathic pulmonary fibrosis. Chest 2008; 133:226–32.
63. Song, J.W.; Hong, S.B.; Lim, C.M.; Koh, Y.; Kim, D.S. Acute exacerbation of idiopathic pulmonary fibrosis: Incidence, risk factors and outcome. Eur. Respir. J. 2011, 37, 356–363.
64. Kishaba, T.; Nei, Y.; Momose, M.; Nagano, H.; Yamashiro, S. Clinical Characteristics Based on the New Criteria of Acute Exacerbation in Patients with Idiopathic Pulmonary Fibrosis. Eurasian J. Med. 2018, 50, 6–10.
65. Enomoto, N.; Oyama, Y.; Enomoto, Y.; Mikamo, M.; Karayama, M.; Hozumi, H.; Suzuki, Y.; Kono, M.; Furuhashi, K.; Fujisawa, T.; et al. Prognostic evaluation of serum ferritin in acute exacerbation of idiopathic pulmonary fibrosis. Clin. Respir. J. 2018, 12, 2378–2389.
66. Collard, H.R.; Moore, B.B.; Flaherty, K.R.; Brown, K.K.; Kaner, R.J.; King, T.E., Jr.; Lasky, J.A.; Loyd, J.E.; Noth, I.; Olman, M.A.; et al. Acute exacerbations of idiopathic pulmonary fibrosis. Am. J. Respir. Crit. Care Med. 2007, 176, 636–643.
67. Saito, S.; Lasky, J.A.; Hagiwara, K.; Kondoh, Y. Ethnic differences in idiopathic pulmonary fibrosis. The Japanese perspective. Respir. Investig. 2018, 56, 375–383.
68. Deng, Y.; Li, Z.; Liu, J.; Wang, Z.; Cao, Y.; Mou, Y.; Fu, B.; Mo, B.; Wei, J.; Cheng, Z.; et al. Targeted resequencing reveals genetic risks in patients with sporadic idiopathic pulmonary fibrosis. Hum. Mutat. 2018, 39, 1238–1245.
69. Akira, M.; Kozuka, T.; Yamamoto, S.; Sakatani, M. Computed tomography findings in acute exacerbation of idiopathic pulmonary fibrosis. Am. J. Respir. Crit. Care Med. 2008, 178, 372–378.
70. Homma, S.; Bando, M.; Azuma, A.; Sakamoto, S.; Sugino, K.; Ishii, Y.; Izumi, S.; Inase, N.; Inoue, Y.; Ebina, M.; et al. Japanese guideline for the treatment of idiopathic pulmonary fibrosis. Respir. Investig. 2018, 56, 268–

291.
71. Novelli, L.; Ruggiero, R.; De Giacomi, F.; Biffi, A.; Faverio, P.; Bilucaglia, L.; Gamberini, S.; Messinesi, G.; Pesci, A. Corticosteroid and cyclophosphamide in acute exacerbation of idiopathic pulmonary fibrosis. A single center experience and literature review. Sarcoidosis Vasc. Diffuse Lung Dis. 2016, 33, 385–391.
72. Tsushima, K.; Yamaguchi, K.; Kono, Y.; Yokoyama, T.; Kubo, K.; Matsumura, T.; Ichimura, Y.; Abe, M.; Terada, J.; Tatsumi, K. Thrombomodulin for acute exacerbations of idiopathic pulmonary fibrosis: A proof of concept study. Pulm. Pharmacol. Ther. 2014, 29, 233–240.
73. Isshiki, T.; Sakamoto, S.; Kinoshita, A.; Sugino, K.; Kurosaki, A.; Homma, S. Recombinant human soluble thrombomodulin treatment for acute exacerbation of idiopathic pulmonary fibrosis: A retrospective study. Respiration 2015, 89, 201–207.
74. Kataoka, K.; Taniguchi, H.; Kondoh, Y.; Nishiyama, O.; Kimura, T.; Matsuda, T.; Yokoyama, T.; Sakamoto, K.; Ando, M. Recombinant Human Thrombomodulin in Acute Exacerbation of Idiopathic Pulmonary Fibrosis. Chest 2015, 148, 436–443.
75. Bräunlich, J.; Beyer, D.; Mai, D.; Hammerschmidt, S.; Seyfarth, H.J.;Wirtz, H. Effects of nasal high flow on ventilation in volunteers, COPD and idiopathic pulmonary fibrosis patients. Respiration 2013, 85, 319–325.
76. Natsuizaka, M.; Chiba, H.; Kuronuma, K.; Otsuka, M.; Kudo, K.; Mori, M.; Bando, M.; Sugiyama, Y.; Takahashi, H. Epidemiologic survey of Japanese patients with idiopathic pulmonary fibrosis and investigation of ethnic differences. Am. J. Respir. Crit. Care Med. 2014, 190, 773–779.
77. A. Tzouvelekis; G. Gomatou; E. Bouros; R. Trigidou; V. Tzilas. Common Pathogenic Mechanisms Between Idiopathic Pulmonary Fibrosis and Lung Cancer. Chest 2019; 19-0582.
78. Iwata T, Yoshida S, Fujiwara T, et al. Effect of perioperative pirfenidone treatment in lung cancer patients with idiopathic pulmonary fibrosis. Ann Thorac Surg. 2016;102(6):1905-1910.
79. Tila Khan, Sanjukta Dasgupta, Nilanjana Ghosh, Koel Chaudhury. Proteomics in idiopathic pulmonary fibrosis: the quest for biomarkers. Molecular Omics- The Royal Society of Chemistry, 2020.
80. A. Hara, N. Sakamoto, Y. Ishimatsu, T. Kakugawa, S. Nakashima, S. Hara, M. Adachi, H. Fujita, H. Mukae and S. Kohno, Respir. Med., 2012, 106, 571–580.
81. A. Carleo, C. Landi, A. Prasse, L. Bergantini, M. D'Alessandro, P. Cameli, S. Janciauskiene, P. Rottoli, L. Bini and E. Bargagli, Monaldi Arch. Chest Dis., 2020, 90, DOI: 10.4081/monaldi. 2020.1231.
82. Y. Zhang, Q. Xin, Z. Wu, C. Wang, Y. Wang, Q. Wu and R. Niu, Med. Sci. Monit., 2018, 24, 4146–4153.
83. Y. P. Moodley, T. J. Corte, B. G. Oliver, I. N. Glaspole, A. Livk, J. Ito, K. Peters, R. Lipscombe, T. Casey and D. B. A. Tan, Respirology, 2019, 24, 1111–1114.
84. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. Clin. Pharmacol. Ther. 2001, 69, 89–95.
85. Whitsett, J.A.;Wert, S.E.;Weaver, T.E. Alveolar Surfactant Homeostasis and the Pathogenesis of Pulmonary Disease. Annu. Rev. Med. 2010, 61, 105–

119.
86. Lawson, W.E.; Grant, S.W.; Ambrosini, V.; Womble, K.E.; Dawson, E.P.; Lane, K.B.; Markin, C.; Renzoni, E.; Lympany, P.; Thomas, A.Q.; et al. Genetic mutations in surfactant protein C are a rare cause of sporadic cases of IPF. Thorax 2004, 59, 977–980.
87. Ishikawa, N.; Hattori, N.; Yokoyama, A.; Kohno, N. Utility of KL-6/MUC1 in the clinical management of interstitial lung diseases. Respir. Investig. 2012, 50, 3–13.
88. Ohtsuki, Y.; Fujita, J.; Hachisuka, Y.; Uomoto, M.; Okada, Y.; Yoshinouchi, T.; Lee, G.-H.; Furihata, M.; Kohno, N. Immunohistochemical and immunoelectron microscopic studies of the localization of KL-6 and epithelial membrane antigen (EMA) inpresumably normal pulmonary tissue and in interstitial pneumonia. Med. Mol. Morphol. 2007, 40, 198–202.
89. Ishii, H.; Mukae, H.; Kadota, J.; Kaida, H.; Nagata, T.; Abe, K.; Matsukura, S.; Kohno, S. High serum concentrations of surfactant protein A in usual interstitial pneumonia compared with non-specific interstitial pneumonia. Thorax 2003, 58, 52–57.
90. Ohnishi, H.; Yokoyama, A.; Kondo, K.; Hamada, H.; Abe, M.; Nishimura, K.; Hiwada, K.; Kohno, N. Comparative Study of KL-6, Surfactant Protein-A, Surfactant Protein-D, and Monocyte Chemoattractant Protein-1 as Serum Markers for Interstitial Lung Diseases. Am. J. Respir. Crit. Care Med. 2002, 165, 378–381.
91. Zhu, C.; Zhao, Y.B.; Kong, L.F.; Li, Z.H.; Kang, J. The expression and clinical role of KL-6 in serum and BALF of patients with different diffuse interstitial lung diseases. Chin. J. Tuberc. Respir. Dis. 2016, 39.
92. Bennett, D.; Salvini, M.; Fui, A.; Cillis, G.; Cameli, P.; Mazzei, M.A.; Fossi, A.; Refini, R.M.; Rottoli, P. Calgranulin B and KL-6 in Bronchoalveolar Lavage of Patients with IPF and NSIP. Inflammation 2019, 42, 463–470.
93. Xue, M.; Guo, Z.; Zhou, L.; Sun, B.; Wang, H. Evaluation of the Diagnostic Efficacies of Serological Markers Kl-6, SP-A, SP-D, CCL2, and CXCL13 in Idiopathic Interstitial Pneumonia A67. Struct. Funct. Relat. 2020, 98, 534–545.
94. Jiang, Y.; Luo, Q.; Han, Q.; Huang, J.; Ou, Y.; Chen, M.; Wen, Y.; Mosha, S.S.; Deng, K.; Chen, R. Sequential changes of serum KL-6 predict the progression of interstitial lung disease. J. Thorac. Dis. 2018, 10, 4705–4714.
95. Pardo, A.; Selman, M. Role of matrix metaloproteases in idiopathic pulmonary fibrosis. Fibrogenes. Tiss. Repair. 2012, 5, S9.
96. Konishi, K.; Gibson, K.F.; Lindell, K.O.; Richards, T.J.; Zhang, Y.; Dhir, R.; Bisceglia, M.; Gilbert, S.; Yousem, S.A.; Song, J.W.; et al. Gene Expression Profiles of Acute Exacerbations of Idiopathic Pulmonary Fibrosis. Am. J. Respir. Crit. Care Med. 2009, 180, 167–175.
97. Morais, A.; Beltrão, M.; Sokhatska, O.; Costa, D.; Melo, N.; Mota, P.; Marques, A.; Delgado, L. Serum metalloproteinases 1 and 7 in the diagnosis of idiopathic pulmonary fibrosis and other interstitial pneumonias. Respir. Med. 2015, 109, 1063–1068.
98. Okamoto, M.; Izuhara, K.; Ohta, S.; Ono, J.; Hoshino, T. Ability of Periostin as a New Biomarker of Idiopathic Pulmonary Fibrosis. Single Mol. Single Cell Seq. 2019, 1132, 79–87.
99. Bonella, F.; Ohshimo, S.; Boerner, E.; Guzman, J.;Wessendorf, T.E.; Costabel, U. Serum Kl-6 Levels Correlate with Response to Pirfenidone in

Idiopathic Pulmonary Fibrosis. Am. J. Respir. Crit. Care Med. 2015, 191, A4398.
100. Bergantini, L.; Bargagli, E.; Cameli, P.; Cekorja, B.; Lanzarone, N.; Pianigiani, L.; Vietri, L.; Bennett, D.; Sestini, P.; Rottoli, P. Serial KL-6 analysis in patients with idiopathic pulmonary fibrosis treated with nintedanib. Respir. Investig. 2019, 57, 290–291.
101. Raghu G, Rochwerg B, Zhang Y, Garcia CAC, Azuma A, Behr J, et al. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline: Treatment of Idiopathic Pulmonary Fibrosis. An Update of the 2011 Clinical Practice Guideline. Am J Respir Crit Care Med. 15 luglio 2015;192(2):e3-19.
102. Idiopathic Pulmonary Fibrosis Clinical Research Network, Raghu G, Anstrom KJ, King TE, Lasky JA, Martinez FJ. Prednisone, azathioprine, and N-acetylcysteine for pulmonary fibrosis. N Engl J Med. 24 maggio 2012;366(21):1968–77.
103. Oldham JM, Ma S-F, Martinez FJ, Anstrom KJ, Raghu G, Schwartz DA, et al. TOLLIP, MUC5B, and the Response to N-Acetylcysteine among Individuals with Idiopathic Pulmonary Fibrosis. Am J Respir Crit Care Med. 15 dicembre 2015;192(12):1475–82.
104. Noth I, Anstrom KJ, Calvert SB, de Andrade J, Flaherty KR, Glazer C, et al. A placebo-controlled randomized trial of warfarin in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 1 luglio 2012;186(1):88–95.
105. Raghu G, Behr J, Brown KK, Egan JJ, Kawut SM, Flaherty KR, et al. Treatment of idiopathic pulmonary fibrosis with ambrisentan: a parallel, randomized trial. Ann Intern Med. 7 maggio 2013;158(9):641–9.
106. Raghu G, Million-Rousseau R, Morganti A, Perchenet L, Behr J, MUSIC Study Group. Macitentan for the treatment of idiopathic pulmonary fibrosis: the randomised controlled MUSIC trial. Eur Respir J. dicembre 2013;42(6):1622–32.
107. King TE, Behr J, Brown KK, du Bois RM, Lancaster L, de Andrade JA, et al. BUILD-1: a randomized placebo-controlled trial of bosentan in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 1 gennaio 2008;177(1):75–81.
108. King TE, Brown KK, Raghu G, du Bois RM, Lynch DA, Martinez F, et al. BUILD-3: a randomized, controlled trial of bosentan in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 1 luglio 2011;184(1):92–9.
109. Nishiyama O, Kondoh Y, Kimura T, Kato K, Kataoka K, Ogawa T, et al. Effects of pulmonary rehabilitation in patients with idiopathic pulmonary fibrosis. Respirol Carlton Vic. maggio 2008;13(3):394–9.
110. Polastri M, Dell'Amore A, Zagnoni G, Nava S. Preoperative physiotherapy in subjects with idiopathic pulmonary fibrosis qualified for lung transplantation: implications on hospital length of stay and clinical outcomes. J Thorac Dis. maggio 2016;8(5):E264-268.
111. Weill D, Benden C, Corris PA, Dark JH, Davis RD, Keshavjee S, et al. A consensus document for the selection of lung transplant candidates: 2014-an update from the Pulmonary Transplantation Council of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant Off Publ Int Soc Heart Transplant. gennaio 2015;34(1):1–15.
112. Brown AW, Kaya H, Nathan SD. Lung transplantation in IIP: A review. Respirol Carlton Vic. ottobre 2016;21(7):1173–84.
113. Kistler KD, Nalysnyk L, Rotella P, Esser D. Lung transplantation in

idiopathic pulmonary fibrosis: a systematic review of the literature. BMC Pulm Med. 16 agosto 2014;14:139.
114. Noble PW, Albera C, Bradford WZ, Costabel U, Glassberg MK, Kardatzke D, et al. Pirfenidone in patients with idiopathic pulmonary fibrosis (CAPACITY): two randomised trials. Lancet Lond Engl. 21 maggio 2011;377(9779):1760–9.
115. King TE, Bradford WZ, Castro-Bernardini S, Fagan EA, Glaspole I, Glassberg MK, et al. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. N Engl J Med. 29 maggio 2014;370(22):2083–92.
116. Richeldi L, Costabel U, Selman M, Kim DS, Hansell DM, Nicholson AG, et al. Efficacy of a tyrosine kinase inhibitor in idiopathic pulmonary fibrosis. N Engl J Med. 22 settembre 2011;365(12):1079– 87.
117. Richeldi L, du Bois RM, Raghu G, Azuma A, Brown KK, Costabel U, et al. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. N Engl J Med. 29 maggio 2014;370(22):2071–82.
118. Wuyts WA, Kolb M, Stowasser S, Stansen W, Huggins JT, Raghu G. First Data on Efficacy and Safety of Nintedanib in Patients with Idiopathic Pulmonary Fibrosis and Forced Vital Capacity of ≤50 % of Predicted Value. Lung. ottobre 2016;194(5):739–43.
119. Paolo Spagnolo, Jonathan A. Kropski, Mark G. Jones, Joyce S. Lee, Giulio Rossi, Theodoros Karampitsakos et al. Idiopathic pulmonary fibrosis: Disease mechanisms and drug development. Pharmacology&Therapeutics, 2021;107798.
120. Waxman A, Restrepo-Jaramillo R, Thenappan T, et al. Inhaled treprostinil in pulmonary hypertension due to interstitial lung disease. N Engl J Med 2021; 384: 325–334.
121. Kreuter M, Lee JS, Tzouvelekis A, et al. Monocyte count as a prognostic biomarker in patients withidiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2021; 204: 74–81.
122. Scott MKD, Quinn K, Li Q, et al. Increased monocyte count as a cellular biomarker for poor outcomes in fibrotic diseases: a retrospective, multicentre cohort study. Lancet Respir Med 2019; 7: 497–508.
123. Karampitsakos T, Torrisi S, Antoniou K, et al. Increased monocyte count and red cell distribution width as prognostic biomarkers in patients with idiopathic pulmonary fibrosis. Respir Res 2021; 22: 140.
124. Maher TM, Stowasser S, Nishioka Y, et al. Biomarkers of extracellular matrix turnover in patients with idiopathic pulmonary fibrosis given nintedanib (INMARK study): a randomised, placebo-controlled study. Lancet Respir Med 2019; 7: 771–779.
125. Armanios M. Telomeres and age-related disease: how telomere biology informs clinical paradigms. J Clin Invest. 2013 Mar;123(3):996-1002.
126. Luca Richeldi, Arata Azuma, Vincent Cottin, Christan Hesslinger, Susanne Stowasser, Claudia Valenzuela, Marlies Wijsenbeek, Donald. F. Zoz. Trial of a Preferential Phosphodiesterase 4B Inhibitor for Idiopathic Pulmonary Fibrosis. The New England Journal of Medicine, 2022; 386:2178-2187.
127. Lacasse Y, Selman M, Costabel U, et al. Clinical Diagnosis of Hypersensitivity Pneumonitis. Am J Respir Crit Care Med. 2003;168(8):952-958. doi:10.1164/rccm.200301-137OC.
128. Mazzei MA, Sartorelli P, Bagnacci G, et al. Occupational Lung Diseases: Underreported Diagnosis in Radiological Practice. Semin Ultrasound, CT

MRI. 2019;40(1):36-50. doi:10.1053/J.SULT.2018.10.019.
129. Salisbury ML, Myers JL, Belloli EA, Kazerooni EA, Martinez FJ, Flaherty KR. Diagnosis and Treatment of Fibrotic Hypersensitivity Pneumonia. Where We Stand and Where We Need to Go. Am J Respir Crit Care Med. 2017;196(6):690-699. doi:10.1164/rccm.201608-1675PP.
130. Vasakova M, Morell F, Walsh S, Leslie K, Raghu G. Hypersensitivity pneumonitis: perspectives in diagnosis and management. Am J Respir Crit Care Med 2017;196:680–689.
131. Vasakova M, Morell F, Walsh S, Leslie K, Raghu G. Hypersensitivity Pneumonitis: Perspectives in Diagnosis and Management. Am J Respir Crit Care Med. 2017;196(6):680-689. doi:10.1164/rccm.201611-2201PP.
132. Elicker BM, Jones KD, Henry TS, Collard HR. Multidisciplinary Approach to Hypersensitivity Pneumonitis. J Thorac Imaging. 2016;31(2):92-103. doi:10.1097/RTI.0000000000000186.
133. Richerson HB, Bernstein IL, Fink JN, et al. Guidelines for the clinical evaluation of hypersensitivity pneumonitis: Report of the Subcommittee on Hypersensitivity Pneumonitis. J Allergy Clin Immunol. 1989;84(5):839-844. doi:10.1016/0091-6749(89)90349-7.
134. Fenoglio C-M, Reboux G, Sudre B, et al. Diagnostic value of serum precipitins to mould antigens in active hypersensitivity pneumonitis. Eur Respir J. 2007;29(4):706-712. doi:10.1183/09031936.00001006.
135. Cormier Y, Bélanger J, Tardif A, Leblanc P, Laviolette M. Relationships between radiographic change, pulmonary function, and bronchoalveolar lavage fluid lymphocytes in farmer's lung disease. Thorax. 1986;41(1):28-33. doi:10.1136/thx.41.1.28.
136. Hanak V, Golbin JM, Hartman TE, Ryu JH. High-Resolution CT Findings of Parenchymal Fibrosis Correlate With Prognosis in Hypersensitivity Pneumonitis*. Chest. 2008;134(1):133-138. doi:10.1378/CHEST.07-3005.
137. Morisset J, Johannson KA, Jones KD, et al. Identification of Diagnostic Criteria for Chronic Hypersensitivity Pneumonitis. An International Modified Delphi Survey. Am J Respir Crit Care Med. 2018;197(8):1036-1044. doi:10.1164/rccm.201710-1986OC.
138. Gruden JF, Webb WR, Warnock M. Centrilobular opacities in the lung on high-resolution CT: diagnostic considerations and pathologic correlation. AJR Am J Roentgenol. 1994;162(3):569-574. doi:10.2214/ajr.162.3.8109498.
139. Hansell DM, Bankier AA, MacMahon H, McLoud TC, Müller NL, Remy J. Fleischner Society: Glossary of Terms for Thoracic Imaging. Radiology. 2008;246(3):697-722. doi:10.1148/radiol.2462070712.
140. Buschman DL, Gamsu G, Waldron JA, Klein JS, King TE. Chronic hypersensitivity pneumonitis: use of CT in diagnosis. AJR Am J Roentgenol. 1992;159(5):957-960. doi:10.2214/ajr.159.5.1414806.
141. Abbritti M, Mazzei MA, Bargagli E, et al. Utility of spiral CAT scan in the follow-up of patients with pulmonary Langerhans cell histiocytosis. Eur J Radiol. 2012;81(8):1907-1912. doi:10.1016/J.EJRAD.2011.04.018.
142. Sergiacomi G, Cicciò C, Boi L, et al. Ground-glass opacity: High-resolution computed tomography and 64-multi-slice computed tomography findings comparison. Eur J Radiol. 2010;74(3):479-483. doi:10.1016/J.EJRAD.2009.03.034.
143. Ganesh Raghu, Martine Remy-Jardin, Christopher J. Ryerson, Jeffrey L.

Myers, Michael Kreuter, Martina Vasakova, Elena Bargagli, Jonathan H. Chung, Bridget F. Collins, Elisabeth Bendstrup, Hassan A. Chami et al. Diagnosis of Hypersensitivity Pneumonitis in Adults. An Official ATS/JRS/ALAT Clinical Practice Guideline. American Journal of Resp Crit Care Med, 2020; Iss 3, pp e36-e69.
144. Reich JM. Chirping rales in bird-fancier's lung. Chest 1993;104:326–327.
145. Lacasse Y, Selman M, Costabel U, Dalphin JC, Ando M, Morell F, et al.; HP Study Group. Clinical diagnosis of hypersensitivity pneumonitis. Am J Respir Crit Care Med 2003;168:952–958.
146. Ohtani Y, Saiki S, Sumi Y, Inase N, Miyake S, Costabel U, et al. Clinical features of recurrent and insidious chronic bird fancier's lung. Ann Allergy Asthma Immunol 2003;90:604–610.
147. Fernandez Perez ER, Kong AM, Raimundo K, Koelsch TL, Kulkarni R, Cole AL. Epidemiology of hypersensitivity pneumonitis among an insured population in the United States: a claims-based cohort analysis. Ann Am Thorac Soc 2018;15:460–469.
148. Hanak V, Golbin JM, Ryu JH. Causes and presenting features in 85 consecutive patients with hypersensitivity pneumonitis. Mayo Clin Proc 2007;82:812–816.
149. De Sadeleer LJ, Hermans F, De Dycker E, Yserbyt J, Verschakelen JA, Verbeken EK, et al. Effects of corticosteroid treatment and antigen avoidance in a large hypersensitivity pneumonitis cohort: a singlecentre cohort study. J Clin Med 2018;8:14.
150. Fernandez Perez ER, Swigris JJ, Forsse´ n AV, Tourin O, Solomon JJ, Huie TJ, et al. Identifying an inciting antigen is associated with improved survival in patients with chronic hypersensitivity pneumonitis. Chest 2013;144:1644–1651.
151. Kokkarinen JI, Tukiainen HO, Terho EO. Effect of corticosteroid treatment on the recovery of pulmonary function in farmer's lung. Am Rev Respir Dis 1992;145:3–5.
152. Salisbury ML, Gu T, Murray S, Gross BH, Chughtai A, Sayyouh M, et al. Hypersensitivity pneumonitis: radiologic phenotypes are associated with distinct survival time and pulmonary function trajectory. Chest 2019;155:699–711.
153. Chiba S, Tsuchiya K, Akashi T, Ishizuka M, Okamoto T, Furusawa H, et al. Chronic hypersensitivity pneumonitis with a usual interstitial pneumonia-like pattern: correlation between histopathologic and clinical findings. Chest 2016;149:1473–1481.
154. Gimenez A, Storrer K, Kuranishi L, Soares MR, Ferreira RG, Pereira CAC. Change in FVC and survival in chronic fibrotic hypersensitivity pneumonitis. Thorax 2018;73:391–392.
155. Churg A, Sin DD, Everett D, Brown K, Cool C. Pathologic patterns and survival in chronic hypersensitivity pneumonitis. Am J Surg Pathol 2009;33:1765–1770.
156. Perez-Padilla R, Salas J, Chapela R, Sa´ nchez M, Carrillo G, Pe´ rez R, et al. Mortality in Mexican patients with chronic pigeon breeder's lung compared with those with usual interstitial pneumonia. Am Rev Respir Dis 1993;148:49–53.
157. Barnett J, Molyneaux PL, Rawal B, Abdullah R, Hare SS, Vancheeswaran R, et al. Variable utility of mosaic attenuation to distinguish fibrotic

hypersensitivity pneumonitis from idiopathic pulmonary fibrosis. Eur Respir J 2019;54:1900531.
158. Coleman A, Colby TV. Histologic diagnosis of extrinsic allergic alveolitis. Am J Surg Pathol 1988;12:514–518.
159. Kitaichi M, Shimizu S, Tamaya M, Takaki M, Inoue Y. Pathology of hypersensitivity pneumonitis. In: Sharma OP, editor. Clinical focus series, hypersensitivity pneumonitis. New Delhi, India: Jaypee Brothers Medical Publishers; 2013. pp. 22–32.
160. Lopez M, Salvaggio JE - Hypersensitivity pneumonitis. In: Murray JF, Nadel JA (Eds): Textbook of respiratory medicine. Second edition Saunders, Philadelphia, 1994, vol 2, cap.68, p. 2018-31.
161. Guler SA, Hur SA, Lear SA, Camp PG, Ryerson CJ. Body composition, muscle function, and physical performance in fibrotic interstitial lung disease: a prospective cohort study. Respir Res 2019;20(1):56.
162. Tonelli R, Cocconcelli E, Lanini B, et al. Effectiveness of pulmonary rehabilitation in patients with interstitial lung disease of different etiology: a multicenter prospective study. BMC Pulm Med. 2017;17(1):130.
163. Spruit MA, Singh SJ, Garvey C, et al. An official American Thoracic Society/European Respiratory Society statement: key concepts and advances in pulmonary rehabilitation. Am J Respir Crit Care Med 2013;188(8):e13-64.
164. Kern RM, Singer JP, Koth L, et al. Lung transplantation for hypersensitivity pneumonitis. Chest 2015;147(6):1558-1565.
165. Egeler RM, D'Angio GJ. Medical Progress: Langerhans cell Histiocytosis. J Pediatr. 1995;127(1):1-11.
166. Weitzman S, Jaffe R. Uncommon histiocytic disorders: the non-Langerhans cell histiocytoses. Pediatr Blood Cancer.2005; 45: 256-264.
167. Favara BE, Feller AC, Pauli M et al. Contemporary classification of histiocytic disorders. The WHO Committee On Histiocytic/Reticulum Cell Proliferations. Reclassification Working Group of the Histiocyte Society. Med Pediatr Oncol. 29:157-166.
168. Badalian-Very G, Vergilio JA, Fleming M, Rollins BJ. Pathogenesis of Langerhans cell histiocytosis. Annu Rev Pathol. 2013;8:1-20.
169. Emile JF, Abla O, Fraitag S, et al; Histiocyte Society. Revised classification of histiocy-toses and neoplasms of the macrophage-dendritic cell lineages. Blood. 2016;127:2672-2681.
170. Frater JL. The histiocytoses: as easy as ABC (or LCMRH). 2016;127:2655-2656.
171. Guyot-Goubin A, Donadieu J, Barkaoui M et al. Descriptive epidemiology of childhood Langerhans cellhistiocytosis in France, 2000-2004. Pediatr Blood Cancer. 2008;51:71-75.
172. Baumgartner I, von Hochstetter A, Baumert B et al. Langerhans'-cell histiocytosis in adults. Med Pediatr Oncol.1997;28:9-14.
173. Miller DR. Familiar reticuloendotheliosis: Concurrence of disease in five siblings. Pediatrics 1996; 38:986-995.
174. Nezelof C, Basset F, Rousseau M. Histiocytosis X histogenetic arguments for a Langerhans cell origin. 1973; 18:365.
175. Zinn DJ,Chakraborty R, Allen CE. Langerhans Cell Histiocytosis: Emerging Insights and Clinical Implications. Oncology. 2016;30:122-139.
176. Geissmann F et al, 2001.

177. Egeler RM,Neglia JP, Aricò M et al.The relation of Langerhans cell histiocytosis to acute leukemia, lymphomas, and other solid tumors. The LCH-Malignancy Study Group of the Histiocyte Society. Hematol Oncol Clin North Am. 1998;12:369-378.
178. Morimoto A, Oh Y, Shioda Y et al. Recent advances in Langerhans cell histiocytosis. Pediatr Int. 2014; 56:451-461.
179. Costa CE, Annels NE, Faaij CM ET AL. Presence of osteoclast-like multinucleated giantcells in the bone and non ostotic lesions of Langerhans cell histiocytosis. Exp. Med. 2005;201:687–693.
180. Nezelof C, Basset F, Rousseau M. Histiocytosis X histogenetic arguments for a Langerhans cell origin. 1973; 18:365.
181. Hayashi T, Rush WL, Travis WD. Immunohistochemical study of matrix metalloproteinases and their tissue inhibitors in pulmonary Langerhans' cell granulomatosis.
182. da Costa CE, Annels NE, Faaij CM ET AL. Presence of osteoclast-like multinucleated giantcells in the bone and non ostotic lesions of Langerhans cell histiocytosis. Exp. Med. 2005;201:687–693.
183. Allen CE, Li L, Peters TL et al. Cell-specific gene expression in Langerhans cell histiocytosis lesions reveals a distinct profile compared with epidermal Langerhans cells. J Immunol. 2010;184:4557-4567.
184. Costa CE, Szuhai K, van Eijk R, et al. No genomic aberrations in Langerhans cell histiocytosis as assessed by diverse molecular technologies. Genes Chromosomes Cancer. 2009;48:239-249.
185. Badalian-Very G, Vergilio JA, Degar BA et al. Recurrent BRAF mutations in Langerhans cell histiocytosis. Blood. 2010;116:1919-1923.
186. TiacciE, Trifonov V, Schiavoni G, et al. BRAF mutations in hairy-cell leukemia. N Engl J Med. 2011;364:2305-2315.
187. Berres ML et al, 2014.
188. Héritier S, Emile JF, Barkaoui MA et al. BRAF Mutation Correlates With High-Risk Langerhans Cell Histiocytosis and Increased Resistance to First-Line Therapy. J Clin Oncol.2016;34:3023-3030.
189. Montagut C e Settleman J, 2009.
190. Laman JD,Leenen PJ, Annels NE, et al. Langerhans-cell histiocytosis 'insight into DC biology'. Trends Immunol. 2003;24:190-196.
191. Stålemark H, Laurencikas E, Karis J, Gavhed D, Fadeel B, Henter JI. Incidence of Langerhans cell histiocytosis in children: a population-based study. Pediatr Blood Cancer. 2008;51:76-81.
192. Lanzkowsky P. Manual of Pediatric Hematology and Oncology.
193. Caputo R: A Text Atlas of Histiocytic Syndromes. Informa HealthCare; 1998.
194. Caux C, Dezutter-Dambuyant C, Schmitt D, Banchereau J: GM-CSF and TNF-alpha cooperate in the generation of dendritic Langerhans cells. Nature 1992, 360(6401):258-261.
195. Churg A, Tai H, Coulthard T, Wang R, Wright JL: Cigarette smoke drives small airway remodeling by induction of growth factors in the airway wall. Am J Respir Crit Care Med 2006, 174(12):1327-1334.
196. Letterio JJ, Roberts AB: Regulation of immune responses by TGF-beta. Annu Rev Immunol 1998, 16:137-161.
197. Mogulkoc N, Veral A, Bishop PW, Bayindir U, Pickering CA, Egan JJ: Pulmonary Langerhans' cell histiocytosis: radiologic resolution following

smoking cessation. Chest 1999, 115(5):1452-1455.
198. Lo WC, et al: Isolated adult Langerhans' cell histiocytosis in cervical lymphnodes: should it be treated? J Laryngol Otol 2009, 123(9):1055–1057.
199. Szturz P, et al: [Lymphoma-like course in aggressive adult multisystem Langerhans cell histiocytosis and the benefit of PET/CT imaging in evaluation of diffuse metabolic activity of lung parenchyma]. Vnitr Lek 2010, 56(11):1177–1193.
200. Singhi AD, Montgomery EA: Gastrointestinal tract langerhans cell histiocytosis: A clinicopathologic study of 12 patients. Am J Surg Pathol 2011, 35(2):305–310.
201. P. Makras, K. I. Alexandraki, G. P. Chrousos, A. B. Grossman, andG. A.Kaltsas, "Endocrinemanifestations in Langerhans cell histiocytosis," Trends in Endocrinology & Metabolism, vol. 18, no. 6, pp. 252–257, 2007.
202. Ematologia - Dipartimento di Biotecnologie Cellulari ed Ematologia - Università Sapienza Roma.
203. Karel Smetana K, Mericka O, et al. Diagnostic relevance of Langherin detection in cells from bronchoalveolar lavage of patients with pulmonary Langerhans cell, sarcoidosis and idiopathic pulmonary fibrosis. Virchows Arch 2004; 444: 171–174.
204. Agarwal KK, Seth R, Behra A, et al. 18F-Fluorodeoxyglucose PET/CT in Langerhans cell histiocytosis: spectrum of manifestations. Jpn J Radiol. 2016;34:267-276.
205. Harari S, Torre O, Cassandro R, et al. Bronchoscopic diagnosis of Langerhans cell histiocytosis and lymphangioleiomyomatosis. Respir Med 2012; 106: 1286–1292.
206. Minkov M, Steiner M, Pötschger U, et al. Reactivations in multisystem Langerhans cell histiocytosis: data of the international LCH registry. J Pediatr. 2008;153:700-705.
207. Abraham A, Alsultan A, Jeng M et al. Clofarabine Salvage therapy for refractory high risk Langerhans cell Histiocytosis. Pediatr Blood Cancer. 2013;60:E19-22.
208. Rosso DA, Amaral D, Latella A, et al. Reduced doses of cladribine and cytarabine regimen was effective and well tolerated in patients with refractory-risk multisystem Langerhans cell histiocytosis. British J Hematol. 2016;172:287-290.
209. Friedman PJ, Liebow AA, Sokoloff J: Eosinophilic granuloma of lung Clinical aspects of primary histiocytosis in the adult. Med (Baltimore) 1981, 60(6):385-396.
210. Girschikofsky M et al, 2013.
211. McClain KL. Drug therapy for the treatment of Langerhans cell histiocytosis. Expert Opin Pharmacother. 2005,6:2435-2441.
212. Appel S, Boehmler AM, Grünebach F, et al. Imatinib mesylate affects the development and function of dendritic cells generated from CD34+ peripheral blood progenitor cells. Blood 2004;103:538–544.
213. Haroche J, Cohen-Aubart F, Emile JF, et al. Reproducible and sustained efficacy of targeted therapy with vemurafenib in patients with BRAFV600E-Mutated Erdheim-Chester disease. J Clin Oncol. 2015;33:411-418.
214. Harari S, Torre O, Moss J. Lymphangioleiomyomatosis: what do we know and what are we looking for? Eur Respir Rev 2011; 20: 34–44.
215. Franz DN, Brody A, Meyer C, et al. Mutational and radiographic analysis of

pulmonary disease consistent with lymphangioleiomyomatosis and micronodular pneumocyte hyperplasia in women with tuberous sclerosis. Am J Respir Crit Care Med 2001; 164: 661–666.
216. Sparagana SP, Roach ES. Tuberous sclerosis complex. Curr Opin Neurol 2000; 13: 115–119.
217. Carsillo T, Astrinidis A, Henske EP. Mutations in the tuberous sclerosis complex gene TSC2 are a cause of sporadic pulmonary lymphangioleiomyomatosis. Proc Natl Acad Sci USA 2000; 97: 6085–6090.
218. Yu J, Astrinidis A, Henske EP. Chromosome 16 loss of heterozygosity in tuberous sclerosis and sporadic lymphangiomyomatosis. Am J Respir Crit Care Med 2001; 164: 1537–1540.
219. Sato T, Seyama K, Fujii H, et al. Mutation analysis of the TSC1 and TSC2 genes in Japanese patients with pulmonary lymphangioleiomyomatosis. J Hum Genet 2002; 47: 20–28.
220. Schmelzle T, Hall MN. TOR, a central controller of cell growth. Cell 2000; 103: 253–262.
221. Inoki K, Li Y, Xu T, et al. Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. Genes Dev 2003; 17: 1829–1834.
222. Saucedo LJ, Gao X, Chiarelli DA, et al. Rheb promotes cell growth as a component of the insulin/TOR signaling network. Nat Cell Biol 2003; 5: 566–571.
223. Matsumoto Y, Horiba K, Usuki J, et al. Markers of cell proliferation and expression of melanosomal antigen in lymphangioleiomyomatosis. Am J Respir Cell Mol Biol 1999; 21: 327–336.
224. Zhe X, Schuger L. Combined smooth muscle and melanocytic differentiation in lymphangioleiomyomatosis. J Histochem Cytochem 2004; 52: 1537–1542.
225. Martignoni G, Pea M, Reghellin D, et al. Molecular pathology of lymphangioleiomyomatosis and other perivascular epithelioid cell tumors. Arch Pathol Lab Med 2010; 134: 33–40.
226. Inoki K, Corradetti MN, Guan KL. Dysregulation of the TSC-mTOR pathway in human disease. Nat Genet 2005; 37: 19–24.
227. Karbowniczek M, Astrinidis A, Balsara BR, et al. Recurrent lymphangiomyomatosis after transplantation: genetic analyses reveal a metastatic mechanism. Am J Respir Crit Care Med 2003; 167: 976–982.
228. Karbowniczek M, Yu J, Henske EP. Renal angiomyolipomas from patients with sporadic lymphangiomyomatosis contain both neoplastic and non-neoplastic vascular structures. Am J Pathol 2003; 162: 491–500.
229. Crooks DM, Pacheco-Rodriguez G, DeCastro RM, et al. Molecular and genetic analysis of disseminated neoplastic cells in lymphangioleiomyomatosis. Proc Natl Acad Sci USA 2004; 101: 17462–17467.
230. Cai X, Pacheco-Rodriguez G, Fan QY, et al. Phenotypic characterization of disseminated cells with TSC2 loss of heterozygosity in patients with lymphangioleiomyomatosis. Am J Respir Crit Care Med 2010; 182: 1410–1418.
231. Pacheco-Rodriguez G, Steagall WK, Crooks DM, et al. TSC2 loss in lymphangioleiomyomatosis cells correlated with expression of CD44v6, a molecular determinant of metastasis. Cancer Res 2007; 67: 10573–10581.
232. Boehlke C, Kotsis F, Patel V, et al. Primary cilia regulate mTORC1 activity

and cell size through Lkb1. Nat Cell Biol 2010; 12: 1115–1122.
233. Goncharova EA, Goncharov DA, Lim PN, et al. Modulation of cell migration and invasiveness by tumor suppressor TSC2 in lymphangioleiomyomatosis. Am J Respir Cell Mol Biol 2006; 34: 473–480.
234. Kumasaka T, Seyama K, Mitani K, et al. Lymphangiogenesis-mediated shedding of LAM cell clusters as a mechanism for dissemination in lymphangioleiomyomatosis. Am J Surg Pathol 2005; 29: 1356–1366.
235. Chilosi M, Pea M, Martignoni G, et al. Cathepsin-K expression in pulmonary lymphangioleiomyomatosis. Mod Pathol 2009; 22: 161–166.
236. Harari S et al, ERJ 2015.
237. Hayashida M, Respirology 2013.
238. Johnson SR et al, ERJ 2010.
239. Seyama K, Kumasaka T, Souma S, et al. Vascular endothelial growth factor-D is increased in serum of patients with lymphangioleiomyomatosis. Lymphat Res Biol 2006; 4: 143–152.
240. Young LR, Inoue Y, McCormack FX. Diagnostic potential of serum VEGF-D for lymphangioleiomyomatosis. N Engl J Med 2008; 358: 199–200.
241. Glasgow CG, Avila NA, Lin JP, et al. Serum vascular endothelial growth factor-D levels in patients with lymphangioleiomyomatosis reflect lymphatic involvement. Chest 2009; 135: 1293–1300.
242. Young LR, Vandyke R, Gulleman PM, et al. Serum vascular endothelial growth factor-D prospectively distinguishes lymphangioleiomyomatosis from other diseases. Chest 2010; 138: 674–681.
243. Radzikowska E, Jaguś P, Skoczylas A, et al. Role of serum vascular endothelial growth factor D in discrimination of patients with polycystic lung diseases. Pol Arch Med Wewn 2013; 123: 533–538.
244. McCormack FX, Gupta N, Finlay GR, et al. Official American Thoracic Society/Japanese Respiratory Society Clinical Practice Guidelines: Lymphangioleiomyomatosis Diagnosis and Management. Am J Respir Crit Care Med 2016; 194: 748–761.
245. Johnson SR, Cordier JF, Lazor R, et al. European Respiratory Society guidelines for the diagnosis and management of lymphangioleiomyomatosis. Eur Respir J 2010; 35: 14–26.
246. Young L, Lee HS, Inoue Y, et al. Serum VEGF-D a concentration as a biomarker of lymphangioleiomyomatosisvseverity and treatment response: a prospective analysis of the Multicenter International Lymphangioleiomyomatosis Efficacy of Sirolimus (MILES) trial. Lancet Respir Med 2013; 1: 445–452.
247. Radzikowska E, Jaguś P, Sobiecka M, et al. Correlation of serum vascular endothelial growth factor-D concentration with clinical presentation and course of lymphangioleiomyomatosis. Respir Med 2015; 109: 1469–1475.
248. Ji RC. Lymphatic endothelial cells, lymphangiogenesis, and extracellular matrix. Lymphat Res Biol 2006; 4: 83–100.
249. Matsui K, Takeda K, Yu Z-X, et al. Role for activation of matrix metalloproteinases in the pathogenesis of pulmonary lymphangioleiomyomatosis. Arch Pathol Lab Med 2000; 124: 267–275.
250. Zhe X, Yang Y, Jakkaraju S, et al. Tissue inhibitor of metalloproteinase-3 downregulation in lymphangioleiomyomatosis. Am J Respir Cell Mol Biol 2003; 28: 504–511.
251. Krymskaya VP, Shipley JM. Lymphangioleiomyomatosis: a complex tale of

serum response factor-mediated tissue inhibitor of metalloproteinase-3 regulation. Am J Respir Cell Mol Biol 2003; 28: 546–550.
252. Lamb RF, Roy C, Diefenbach TJ, et al. The TSC1 tumour suppressor hamartin regulates cell adhesion through ERM proteins and the GTPase Rho. Nat Cell Biol 2000; 2: 281–287.
253. Moses MA, Wiederschain D, Loughlin KR, et al. Increased incidence of matrix metalloproteinases in urine of cancer patients. Cancer Res 1998; 58: 1395–1399.
254. Roy R, Yang J, Moses MA. Matrix metalloproteinases as novel biomarkers and potential therapeutic targets in human cancer. J Clin Oncol 2009; 27: 5287–5297.
255. Moses MA, Harper J, Folkman J. Doxycycline treatment for lymphangioleiomyomatosis with urinary monitoring for MMPs. N Engl J Med 2006; 324: 2621–2622.
256. Ryu JH, Moss J, Beck GJ, et al. The NHLBI lymphangioleiomyomatosis registry: characteristics of 230 patients at enrollment. Am J Respir Crit Care Med 2006; 173: 105–111.
257. Cohen MM, Pollock-BarZiv S, Johnson SR. Emerging clinical picture of lymphangioleiomyomatosis. Thorax 2005; 60: 875–879.
258. Taveira-DaSilva AM, Stylianou MP, Hedin CJ, et al. Maximal oxygen uptake and severity of disease in lymphangioleiomyomatosis. Am J Respir Crit Care Med 2003; 168: 1427–1431.
259. Hayashida M, Seyama K, Inoue Y, et al. The epidemiology of lymphangioleiomyomatosis in Japan: a nationwide cross-sectional study of presenting features and prognostic factors. Respirology 2007; 12: 523–530.
260. Avila NA, Chen CC, Chu SC, et al. Pulmonary lymphangioleiomyomatosis: correlation of ventilation-perfusion scintigraphy, chest radiography, and CT with pulmonary function tests. Radiology 2000; 214: 441–446.
261. Paciocco G, Uslenghi E, Bianchi A, et al. Diffuse cystic lung diseases: correlation between radiologic and functional status. Chest 2004; 125: 135–142.
262. Chang WY, Cane JL, Kumaran M, et al. A 2-year randomised placebo-controlled trial of doxycycline for lymphangioleiomyomatosis. Eur Respir J 2014; 43: 1114–1123.
263. Baldi BG, Freitas CS, Araujo MS, et al. Clinical course and characterisation of lymphangioleiomyomatosis in a Brazilian reference centre. Sarcoidosis Vasc Diffuse Lung Dis 2014; 31: 129–135.
264. McCormack FX, Inoue Y, Moss J, et al. Efficacy and safety of sirolimus in lymphangioleiomyomatosis. N Engl J Med 2011; 364: 1595–1606.
265. Baldi BG, de Albuquerque AL, Pimenta SP, et al. A pilot study assessing the effect of bronchodilator on dynamic hyperinflation in LAM. Respir Med 2013; 107: 1773–1780.
266. Krymskaya VP, Goncharova EA. PI3K/mTORC1 activation in hamartoma syndromes: therapeutic prospects. Cell Cycle 2009; 8: 403–413.
267. Bissler JJ, McCormack FX, Young LR, et al. Sirolimus for angiomyolipoma in tuberous sclerosis complex or lymphangioleiomyomatosis. N Engl J Med 2008; 358: 140–151.
268. Taveira-DaSilva AM, Hathaway O, Stylianou M, et al. Changes in lung function and chylous effusions in patients with lymphangioleiomyomatosis treated with sirolimus. Ann Intern Med 2011; 154: 797–805.

269. Ando K, Kurihara M, Kataoka H, et al. Efficacy and safety of low-dose sirolimus for treatment of lymphangioleiomyomatosis. Respir Investig 2013; 51: 175–183.
270. Franz DN, Belousova E, Sparagana S, et al. Efficacy and safety of everolimus for subependymal giant cell astrocytomas associated with tuberous sclerosis complex (EXIST-1): a multicentre, randomised, placebo-controlled phase 3 trial. Lancet 2013; 381: 125–132.
271. Bissler JJ, Kingswood JC, Radzikowska E, et al. Everolimus for angiomyolipoma associated with tuberous sclerosis complex or sporadic lymphangioleiomyomatosis (EXIST-2): a multicentre, randomised, double-blind, placebo-controlled trial. Lancet 2013; 381: 817–824.
272. Goldberg HJ, Harari S, Cottin V, et al. Everolimus for the treatment of lymphangioleiomyomatosis: a phase II study. Eur Respir J 2015; 46: 783–794.
273. Adamson D, Heinrichs WL, Raybin DM, et al. Successful treatment of pulmonary lymphangiomyomatosis with oophorectomy and progesterone. Am Rev Respir Dis 1985; 132: 916–921.
274. Baldi BG, Medeiros Junior P, Pimenta SP, et al. Evolution of pulmonary function after treatment with goserelin in patients with lymphangioleiomyomatosis. J Bras Pneumol 2011; 37: 375–379.
275. El-Chemaly S, Taveira-Dasilva A, Goldberg HJ, et al. Sirolimus and Autophagy Inhibition in Lymphangioleiomyomatosis: Results of a Phase I Clinical Trial. Chest 2017; 151: 1302–1310.
276. Goncharova EA, Goncharova DA, Li H, et al. mTORC2 is required for proliferation and survival of TSC2-null cells. Mol Cell Biol 2011; 31: 2484–2498.
277. Taveira-DaSilva AM, Jones AM, Julien-Williams PJ, et al. Retrospective review of combined sirolimus and simvastatin therapy in lymphangioleiomyomatosis. Chest;2015; 147: 180–187.
278. Grunewald, J. & Eklund, A. Sex- specific manifestations of Lofgren's syndrome. Am. J. Respir. Crit. Care Med. 2007; 175, 40–44.
279. Löfgren, S. Erythema nodosum: studies on etiology and pathogenesis in 185 adult cases. Acta Med. Scand. 1946; 124, 1–197.
280. Johan Grunewald, Jan C. Grutters, Elizabeth V. Arkema et al. Sarcoidosis. Primer (2019).
281. Dubaniewicz, A. Mycobacterium tuberculosis heat shock proteins and autoimmunity in sarcoidosis. Autoimmun. Rev. 2010; 9, 419–424.
282. Judson, M. A., Boan, A. D. & Lackland, D. T. The clinical course of sarcoidosis: presentation, diagnosis, and treatment in a large white and black cohort in the United States. Sarcoidosis Vasc. Diffuse Lung Dis.2012; 29, 119–127.
283. Arkema, E. V., Grunewald, J., Kullberg, S., Eklund, A. & Askling, J. Sarcoidosis incidence and prevalence: a nationwide register- based assessment in Sweden. Eur. Respir. J. 2016; 48, 1690–1699.
284. Byg, K. E., Milman, N. & Hansen, S. Sarcoidosis in Denmark 1980–1994. A registry- based incidence study comprising 5536 patients. Sarcoidosis Vasc. Diffuse Lung Dis. 2003; 20, 46–52.
285. Milman, N. & Selroos, O. Pulmonary sarcoidosis in the Nordic countries 1950–1982. Epidemiology and clinical picture. Sarcoidosis 7, 50–57 (1990).

286. Baughman, R. P. et al. Sarcoidosis in America. Analysis based on health care use. Ann. Am. Thorac. Soc. 2016; 13, 1244–1252.
287. Cozier, Y. C. et al. Sarcoidosis in black women in the United States: data from the Black Women's Health Study. Chest 139, 2011; 144–150.
288. Dumas, O., Abramovitz, L., Wiley, A. S., Cozier, Y. C. & Camargo, C. A. Jr Epidemiology of sarcoidosis in a prospective cohort study of U.S. women. Ann. Am. Thorac. Soc. 2016; 13, 67–71.
289. Morimoto, T. et al. Epidemiology of sarcoidosis in Japan. Eur. Respir. J. 2008;31, 372–379.
290. Yoon, H. Y., Kim, H. M., Kim, Y. J. & Song, J. W. Prevalence and incidence of sarcoidosis in Korea: a nationwide population- based study. Respir. Res. 2018;19, 158.
291. Beghe, D. et al. Sarcoidosis in an Italian province. Prevalence and environmental risk factors. PLOS ONE 12, 2017; e0176859.
292. Kowalska, M., Niewiadomska, E. & Zejda, J. E. Epidemiology of sarcoidosis recorded in 2006–2010 in the Silesian voivodeship on the basis of routine medical reporting. Ann. Agric. Environ. Med. 2014; 21, 55–58.
293. Deubelbeiss, U., Gemperli, A., Schindler, C., Baty, F. & Brutsche, M. H. Prevalence of sarcoidosis in Switzerland is associated with environmental factors. Eur. Respir. J.2010; 35, 1088–1097.
294. Grunewald, J. & Eklund, A. Lofgren's syndrome: human leukocyte antigen strongly influences the disease course. Am. J. Respir. Crit. Care Med. 2009; 179, 307–312.
295. Rivera, N. V. et al. High- density genetic mapping identifies new susceptibility variants in sarcoidosis phenotypes and shows genomic- driven phenotypic differences. Am. J. Respir. Crit. Care Med. 2016; 193, 1008–1022.
296. Schurmann, M. et al. Results from a genome- wide search for predisposing genes in sarcoidosis. Am. J. Respir. Crit. Care Med. 2001;164, 840–846.
297. Grunewald, J., Spagnolo, P., Wahlstrom, J. & Eklund, A. Immunogenetics of disease- causing inflammation in sarcoidosis. Clin. Rev. Allergy Immunol. 2015; 49, 19–35.
298. Rybicki, B. A. et al. Familial aggregation of sarcoidosis. A case- control etiologic study of sarcoidosis (ACCESS). Am. J. Respir. Crit. Care Med. 2001; 164, 2085–2091.
299. Rossides, M. et al. Familial aggregation and heritability of sarcoidosis: a Swedish nested case- control study. Eur. Respir. J. 2018; 52, 1800385.
300. Sverrild, A. et al. Heredity in sarcoidosis: a registry based twin study. Thorax 2008; 63, 894–896.
301. Headings, V. E., Weston, D., Young, R. C. Jr & Hackney, R. L. Jr Familial sarcoidosis with multiple occurrences in eleven families: a possible mechanism of inheritance. Ann. NY Acad. Sci. 1976; 278, 377–385.
302. Newman, L. S. et al. A case control etiologic study of sarcoidosis: environmental and occupational risk factors. Am. J. Respir. Crit. Care Med. 2004; 170, 1324–1330.
303. Vihlborg, P., Bryngelsson, I. L., Andersson, L. & Graff, P. Risk of sarcoidosis and seropositive rheumatoid arthritis from occupational silica exposure in Swedish iron foundries: a retrospective cohort study. BMJ Open 7, 2017; e016839.

304. Kern, D. G., Neill, M. A., Wrenn, D. S. & Varone, J. C. Investigation of a unique time- space cluster of sarcoidosis in firefighters. Am. Rev. Respir. Dis. 1993;148, 974–980.
305. Chiang, C. H. & Lai, F. J. Sarcoidosis on the injection sites following treatment of interferon- alpha and ribavirin for hepatitis C. J. Formos. Med. Assoc. 2014; 113, 981–982.
306. Jeon, E. K. et al. First reported case of interferon alpha- induced sarcoidosis in an Asian patient with malignant melanoma. Asia Pac. J. Clin. Oncol. 2016; 12, e347–e349.
307. Viana de Andrade, A. C. et al. Development of systemic sarcoidosis and xanthoma planum during multiple sclerosis treatment with interferon- beta 1a: case report. Int. J. Dermatol. 2015; 54, e140–e145.
308. Berthod, G. et al. Pulmonary sarcoid- like granulomatosis induced by ipilimumab. J. Clin. Oncol. 2012; 30, e156–e159.
309. Spain, L., Diem, S. & Larkin, J. Management of toxicities of immune checkpoint inhibitors. Cancer Treat. Rev. 2016; 44, 51–60.
310. Abdel- Wahab, N., Shah, M. & Suarez- Almazor, M. E. Adverse events associated with immune checkpoint blockade in patients with cancer: a systematic review of case reports. PLOS ONE 11, 2016; e0160221.
311. Heyll, A. et al. Possible transmission of sarcoidosis via allogeneic bone marrow transplantation. Bone Marrow Transplant. 1994; 14, 161–164.
312. Gerke, A. K., Judson, M. A., Cozier, Y. C., Culver, D. A. & Koth, L. L. Disease burden and variability in sarcoidosis. Ann. Am. Thorac. Soc. 2017; 14, S421–S428.
313. Gribbin, J. et al. Incidence and mortality of idiopathic pulmonary fibrosis and sarcoidosis in the UK. Thorax 61, 2006; 980–985.
314. Park, J. E. et al. Prevalence, incidence, and mortality of sarcoidosis in Korea, 2003-2015: A nationwide population- based study. Respir. Med. 2018; 144S, S28–S34.
315. Rossides, M. et al. Sarcoidosis mortality in Sweden: a bennpopulation-based cohort study. Eur. Respir. J. 2018; 51, 1701815.
316. Tukey, M. H. et al. Mortality among African American women with sarcoidosis: data from the Black Women's Health Study. Sarcoidosis Vasc. Diffuse Lung Dis. 2013; 30, 128–133.
317. Ungprasert, P., Crowson, C. S. & Matteson, E. L. Sarcoidosis increases risk of hospitalized infection. A population- based study, 1976–2013. Ann. Am. Thorac. Soc. 2017;14, 676–681.
318. Crawshaw, A. P., Wotton, C. J., Yeates, D. G., Goldacre, M. J. & Ho, L. P. Evidence for association between sarcoidosis and pulmonary embolism from 35-year record linkage study. Thorax 66, 2011;447–448.
319. Ungprasert, P., Crowson, C. S. & Matteson, E. L. Risk of cardiovascular disease among patients with sarcoidosis: a population- based retrospective cohort study, 1976–2013. Eur. Respir. J. 2017; 49, 1601290.
320. Yaqoob, Z. J., Al- Kindi, S. G. & Zein, J. G. Sarcoidosis and risk of VTE: validation with big data. Chest 2017;151- 1398–1399.
321. Wu, C. H. et al. Comorbid autoimmune diseases in patients with sarcoidosis: a nationwide case- control study in Taiwan. J. Dermatol. 2017; 44, 423–430.
322. Bonifazi, M. et al. Sarcoidosis and cancer risk: systematic review and meta-analysis of observational studies. Chest 2015; 147, 778–791.

323. Hunninghake, G. W. et al. ATS/ERS/WASOG statement on sarcoidosis. American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders. Sarcoidosis Vasc. Diffuse Lung Dis. 1999; 16, 149–173.
324. Facco, M. et al. Sarcoidosis is a Th1/Th17 multisystem disorder. Thorax 2011; 66, 144–150.
325. Ramstein, J. et al. Interferon- gamma-producing Th17.1 Cells are increased in sarcoidosis and more prevalent than Th1 cells. Am. J. Respir. Crit. Care Med. 2016; 193, 1281–1291.
326. Broos, C. E. et al. Increased T- helper 17.1 cells in sarcoidosis mediastinal lymph nodes. Eur. Respir. J. 2018; 51, 1701124.
327. Ostadkarampour, M. et al. Higher levels of interleukin IL-17 and antigen-specific IL-17 responses in pulmonary sarcoidosis patients with Lofgren's syndrome. Clin. Exp. Immunol. 2014; 178, 342–352.
328. Kaiser, Y. et al. Expanded lung T- bet+RORgammaT+ CD4+ T cells in sarcoidosis patients with a favourable disease phenotype. Eur. Respir. J. 2016; 48, 484–494.
329. Chen, E. S. & Moller, D. R. Sarcoidosis—scientific progress and clinical challenges. Nat. Rev. Rheumatol. 2011; 7, 457–467.
330. Chen, E. S. et al. Serum amyloid A regulates granulomatous inflammation in sarcoidosis through Toll- like receptor-2. Am. J. Respir. Crit. Care Med.2010; 181, 360–373.
331. Chen, E. S. & Moller, D. R. Etiology of sarcoidosis. Clin. Chest Med. 2008; 29, 365–377.
332. Valeyre, D. et al. Sarcoidosis. Lancet 2014; 383, 1155–1167.
333. Demirkok, S. S., Basaranoglu, M. & Akbilgic, O. Seasonal variation of the onset of presentations in stage 1 sarcoidosis. Int. J. Clin. Pract. 2006; 60, 1443–1450.
334. Glennas, A. et al. Acute sarcoid arthritis: occurrence, seasonal onset, clinical features and outcome. Br. J. Rheumatol. 1995; 34, 45–50.
335. Wilsher, M. L. Seasonal clustering of sarcoidosis presenting with erythema nodosum. Eur. Respir. J. 1998; 12, 1197–1199.
336. Heerfordt, C. Uber eine febris uveo- parotidea subchronica. Albrecht Von Graefes Arch. Ophthalmol. 1909; 70, 254–258.
337. Abehsera, M. et al. Sarcoidosis with pulmonary fibrosis: CT patterns and correlation with pulmonary function. Am. J. Roentgenol. 2000; 174, 1751–1757.
338. Sayah, D. M., Bradfield, J. S., Moriarty, J. M., Belperio, J. A. & Lynch, J. P. 3rd Cardiac involvement in sarcoidosis: evolving concepts in diagnosis and treatment. Semin. Respir. Crit. Care Med. 2017; 38, 477–498.
339. Birnie, D. H., Nery, P. B., Ha, A. C. & Beanlands, R. S. Cardiac sarcoidosis. J. Am. Coll. Cardiol. 2016; 68, 411–421.
340. Chau, E. M., Fan, K. Y. & Chow, W. H. Cardiac sarcoidosis: a potentially fatal but treatable form of infiltrative heart disease. Hong Kong Med. J. 2006; 12, 65–67.
341. Rabin, D. L., Richardson, M. S., Stein, S. R. & Yeager, H. Jr Sarcoidosis severity and socioeconomic status. Eur. Respir. J. 2001;18, 499–506.
342. Culver, D. A., Ribeiro Neto, M. L., Moss, B. P. & Willis, M. A. Neurosarcoidosis. Semin. Respir. Crit. Care Med. 2017; 38, 499–513.

343. Tabuena, R. P. et al. Diabetes insipidus from neurosarcoidosis: long- term follow- up for more than eight years. Intern. Med. 2004; 43, 960–966.
344. Hoitsma, E. et al. Small fibre neuropathy in sarcoidosis. Lancet 2002; 359, 2085–2086.
345. Costabel, U. & Hunninghake, G. W. ATS/ERS/WASOG statement on sarcoidosis. Sarcoidosis Statement Committee. American Thoracic Society. European Respiratory Society. World Association for Sarcoidosis and Other Granulomatous Disorders. Eur. Respir. J. 1999; 14, 735–737.
346. Bargagli, E. & Prasse, A. Sarcoidosis: a review for the internist. Intern. Emerg. Med. 2018; 13, 325–331.
347. Judson, M. A. et al. Two year prognosis of sarcoidosis: the ACCESS experience. Sarcoidosis Vasc. Diffuse Lung Dis. 2003; 20, 204–211.
348. Gilbert, S., Steinbrech, D. S., Landas, S. K. & Hunninghake, G. W. Amounts of angiotensinconverting enzyme mRNA reflect the burden of granulomas in granulomatous lung disease. Am. Rev. Respir. Dis. 1993; 148, 483–486.
349. Huitema, M. P. et al. Pulmonary artery diameter to predict pulmonary hypertension in pulmonary sarcoidosis. Eur. Respir. J. 2016; 47, 673–676.
350. Soussan, M. et al. Clinical value of a high- fat and low carbohydrate diet before FDG- PET/CT for evaluation of patients with suspected cardiac sarcoidosis. J. Nucl. Cardiol. 2013; 20, 120–127.
351. Goktalay, T. et al. The role of endobronchial biopsy in the diagnosis of pulmonary sarcoidosis. Turk. Thorac. J. 2016; 17, 22–27.
352. Heron, M. et al. Evaluation of CD103 as a cellular marker for the diagnosis of pulmonary sarcoidosis. Clin. Immunol. 2008; 126, 338–344.
353. Bonifazi, M. et al. Conventional versus ultrasound guided transbronchial needle aspiration for the diagnosis of hilar/mediastinal lymphadenopathies: a randomized controlled trial. Respiration 2017; 94, 216–223.
354. Saygin, D., Karunamurthy, A., English, J. & Aggarwal, R. Tattoo reaction as a presenting manifestation of systemic sarcoidosis. Rheumatology 2018; 58, 927.
355. Baughman, R. P. et al. Defining the clinical outcome status (COS) in sarcoidosis: results of WASOG Task Force. Sarcoidosis Vasc. Diffuse Lung Dis. 2011; 28, 56–64.
356. Dekhuijzen, P. N. & Decramer, M. Steroid- induced myopathy and its significance to respiratory disease: a known disease rediscovered. Eur. Respir. J. 1992; 5, 997–1003.
357. James, W. E. & Baughman, R. Treatment of sarcoidosis: grading the evidence. Expert Rev. Clin. Pharmacol. 2018; 11, 677–687.
358. Hunninghake, G. W. et al. Outcome of the treatment of sarcoidosis. Am. J. Respir. Crit. Care Med. 1994;149, 893–898.
359. Gibson, G. J. et al. British Thoracic Society Sarcoidosis study: effects of long term corticosteroid treatment. Thorax 1996; 51, 238–247.
360. Drent, M. et al. Does the cellular bronchoalveolar lavage fluid profile reflect the severity of sarcoidosis? Eur. Respir. J. 1999; 13, 1338–1344.
361. Ziegenhagen, M. W., Rothe, M. E., Schlaak, M. & Müller- Quernheim, J. Bronchoalveolar and serological parameters reflecting the severity of sarcoidosis. Eur. Respir. J. 2003; 21, 407–413.

362. Broos, C. E. et al. No evidence found for an association between prednisone dose and FVC change in newlytreated pulmonary sarcoidosis. Respir. Med. 2018; 138S, S31–S37.
363. Baughman, R. P., Winget, D. B. & Lower, E. E. Methotrexate is steroid sparing in acute sarcoidosis: results of a double blind, randomized trial. Sarcoidosis Vasc. Diffuse Lung Dis. 2000; 17, 60–66.
364. Müller- Quernheim, J., Kienast, K., Held, M., Pfeifer, S. & Costabel, U. Treatment of chronic sarcoidosis with an azathioprine/prednisolone regimen. Eur. Respir. J. 1999; 14, 1117–1122.
365. Baughman, R. P., Janovcik, J., Ray, M., Sweiss, N. & Lower, E. E. Calcium and vitamin D metabolism in sarcoidosis. Sarcoidosis Vasc. Diffuse Lung Dis. 2013; 30, 113–120.
366. Burke, R. R., Rybicki, B. A. & Rao, D. S. Calcium and vitamin D in sarcoidosis: how to assess and manage. Semin. Respir. Crit. Care Med. 2010; 31, 474–484.
367. Baughman, R. P., Nunes, H., Sweiss, N. J. & Lower, E. E. Established and experimental medical therapy of pulmonary sarcoidosis. Eur. Respir. J. 2013; 41, 1424–1438.
368. Ziegenhagen, M. W., Rothe, M. E., Zissel, G. & Muller- Quernheim, J. Exaggerated TNFalpha release of alveolar macrophages in corticosteroid resistant sarcoidosis. Sarcoidosis Vasc. Diffuse Lung Dis. 2002; 19, 185–190.
369. Hostettler, K. E., Studler, U., Tamm, M. & Brutsche, M. H. Long- term treatment with infliximab in patients with sarcoidosis. Respiration 2012; 83, 218–224.
370. Shlobin, O. A. & Nathan, S. D. Management of endstage sarcoidosis: pulmonary hypertension and lung transplantation. Eur. Respir. J. 2012; 39, 1520–1533.
371. Elliot. D. Crouser, Lisa A. Maier, Kevin C. Wilson, Catherine A. Bonham, et al.Diagnosis and Detection of Sarcoidosis. An official American Thoracic Society Clinical Practice Guideline. American Journal Respiratory Critical Care Medicine 2020; Vol 201, Iss 8, pp e26-e51.
372. Baughman RP, Valeyre D, Korsten P, Mathioudakis AG, Wuyts WA, Wells A, et al. ERS clinical practice guidelines on treatment of sarcoidosis. Eur Respir J. 2021; 58:2004079.
373. Rahaghi FF, Baughman RP, Saketkoo LA, Sweiss NJ, Barney JB, Birring SS, et al. Delphi consensus recommendations for a treatment algorithm in pulmonary sarcoidosis. Eur Respir Rev. 2020; 29:190146.
374. Brito-Zeron P, Perez-Alvarez R, Pallares L, Retamozo S, Baughman RP, Ramos-Casals M, et al. Sarcoidosis: an update on current pharmacotherapy options and future directions. Expert Opin Pharmacother 2016: 17:2431–48.
375. Schutt AC, Bullington WM, Judson MA. Pharmacotherapy for pulmonary sarcoidosis: a Delphi consensus study. Respir Med. 2010; 104:717–23.
376. Baughman RP, Drent M, Kavuru M, Judson MA, Costabel U, du Bois R, et al. Infliximab therapy in patients with chronic sarcoidosis and pulmonary involvement. Am J Respir Crit Care Med 2006: 174:795–802.
377. Baughman RP, Shipley R, Desai S, Drent M, Judson MA, Costabel U, et al. Changes in chest roentgenogram of sarcoidosis patients during a clinical trial of infliximab therapy: comparison of different methods of evaluation. Chest 2009:136:526–35.

378. Denton CP, Khanna D. Systemic sclerosis. Lancet 2017; 390: 1685–99.
379. van den Hoogen F, Khanna D, Fransen J, et al. 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European League against Rheumatism collaborative initiative. Arthritis Rheum 2013; 65: 2737–47.
380. Tyndall AJ, Bannert B, Vonk M, et al. Causes and risk factors for death in systemic sclerosis: a study from the EULAR Scleroderma Trials and Research (EUSTAR) database. Ann Rheum Dis 2010; 69: 1809–15.
381. Benan M, Hande I, Gul O. The natural course of progressive systemic sclerosis patients with interstitial lung involvement. Clin Rheumatol 2007; 26: 349–54.
382. Wangkaew S, Euathrongchit J, Wattanawittawas P, Kasitanon N, Louthrenoo W. Incidence and predictors of interstitial lung disease (ILD) in Thai patients with early systemic sclerosis: inception cohort study. Mod Rheumatol 2016; 26: 588–93.
383. Mayes MD, Lacey JV Jr, Beebe-Dimmer J, et al. Prevalence, incidence, survival, and disease characteristics of systemic sclerosis in a large US population. Arthritis Rheum 2003; 48: 2246–55.
384. Jung E, Suh CH, Kim HA, Jung JY. Clinical characteristics of systemic sclerosis with interstitial lung disease. Arch Rheumatol 2018; 33: 322–27.
385. ck CJW, Renzoni EA. Genetic predictors of systemic sclerosisassociated interstitial lung disease: a review of recent literature. Eur J Hum Genet 2018; 26: 765–77.
386. Akter T, Silver RM, Bogatkevich GS. Recent advances in understanding the pathogenesis of scleroderma-interstitial lung disease. Curr Rheumatol Rep 2014; 16: 411.
387. Suliman YA, Dobrota R, Huscher D, et al. Brief report: pulmonary function tests: high rate of false-negative results in the early detection and screening of scleroderma-related interstitial lung disease. Arthritis Rheumatol 2015; 67: 3256–61.
388. Daimon T, Johkoh T, Honda O, et al. Nonspecific interstitial pneumonia associated with collagen vascular disease: analysis of CT features to distinguish the various types. Intern Med 2009; 48: 753–61.
389. Desai SR, Veeraraghavan S, Hansell DM, et al. CT features of lung disease in patients with systemic sclerosis: comparison with idiopathic pulmonary fibrosis and nonspecific interstitial pneumonia. Radiology 2004; 232: 560–67.
390. Bouros D, Wells AU, Nicholson AG, et al. Histopathologic subsets of fibrosing alveolitis in patients with systemic sclerosis and their relationship to outcome. Am J Respir Crit Care Med 2002;165: 1581–86.
391. Winstone TA, Assayag D, Wilcox PG, et al. Predictors of mortality and progression in scleroderma-associated interstitial lung disease: a systematic review. Chest 2014; 146: 422–36.
392. Santosa A, Tan CS, Teng GG, et al. Lung and gastrointestinal complications are leading causes of death in SCORE, a multi-ethnic Singapore systemic sclerosis cohort. Scand J Rheumatol 2016; 45: 499–506.
393. Hoyles RK, Ellis RW, Wellsbury J, et al. A multicenter, prospective, randomized, double-blind, placebo-controlled trial of corticosteroids and intravenous cyclophosphamide followed by oral azathioprine for the

treatment of pulmonary fibrosis in scleroderma. Arthritis Rheum 2006; 54: 3962–70.
394. Poormoghim H, Rezaei N, Sheidaie Z, et al. Systemic sclerosis: comparison of efficacy of oral cyclophosphamide and azathioprine on skin score and pulmonary involvement-a retrospective study. Rheumatol Int 2014; 34: 1691–99.
395. Daoussis D, Liossis SN, Tsamandas AC, et al. Experience with rituximab in scleroderma: results from a 1-year, proof-of-principle study. Rheumatology (Oxford) 2010; 49: 271–80.
396. Keating GM. Nintedanib: a review of its use in patients with idiopathic pulmonary fibrosis. Drugs 2015; 75: 1131–40.
397. Huang J, Beyer C, Palumbo-Zerr K, et al. Nintedanib inhibits fibroblast activation and ameliorates fibrosis in preclinical models of systemic sclerosis. Ann Rheum Dis 2016; 75: 883–90.
398. Distler O, Highland KB, Gahlemann M, et al. Nintedanib for systemic sclerosis-associated interstitial lung disease. N Engl J Med 2019; 380: 2518–28.
399. Flaherty KR, Wells AU, Cottin V, et al. Nintedanib in progressive fibrosing interstitial lung diseases. N Engl J Med 2019; 381: 1718–27.
400. Shah RJ, Boin F. Lung transplantation in patients with systemic sclerosis. Curr Rheumatol Rep 2017; 19: 23.
401. Khan IY, Singer LG, de Perrot M, et al. Survival after lung transplantation in systemic sclerosis: a systematic review. Respir Med 2013; 107: 2081–87.
402. Apostolos Perelas, Richard M. Silver, Andrea V. Arrossi, Kristin B. Highland. Systemic sclerosis-associated interstitial lung diseas. Lancet Resp Med 2020. S2213-2600(20)30012-6.
403. Katzenstein AL, Fiorelli RF. Nonspecific interstitial pneumonia/ fibrosis. Histologic features and clinical significance. Am. J. Surg. Pathol. 1994; 18: 136–47.
404. Arakawa H, Yamada H, Kurihara Y, Nakajima Y, Takeda A, Fukushima Y, Fujioka M. Nonspecific interstitial pneumonia associated with polymyositis and dermatomyositis: serial high resolution CT findings and functional correlation. Chest 2003; 123: 1096–103.
405. Yamadori I, Fujita J, Bandoh S, Tokuda M, Tanimoto Y, Kataoka M, Yamasaki Y, Yoshinouchi T, Ohtsuki Y, Ishida T. Non specific interstitial pneumonia as pulmonary involvement of primary Sjogren's syndrome. Rheumatol. Int. 2002; 22: 89–92.
406. Steele MP, Speer MC, Loyd JE, Brown KK, Herron A, Slifer SH, Burch LH, Wahidi MM, Phillips JA 3rd, Sporn TA et al. Clinical and pathologic features of familial interstitial pneumonia. Am. J. Respir. Crit. Care Med. 2005; 172: 1146–52.
407. Borie R, Kannengiesser C, Crestani B. Familial forms of nonspecific interstitial pneumonia/idiopathic pulmonary fibrosis: clinical course and genetic background. Curr.Opin. Pulm.Med. 2012; 18: 455–61.
408. Travis WD, Hunninghake G, King TE Jr, Lynch DA, Colby TV, Galvin JR, Brown KK, Chung MP, Cordier JF, du Bois RM et al. Idiopathic nonspecific interstitial pneumonia: report of an American Thoracic Society project. Am. J. Respir. Crit. Care Med. 2008; 177: 1338–47.
409. Fujita J, Ohtsuki Y, Yoshinouchi T, Yamadori I, Bandoh S, Tokuda M, Miyawaki H, Kishimoto N, Ishida T. Idiopathic non-specific interstitial

pneumonia: as an ‘autoimmune interstitial pneumonia. Respir.Med. 2005; 99: 234–40.
410. Cottin V, Donsbeck AV, Revel D, Loire R, Cordier JF. Nonspecific interstitial pneumonia. Individualization of a clinicopathologic entity in a series of 12 patients. Am. J.Respir. Crit.CareMed. 1998; 158: 1286–93.
411. Bjoraker JA, Ryu JH, Edwin MK, Myers JL, Tazelaar HD, Schroeder DR, Offord KP. Prognostic significance of histopathologic subsets in idiopathic pulmonary fibrosis. Am. J. Respir. Crit. Care Med. 1998; 157: 199–203.
412. Hamada K, Nagai S, Tanaka S, Handa T, Shigematsu M, Nagao T, Mishima M, Kitaichi M, Izumi T. Significance of pulmonary arterial pressure and diffusion capacity of the lung as prognosticator in patients with idiopathic pulmonary fibrosis. Chest 2007; 131: 650–6.
413. Nagai S, Kitaichi M, Itoh H, Nishimura K, Izumi T, Colby TV. Idiopathic nonspecific interstitial pneumonia/fibrosis: comparison with idiopathic pulmonary fibrosis and BOOP. Eur. Respir. J. 1998; 12: 1010–19.
414. Silva CI, Muller NL, Lynch DA, Curran-Everett D, Brown KK, Lee KS, Chung MP, Churg A. Chronic hypersensitivity pneumonitis: differentiation from idiopathic pulmonary fibrosis and nonspecific interstitial pneumonia by using thin-section CT. Radiology 2008; 246: 288–97.
415. Park IN, Jegal Y, KimDS, Do KH, Yoo B, Shim TS, Lim CM, Lee SD, Koh Y, KimWS et al. Clinical course and lung function change of idiopathic nonspecific interstitial pneumonia. Eur. Respir. J. 2009; 33: 68–76.
416. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, Colby TV, Cordier JF, Flaherty KR, Lasky JA et al. An official ATS/ ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am. J. Respir. Crit. Care Med. 2011; 183: 788–824.
417. Jegal Y, KimDS, Shim TS, Lim CM, Do Lee S, Koh Y, Kim WS, Kim WD, Lee JS, Travis WD et al. Physiology is a stronger predictor of survival than pathology in fibrotic interstitial pneumonia. Am. J. Respir. Crit. Care Med. 2005; 171: 639–44.
418. Park JH, Kim DS, Park IN, Jang SJ, Kitaichi M, Nicholson AG, Colby TV. Prognosis of fibrotic interstitial pneumonia: idiopathic versus collagen vascular disease-related subtypes. Am. J. Respir. Crit. Care Med. 2007; 175: 705–11.
419. Travis WD, Matsui K, Moss J, Ferrans VJ. Idiopathic nonspecific interstitial pneumonia: prognostic significance of cellular and fibrosing patterns: survival comparison with usual interstitial pneumonia and desquamative interstitial pneumonia. Am. J. Surg. Pathol. 2000; 24: 19–33.
420. Bradley B, Branley HM, Egan JJ, Greaves MS, Hansell DM, Harrison NK, Hirani N, Hubbard R, Lake F,Millar AB et al. Interstitial lung disease guideline: the British Thoracic Society in collaboration with the Thoracic Society of Australia and New Zealand and the Irish Thoracic Society. Thorax 2008; 63(Suppl.5): v1–58.
421. Suda T, Kono M, Nakamura Y, Enomoto N, Kaida Y, Fujisawa T, Imokawa S, Yasuda K, Hashizume H, Yokomura K et al. Distinct prognosis of idiopathic nonspecific interstitial pneumonia (NSIP) fulfilling criteria for undifferentiated connective tissue disease (UCTD). Respir.Med. 2010; 104: 1527–34.
422. Lee JY, Jin SM, Lee BJ, Chung DH, Jang BG, Park HS, Lee SM, Yim

JJ, Yang SC, Yoo CG et al. Treatment response and long term follow-up results of nonspecific interstitial pneumonia. J. Korean Med. Sci. 2012; 27: 661–7.
423. Xu W, Xiao Y, Liu H, Qin M, Zheng W, Shi J. Nonspecific interstitial pneumonia: clinical associations and outcomes. BMC Pulm. Med. 2014; 14: 175.
424. Kinder BW. Nonspecific interstitial pneumonia. Clin. Chest Med. 2012; 33: 111–21.
425. Corte TJ, Ellis R, Renzoni EA, Hansell DM, Nicholson AG, du Bois RM, Wells AU. Use of intravenous cyclophosphamide in known or suspected, advanced non-specific interstitial pneumonia. Sarcoidosis Vasc. Diffuse Lung Dis. 2009; 26: 132–8.
426. Patel H, Fellowes R, Coade S, Woo P. Human serum amyloid A has cytokine-like properties. Scand J Immunol. 1998 Oct;48(4):410-8.
427. Yamada T. Serum amyloid A (SAA): a concise review of biology, assay methods and clinical usefulness. Clin Chem Lab Med. 1999 Apr;37(4):381-8.
428. Westermark GT, Engstrom U, Westermark P. The N-terminal segment of protein AA determines its fibrillogenic property. Biochem Biophys Res Commun. 1992 Jan 15;182(1):27-33.
429. Jensen LE, Whitehead AS. Regulation of serum amyloid A protein expression during the acute-phase response. Biochem J. 1998 Sep 15;334 (Pt 3):489-503.
430. Dwulet FE, Wallace DK, Benson MD. Amino acid structures of multiple forms of amyloid-related serum protein SAA from a single individual. Biochemistry. 1988 Mar 8;27(5):1677-82.
431. Patel H, Bramall J, Waters H, De Beer MC, Woo P. Expression of recombinant human serum amyloid A in mammalian cells and demonstration of the region necessary for high-density lipoprotein binding and amyloid fibril formation by site-directed mutagenesis. Biochem J. 1996 Sep 15;318 (Pt 3):1041-9.
432. Urieli-Shoval S, Linke RP, Matzner Y. Expression and function of serum amyloid A, a major acute-phase protein, in normal and disease states. Curr Opin Hematol. 2000 Jan;7(1):64-9.
433. Kluve-Beckerman B, Yamada T, Hardwick J, Liepnieks JJ, Benson MD. Differential plasma clearance of murine acute-phase serum amyloid A proteins SAA1 and SAA2. Biochem J. 1997 Mar 1;322 (Pt 2):663-9.
434. Lavie G, Zucker-Franklin D, Franklin EC. Degradation of serum amyloid A protein by surface-associated enzymes of human blood monocytes. J Exp Med. 1978 Oct 1;148(4):1020-31.
435. Gollaher CJ, Bausserman LL. Hepatic catabolism of serum amyloid A during an acute phase response and chronic inflammation. Proc Soc Exp Biol Med. 1990 Jul;194(3):245-50.
436. F. Pallotti AB, G. Melzi d'Eril. La siero amiloide A: biologia ed applicazioni cliniche. Riv Med Lab - JLM. 2003;Vol. 4.
437. Brandwein SR, Medsger TA, Jr., Skinner M, Sipe JD, Rodnan GP, Cohen AS. Serum amyloid A protein concentration in progressive systemic sclerosis (scleroderma). Ann Rheum Dis. 1984 Aug;43(4):586-9.

438. Lakota K, Carns M, Podlusky S, Mrak-Poljsak K, Hinchcliff M, Lee J, et al. Serum amyloid A is a marker for pulmonary involvement in systemic sclerosis. PLoS One. 2015;10(1):e0110820.
439. Lis-Swiety A, Widuchowska M, Brzezinska-Wcislo L, Kucharz E. High acute phase protein levels correlate with pulmonary and skin involvement in patients with diffuse systemic sclerosis. J Int Med Res. 2018 Apr;46(4):1634-9.
440. Vallon R, Freuler F, Desta-Tsedu N, Robeva A, Dawson J, Wenner P, et al. Serum amyloid A (apoSAA) expression is up-regulated in rheumatoid arthritis and induces transcription of matrix metalloproteinases. J Immunol. 2001 Feb 15;166(4):2801-7.
441. O'Hara R, Murphy EP, Whitehead AS, FitzGerald O, Bresnihan B. Local expression of the serum amyloid A and formyl peptide receptor-like 1 genes in synovial tissue is associated with matrix metalloproteinase production in patients with inflammatory arthritis. Arthritis Rheum. 2004 Jun;50(6):1788-99.
442. Mullan RH, Bresnihan B. Disease-modifying anti-rheumatic drug therapy and structural damage in early rheumatoid arthritis. Clin Exp Rheumatol. 2003 Sep-Oct;21(5 Suppl 31):S158-64.
443. Mirza S, Clay RD, Koslow MA, Scanlon PD. COPD Guidelines: A Review of the 2018 GOLD Report. Mayo Clin Proc. 2018 Oct;93(10):1488-502.
444. Bozinovski S, Hutchinson A, Thompson M, Macgregor L, Black J, Giannakis E, et al. Serum amyloid a is a biomarker of acute exacerbations of chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2008 Feb 1;177(3):269-78.
445. Bozinovski S, Uddin M, Vlahos R, Thompson M, McQualter JL, Merritt AS, et al. Serum amyloid A opposes lipoxin A(4) to mediate glucocorticoid refractory lung inflammation in chronic obstructive pulmonary disease. Proc Natl Acad Sci U S A. 2012 Jan 17;109(3):935-40.
446. Svatikova A, Wolk R, Shamsuzzaman AS, Kara T, Olson EJ, Somers VK. Serum amyloid a in obstructive sleep apnea. Circulation. 2003 Sep 23;108(12):1451-4.
447. Strohl KP, Brown DB, Collop N, George C, Grunstein R, Han F, et al. An official American Thoracic Society Clinical Practice Guideline: sleep apnea, sleepiness, and driving risk in noncommercial drivers. An update of a 1994 Statement. Am J Respir Crit Care Med. 2013 Jun 1;187(11):1259-66.
448. Chen SS, Chen FP, Chen XJ. [Clinical implications of serum amyloid A level in patients with obstructive sleep apnea]. Nan Fang Yi Ke Da Xue Xue Bao. 2009 Aug;29(8):1599-601.
449. Bargagli E, Magi B, Olivieri C, Bianchi N, Landi C, Rottoli P. Analysis of serum amyloid A in sarcoidosis patients. Respir Med. 2011 May;105(5):775-80.
450. Miyoshi S, Hamada H, Kadowaki T, Hamaguchi N, Ito R, Irifune K, et al. Comparative evaluation of serum markers in pulmonary sarcoidosis. Chest. 2010 Jun;137(6):1391-7.
451. Chen ES, Song Z, Willett MH, Heine S, Yung RC, Liu MC, et al. Serum amyloid A regulates granulomatous inflammation in sarcoidosis through Toll-like receptor-2. Am J Respir Crit Care Med. 2010 Feb 15;181(4):360-73.

452. Chen ES, Moller DR. Etiologies of Sarcoidosis. Clin Rev Allergy Immunol. 2015 Aug;49(1):6-18.
453. Salazar A, Pinto X, Mana J. Serum amyloid A and high-density lipoprotein cholesterol: serum markers of inflammation in sarcoidosis and other systemic disorders. Eur J Clin Invest. 2001 Dec;31(12):1070-7.
454. Landi C, Carleo A, Cillis G, Rottoli P. Sarcoidosis: proteomics and new perspectives for improving personalized medicine. Expert Rev Proteomics. 2018 Oct;15(10):829-35.
455. Hekking PP, Bel EH. Developing and emerging clinical asthma phenotypes. J Allergy Clin Immunol Pract. 2014 Nov-Dec;2(6):671-80; quiz 81.
456. He R, Shepard LW, Chen J, Pan ZK, Ye RD. Serum amyloid A is an endogenous ligand that differentially induces IL-12 and IL-23. J Immunol. 2006 Sep 15;177(6):4072-9.
457. Cheng N, He R, Tian J, Ye PP, Ye RD. Cutting edge: TLR2 is a functional receptor for acute-phase serum amyloid A. J Immunol. 2008 Jul 1;181(1):22-6.
458. Ather JL, Ckless K, Martin R, Foley KL, Suratt BT, Boyson JE, et al. Serum amyloid A activates the NLRP3 inflammasome and promotes Th17 allergic asthma in mice. J Immunol. 2011 Jul 1;187(1):64-73.
459. Zhang XY, Zhang G, Jiang Y, Liu D, Li MZ, Zhong Q, et al. The prognostic value of serum C-reactive protein-bound serum amyloid A in early-stage lung cancer. Chin J Cancer. 2015 Aug 10;34(8):335-49.
460. Sung HJ, Ahn JM, Yoon YH, Rhim TY, Park CS, Park JY, et al. Identification and validation of SAA as a potential lung cancer biomarker and its involvement in metastatic pathogenesis of lung cancer. J Proteome Res. 2011 Mar 4;10(3):1383-95.
461. Lin HY, Tan GQ, Liu Y, Lin SQ. The prognostic value of serum amyloid A in solid tumors: a meta-analysis. Cancer Cell Int. 2019;19:62.
462. Zhou J, Sheng J, Fan Y, Zhu X, Tao Q, He Y, et al. Association between serum amyloid A levels and cancers: a systematic review and meta-analysis. Postgrad Med J. 2018 Sep;94(1115):499-507.
463. Malle E, Sodin-Semrl S, Kovacevic A. Serum amyloid A: an acute-phase protein involved in tumour pathogenesis. Cell Mol Life Sci. 2009 Jan;66(1):9-26.
464. Cho WC, Yip TT, Cheng WW, Au JS. Serum amyloid A is elevated in the serum of lung cancer patients with poor prognosis. Br J Cancer. 2010 Jun 8;102(12):1731-5.
465. Kanoh Y, Abe T, Masuda N, Akahoshi T. Progression of non-small cell lung cancer: diagnostic and prognostic utility of matrix metalloproteinase-2, C-reactive protein and serum amyloid A. Oncol Rep. 2013 Feb;29(2):469-73.
466. Vietri L, Bennett D, Cameli P, Bergantini L, Cillis G, Sestini P, et al. Serum amyloid A in patients with idiopathic pulmonary fibrosis. Respir Investig. 2019 Apr 26.
467. de Beer FC, Nel AE, Gie RP, Donald PR, Strachan AF. Serum amyloid A protein and C-reactive protein levels in pulmonary tuberculosis: relationship to amyloidosis. Thorax. 1984 Mar;39(3):196-200.
468. Wang YS, Chang HJ, Chang YC, Huang SC, Ko HL, Chang CC, et al. Serum amyloid a as a predictive marker for radiation pneumonitis in lung cancer patients. Int J Radiat Oncol Biol Phys. 2013 Mar 1;85(3):791-7.

469. Muhlebach MS, Clancy JP, Heltshe SL, Ziady A, Kelley T, Accurso F, et al. Biomarkers for cystic fibrosis drug development. J Cyst Fibros. 2016 Nov;15(6):714-23.
470. Zhang Y, Zhang J, Sheng H, Li H, Wang R. Acute phase reactant serum amyloid A in inflammation and other diseases. Adv Clin Chem. 2019;90:25-80.
471. Asis K. Jana, Augustus B. Greenwood and Ulrich H.E. Hansmann. Presence of a SARS-COV-2 protein enhances Amyloid Formation of Serum Amyloid A.2021.
472. D. Zhang, W.J. Huang, M.Q. Lan, F.R. Gan et al. Association between serum amyloid A levels and predicting disease severity in COVID-19 patients: a systematic review and meta-analysis. European Review for Medical and Pharmacological Sciences; 2021; 25:4627-4638.
473. Phil Bardin, Paul Reynolds. Serum amyloid A is a predictor for prognosis of COVID-19. Respirology (scientific letter), 2020.
474. Lucia Vietri, David Bennett, Paolo Cameli, Laura Bergantini, Giuseppe Cillis, Piersante Sestini, Elena Bargagli, Paola Rottoli. Serum amyloid A in patients with idiopathic pulmonary fibrosis. Respiratory Investigation, 2019; 2212-5345.
475. Lucia Vietri, Miriana d'Alessandro, Laura Bergantini, Alfonso Carleo, Paolo Cameli, Maria Antonietta Mazzei, Piersante Sestini, Elena Bargagli. Specificity of serum amyloid A as a biomarker of idiopathic pulmonary fibrosis. Internal Medicine Journal, 2020; 1571-1574.
476. Yanjin Jegal. The role of neutrophils in the pathogenesis of IPF. Korean J Intern Med 2022;37:945-946.
477. Mehrnaz Gharaee-kermani, Sem H.Phan. The role of eosinophils in pulmonary fibrosis (Review). International Journal of Molecular Medicine 1997; 43-53.
478. Peterson MW, Monick M and Hunninghake GW. Prognostic role of eosinophils in pulmonary fibrosis. Chest 1987; 92:51-56.
479. Vidyani Suryadevara, Ramaswamy Ramchandran, David W.Kamp et al. Lipid Mediators Regulate Pulmonary Fibrosis: Potential Mechanisms and Signaling Pathways. International Journal of Molecular Sciences, 2020;21-4257.
480. Tae Hoon Kim, Yoo Hoon Lee, Kyung Hun Kim et al. Role of Lung Apolipoprotein A1 in Idiopathic Pulmonary Fibrosis. Antinflammatory and Antifibrotic Effect on experimental Lung Injury and Fibrosis. American Journal of Respiratory Critical Care Medicine, 2010; 182, 633-642.
481. Yen-Ju Lin, Martina Anzaghe, Stefan Schulke. Update on the Pathomechanism, Diagnosis and Treatment Options for Rheumatoid Arthritis. Cells, 2020; 9(4):880.
482. Mitsuhiro Akiyama, Yuko Kaneko. Pathogenesis, clinical features and treatment strategy for rheumatoid arthritis-associated interstitial lung disease. Autoimmunity Reviews, 2022; 103056.
483. Georgiana Dinache, Claudiu Costinel Popescu, Corina Mogosan et al. Lung Damage in Rheumatoid Arthritis- A retrospective study. International Journal of Molecular Sciences, 2022; 24 (1):28.
484. Ligia Fernandes, Mouhamad Nasser, Kais Ahmad et al. Interstitial Pneumoniae With Autoimmune Features (IPAF). Frontiers in Medicine, 2019; 6:209.

485. John A. Mackintosh, Athol U. Wells, Vincent Cottin, Andrew G. Nicholson and Elisabetta A. Renzoni. Interstitial pneumoniae with autoimmune features: challenges and controversies. European Respiratory Review, 2021; 30 (162): 210177.
486. Francesco Bozzao, Paola Tomietto, Elisa Baratella et al. Clinical Characterization and Predictive Factors for Progression in a Cohort of Patients with Interstitial Lung Disease and Features of Autoimmunity: The Need for a Revision of IPAF Classification Criteria. Medicina, 2023; 59 (4):794.
487. Badreeddine Alami, Bouchra Amara, Meryem Haloua, Moulay Youssef Alaoui Lmrani et al. Diffuse pulmonary ossification associated with fibrosing interstitial lung disease. Respiratory Medicine case reports, 2019; 28:100868.
488. Haruhiko Shiiya, Masaaki Sato. Lung Transplantation for Pleuroparenchymal Fibroelastosis. Journal of Clinical Medicine, 2021; 10(5):957.
489. Felix Chua, Sujal R. Desai, Andrew G. Nicholson, Anand Devaraj et al. Pleuroparenchymal Fibroelastosis. Annals of the American Thoracic Society, 2019; 16(11):1351-1359.
490. Vincent Cottin, Salim Si-Mohamed, Remi Diesler, Philippe Bonniaud, Claudia Valenzuela. Pleuroparenchymal fibroelastosis. Current opinion in pulmonary medicine, 2022; 28(5):432-440.
491. Kentaro Watanabe, Hiroshi Ishii, Fumiaki Kiyomi, Yasuhiro Terasaki, Akira Hebisawa et al. Criteria for the diagnosis of idiopathic pleuroparenchymal fibroelastosis: A proposal. Respiratory Investigation, 2019; 57 (4): 312-320.

Figures and tables

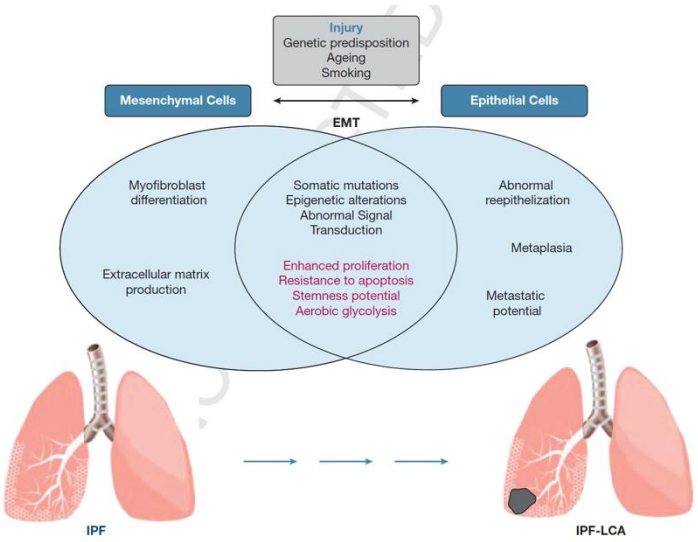

Fig1: Mechanisms of tumorigenesis in Idiopathic pulmonary fibrosis (IPF). Repetitive injurious stimuli of unknown origin in genetically predisposed individuals may lead to activation of fibroblasts, aberrant accumulation of extracellular matrix, and abnormal bronchiolization of alveolar epithelium mediating development of honeycomb cysts. An ongoing interaction between metaplastic epithelial cells with accumulated genetic alterations and activated mesenchymal cells triggers cancer initiation and progression. (EMT: epithelial-mesenchymal transition; LCA: lung cancer) (76).

IPF suspected*		Histopathology pattern†			
		UIP	Probable UIP	Indeterminate for UIP or biopsy not performed	Alternative diagnosis
HRCT pattern	UIP	IPF	IPF	IPF	Non-IPF dx
	Probable UIP	IPF	IPF	IPF (Likely)‡	Non-IPF dx
	Indeterminate	IPF	IPF (Likely)‡	Indeterminate§	Non-IPF dx
	Alternative diagnosis	IPF (Likely)‡	Indeterminate§	Non-IPF dx	Non-IPF dx

Fig2: Diagnosis of Idiopathic pulmonary fibrosis (IPF) through radiological and histological patterns- ATS 2022 (1).
** UIP= usual interstitial pneumoniae; HRCT= high resolution computed tomography*

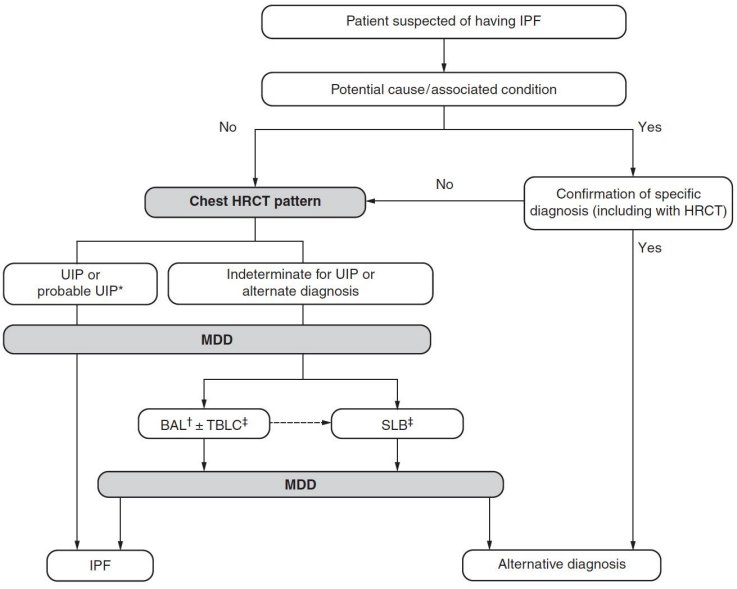

Fig3: Diagnostic algorithm of Idiopathic pulmonary fibrosis (IPF). ATS 2022 (1).
** UIP= usual interstitial pneumoniae; MDD=multidisciplinary discussion;*
BAL=bronchoalveolar lavage; TBLC= transbronchial lung cryobiopsy;
SLB= surgical lung biopsy.

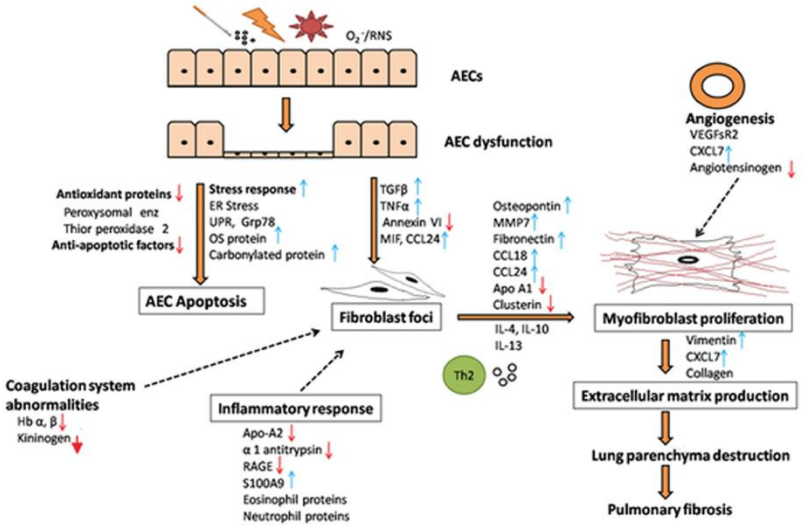

Fig4: Mechanisms of Idiopathic pulmonary fibrosis (IPF) pathogenesis based on proteomics data (78).
**AEC= alveolar epithelial cells*

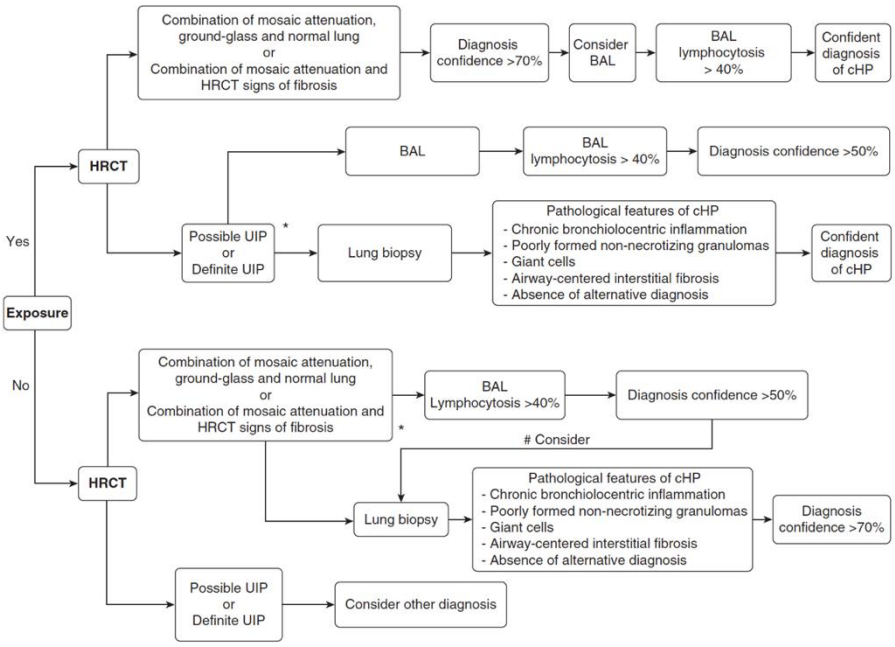

Fig5: Diagnostic algorithm of Hypersensitivity Pneumonitis (HP) according to Delphi consensus 2017 (136).
** BAL= bronchoalveolar lavage; HRCT= high resolution computed tomography; UIP= usual interstitial pneumoniae; cHP= chronic-HP*

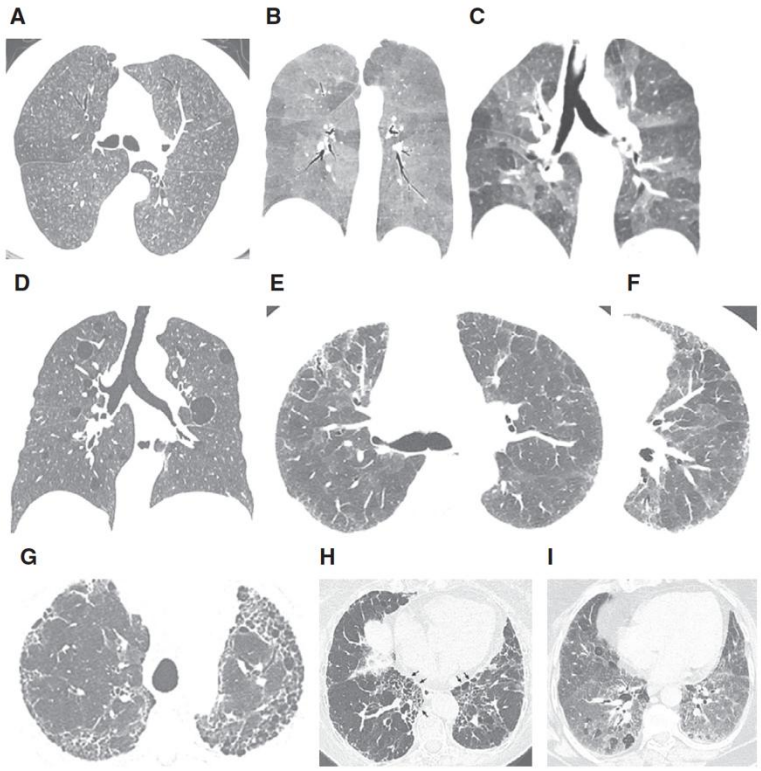

Fig6: Radiological patterns of Hypersensitivity Pneumonitis (HP) (142).
The nonfibrotic typical HP pattern is characterized by (A) centrilobular nodules, (B) mosaic attenuation on an inspiratory scan, and (C) air trapping on an expiratory scan.
The nonfibrotic compatible-with-HP pattern (D) is exemplified by uniform and subtle ground-glass opacity and cysts.
The fibrotic typical HP pattern consists of (E) coarse reticulation and minimal honeycombing in a random axial distribution with no zonal predominance in association with (F) small airway disease.
The fibrotic compatible-with-HP pattern varies in the patterns and/or distribution of lung fibrosis (e.g., basal and subpleural predominance, [G] upper-lung-zone predominance, [H] central [or peribronchovascular] predominance, or [I] fibrotic ground-glass attenuation seen alone or in association with small airway disease).
The fibrotic indeterminate-for-HP pattern includes the usual interstitial pneumonia pattern, nonspecific interstitial pneumonia pattern, organizing pneumonia–like pattern, or truly indeterminate findings.

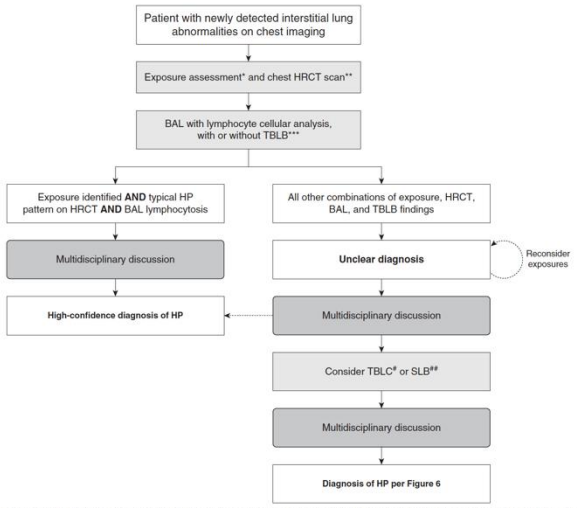

Fig7: Diagnostic algorithm of Hypersensitivity Pneumonitis (HP) according with ATS guidelines 2020 (142).
** HRCT= high resolution computed tomography; TBLB= transbronchial lung biopsy; TBLC= transbronchial lung cryobiopsy; SLB= surgical lung biopsy*

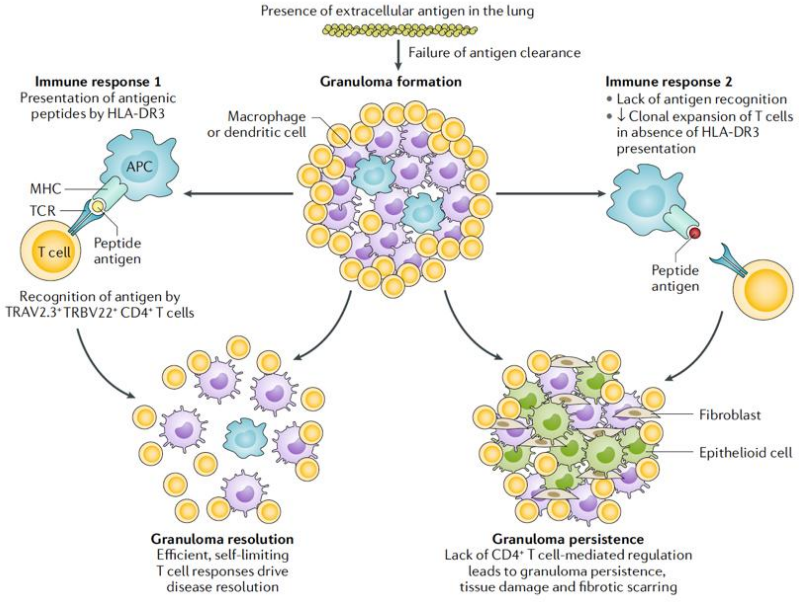

Fig8: Diagnosis and pathogenesis of sarcoidosis granuloma (330)

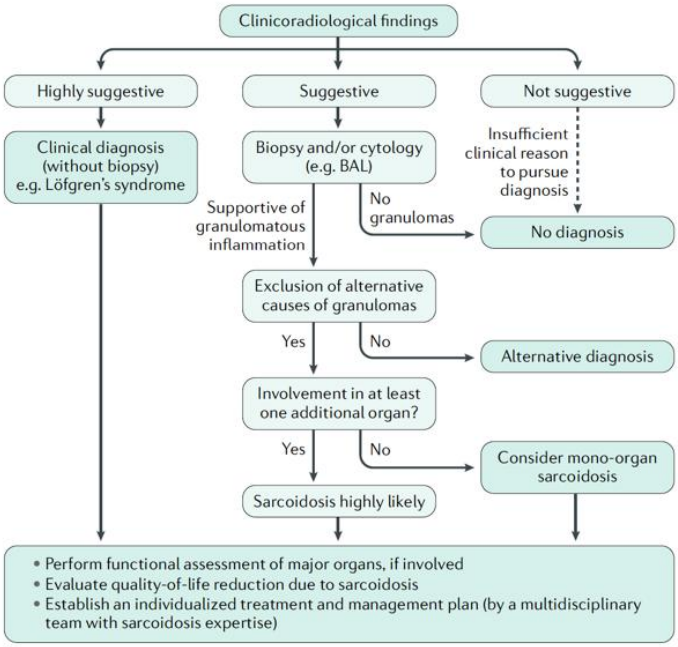

Fig9: Diagnostic algorithm of Sarcoidosis (352)

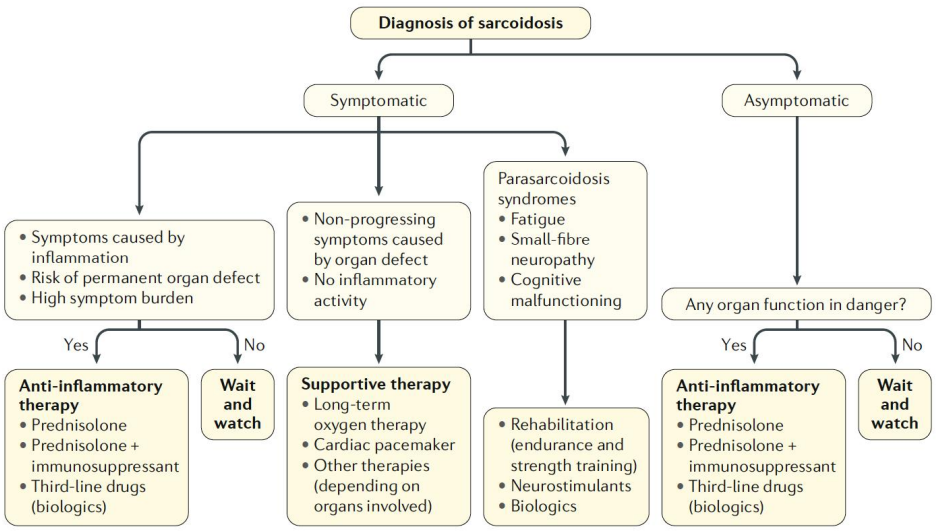

Fig10: Treatment algorithm of Sarcoidosis (369)

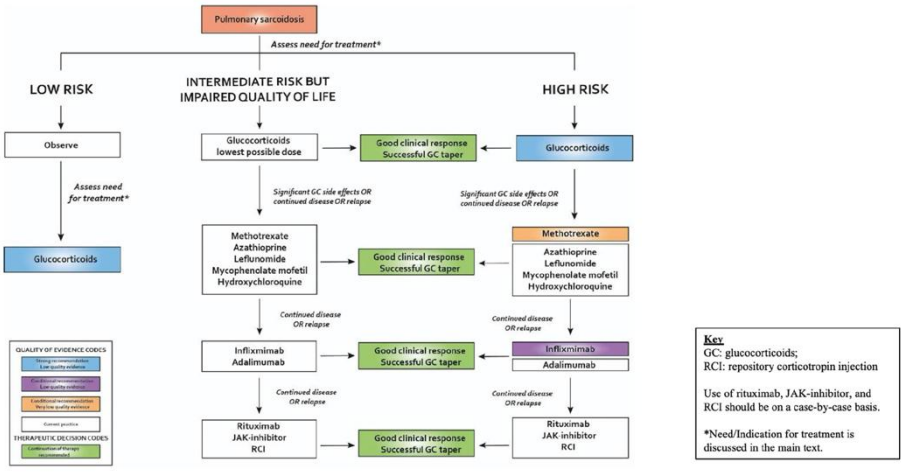

Fig11: New approach to management of pulmonary sarcoidosis (371)

Epidemiology
- African American race
- Male sex
- Genetic polymorphisms

Clinical features
- Diffuse cutaneous scleroderma variant
- Nailfold capillary abnormalities
- Digital ulcers
- Longer disease duration
- Pulmonary hypertension

Autoantibodies
- Anti-topoisomerase I
- Anti-neutrophil cytoplasmic antibody
- Anticardiolipin
- Anti-Ro52
- Anti-NOR90
- Anti-U11/U12
- Anti-Th/To
- Anti-polymyositis-scleroderma

Novel Biomarkers
- Interleukin-6, interleukin-34
- chemokine (C-X-C motif) ligand 4
- chemokine (C-C motif) ligand 18
- Carbohydrate antigen 15-3
- Lysyl oxidase
- Tenascin-C
- Serum amyloid A
- Surfactant protein D
- Chitinase 1
- Krebs von den Lungen-6
- Cartilage oligomeric matrix protein

Fig12: Factors associated to the risk of developing interstitial lung disease in Systemic Sclerosis (384).

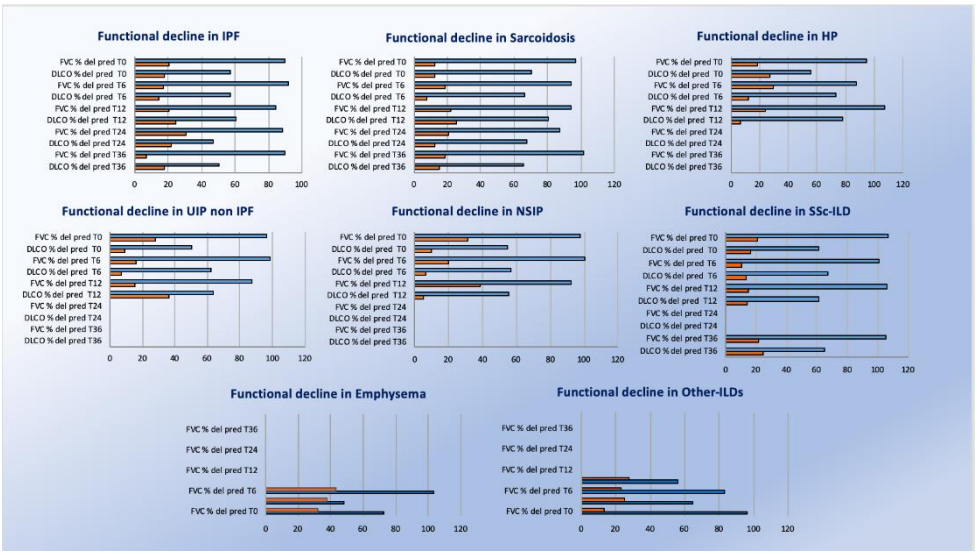

Fig.13: Functional decline of FVC and DLCO percentage at T0 (time at diagnosis and starting treatment), T6 (6 months after diagnosis), T12 (12 months after diagnosis), T24 (24 months after diagnosis), T36 (36 months after diagnosis) in the study population.

Pairwise comparisons - SAA

		W	p
IPF	UIP nonIPF	-5.1209	0.0155
IPF	fHP	-6.4836	0.0003
IPF	SSc-ILD	-6.1397	0.0009
IPF	HC	-6.5402	0.0002
UIP nonIPF	HC	-5.4374	0.0068
fHP	HC	-7.5151	< .0001
NSIP	HC	-5.0498	0.0185
SARCOIDOSI	HC	-6.8712	< .0001
ENFISEMA	HC	-4.7089	0.0413
SSc-ILD	HC	-6.9282	< .0001
LAM	HC	-4.7089	0.0413
PLCH	HC	-6.2201	0.0007
AE-IPF	HC	-5.6022	0.0043

Fig14: Statistical analysis comparing SAA serum levels in the patient groups

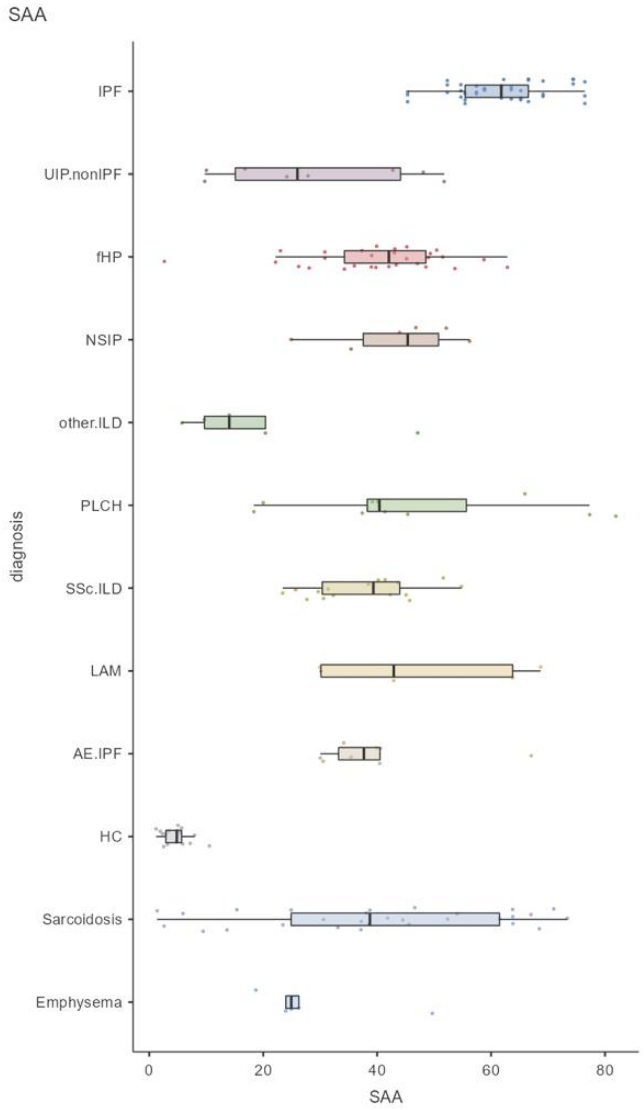

Fig15: Graphical representation of statistical analysis comparing SAA serum levels in the study population

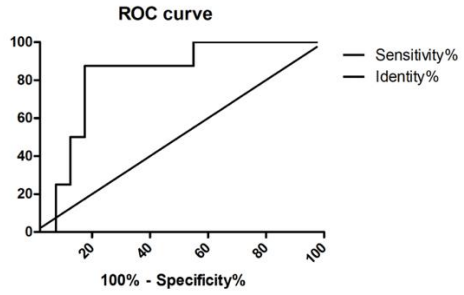

Fig16: ROC-curve analysis beetween IPF and AE-IPF according to SAA serum levels.

Pairwise comparisons - Age

		W	p
IPF	SARCOIDOSI	-5.221	0.0120
IPF	LAM	-4.842	0.0304
IPF	PLCH	-4.714	0.0408
IPF	HC	-6.372	0.0004
UIP nonIPF	fHP	-5.004	0.0207
UIP nonIPF	SARCOIDOSI	-5.565	0.0048
UIP nonIPF	PLCH	-5.052	0.0184
UIP nonIPF	HC	-5.841	0.0021
fHP	LAM	-5.408	0.0073
fHP	HC	-6.722	0.0001
SARCOIDOSI	LAM	-4.926	0.0250
SARCOIDOSI	HC	-5.124	0.0154
SSc-ILD	LAM	-5.011	0.0203
SSc-ILD	HC	-5.434	0.0068
AE-IPF	HC	-5.162	0.0139

Fig17: Statistical analysis comparing age in the ILDs groups.

Pairwise comparisons - mono x10^3/mcrl

		W	p
IPF	UIP nonIPF	-5.331	0.0063
UIP nonIPF	fHP	5.876	0.0013
UIP nonIPF	PLCH	5.034	0.0137

Pairwise comparisons - eos x10^3/mcrl

		W	p
IPF	fHP	6.173	0.0005
IPF	NSIP	4.533	0.0441
IPF	PLCH	4.651	0.0340
UIP nonIPF	fHP	4.960	0.0164

Fig18: Statistical analysis comparing eosinophil and monocyte blood counts among ILDs groups

Radiographic type	Radiographic characteristics	Prognosis
0	No visible findings	Not applicable
I	Bilateral hilar lymphadenopathy	Spontaneous resolution in most cases
II	Bilateral hilar lymphadenopathy and parenchymal infiltration	Spontaneous resolution possible
III	Parenchymal infiltration without hilar adenopathy in regular chest radiography	Spontaneous resolution in rare cases
IV	Advanced fibrosis with evidence of honeycombing bronchiectasis, hilar retraction, bulla and cysts	Permanent organ damage

Table1: Radiological Scadding classification of Sarcoidosis (336)

Affected organ	Examples of related symptoms	Prevalence of organ involvement (%)[a]
Lung	Cough, dyspnoea, wheezing and stridor	89–99
Skin	Lupus pernio, papules, nodules, plaques and infiltrated scars and tattoos	16–32
Eyes	Painful and/or red eye and vision loss	5–23
Liver	Abdominal pain and elevated liver functions	12–20
Lymph nodes	Peripheral lymphadenopathy	13–15
Spleen	Abdominal pain	5–10
Nervous system	Facial palsy, fatigue (for example, pituitary insufficiency), gait disturbance, headache, hearing loss, numbness or paraesthesia, seizure, trigeminal neuralgia, vertigo, visual loss and weakness and/or paresis	3–9
Heart	Conductance disturbances, arrhythmias, dyspnoea, fatigue (for example, cardiomyopathy) and syncope	2–5

Table2: Organs involvement of Sarcoidosis (331)

	Score
Skin thickening of the fingers of both hands extending proximally to the metacarpophalangeal joints	9
Telangiectasia	2
Abnormal nailfold capillaries	2
Pulmonary arterial hypertension or interstitial lung disease, or both	2
Raynaud's phenomenon	3
Skin thickening of the fingers (only count highest score)	
Puffy fingers	2
Sclerodactyly of the fingers	4
Fingertip lesions (only count highest score)	
Digital tip ulcers	2
Fingertip pitting scars	3
Scleroderma-related autoantibodies (eg, anticentromere, anti-topoisomerase 1, or anti-RNA polymerase 3)	3

Patients with a total score greater than 9 are classified as having definite systemic sclerosis.

Table3: Criteria for the classification of Systemic Sclerosis according to European League Against Rheumatism and the American College of Rheumatology (378).

Parameters	IPF (n= 40)	AE-IPF (n=8)	Sarco (n=30)	HP (n=30)	PLCH+ LAM (n=17)	NSIP (n=6)	UIP-non IPF (n=9)	SSc-ILD (n=16)	Emphysema (n=6)	Other - ILDs (n=6)	HC (n=17)
Age (mean ± SD)	73,32 ± 6,25	69,38 ± 7,90	59,27 ± 11,35	66,72 ± 8,44	50,94 ± 12,08	66 ± 12,09	78 ± 5,61	65,25 ± 10,35	69,8 ± 6,3	74,4 ± 8,01	43,35 ± 13,03
Male (%)	32 (80%)	6 (75%)	6 (20%)	18 (60%)	3 (17%)	0	7 (77%)	0	4 (66%)	3 (50%)	7 (41%)
Smoking habit former/ current	12/0	8/0	2/0	16 /0	7/4	2/0	7/0	4/0	4/1	3/0	8/0

Table4: Demographic characteristics of the study population.

	IPF	AE-IPF	Sarco	HP	PLCH+ LAM	NSIP	UIP non IPF	SSc-ILD	Emphysema	Other-ILDs
Hypertension	20 pts (50%)	3 pts (37%)	7 pts (23%)	9 pts (30%)	4 pts (23%)	2 pts (33%)	5 pts (55%)	4 pts (25%)	6 pts (100%)	5 pts (83%)
Hypercholesterolemia	8 pts (20%)	2 pts (25%)	5 pts (16%)	3 pts (10%)	2 pts (11%)	1 pts (16%)	2 pts (22%)	0 pts	2 pts (33%)	4 pts (66%)
Ischemic heart disease	16 pts (40%)	2 pts (25%)	0 pts	3 pts (10%)	2 pts (11%)	1 pts (16%)	3 pts (33%)	0 pts	3 pts (50%)	1 pt (16%)
ATS	12 pts (30%)	3 pts (37%)	2 pts (6%)	2 pts (6%)	2 pts (11%)	1 pts (16%)	0 pts	2 pts (12%)	4 pts (66%)	1 pt (16%)
Hepatic steatosis	3 pts (7%)	0 pts	2 pts (6%)	2 pts (6%)	0 pts	0 pts	0 pts	1 pts (6%)	2 pts (33%)	2 pts (33%)
Mild OSAS	2 pts (5%)	0 pts	0 pts	1 pts (3%)	0 pts	0 pts	1 pts (11%)	0 pts	1 pt (16%)	0 pts
Diabetes mellitus type II	6 pts (15%)	1 pts (12%)	5 pts (16%)	2 pts (6%)	0 pts	2 pts (33%)	3 pts (33%)	0 pts	4 pts (66%)	2 pts (33%)
Atrial fibrillation	8 pts (20%)	0 pts	0 pts	2 pts (6%)	0 pts	0 pts	2 pts (22%)	0 pts	2 pts (33%)	2 pts (33%)
Visceral obesity	1 pts (2%)	1 pts (12%)	3 pts (10%)	2 pts (6%)	0 pts	1 pts (16%)	0 pts	0 pts	0 pts	0 pts
Bronchial Asthma	0 pts	0 pts	0 pts	0 pts	0 pts	0 pts	2 pts (22%)	0 pts	0 pts	0 pts
GER	8 pts (20%)	2 pts (25%)	0 pts	3 pts (10%)	1 pts (6%)	2 pts (33%)	0 pts	12 pts (75%)	0 pts	0 pts

Table5: Main comorbidities in the study population.

Sarcoidosis	n° pts
Acute onset	10 pts (33%)
Subacute onset	14 pts (46%)
Steroid therapy	22 pts (73%)
MTX therapy	2 pts (6%)
Azathioprine therapy	5 pts (16%)
Stage I	1 pts (3%)
Stage II	15 pts (50%)
Stage III	7 pts (23%)
Stage IV	3 pts (10%)

Table6: Type of onset, therapy and radiological staging in patients with sarcoidosis.

Extrathoracic involvement	n° pts
Subcutaneous nodules	2 pts (6%)
Retropancreatic lymphadenopathy	2 pts (6%)
Uveitis	3 pts (10%)
Meningeal granuloma	2 pts (6%)

Table7: Extrathoracic involvement in sarcoidosis patients.

	Nintedanib	Pirfenidon
IPF	28 pts (70%)	12 pts (30%)
AE-IPF	1 pts (13%)	7 pts (87%)
Sarco	0 pts	0 pts
HP	1 pts (3%)	0 pts
PLCH+LAM	0 pts	0 pts
NSIP	1 pts (16%)	0 pts
UIP non IPF	3 pts (33%)	0 pts
SSc-ILD	2 pts (12%)	0 pts
Emphysema	0 pts	0 pts
Other-ILDs	2 pts (33%)	0 pts

Table8: Patients on antifibrotic treatment.

	IPF	AE-IPF	Sarco	HP	PLCH+ LAM	NSIP	UIP non IPF	SSc-ILD	Emphysema	Other-ILDs
FVC % del pred T0	89,83 ± 20,17	51,36 ± 12,72	96,67 ± 12,42	94,8 ± 18,53	101,87 ± 27,54	97,4 ± 31,29	96,56 ± 28,23	106,33 ± 21	72,33 ± 31,72	96,00 ± 13,53
FVC % del pred T6	91,58 ± 17,15	//	94,25 ± 18,70	87,25 ± 29,76	//	100,33 ± 19,55	98,67 ± 15,93	100,8 ± 10,18	103,5 ± 43,13	83,33 ± 23,29
FVC % del pred T12	84,10 ± 20,21	//	94,40 ± 22,09	107 ± 24,04	//	92,25 ± 38,74	87,25 ± 15,56	105,89 ± 15	//	//
FVC % del pred T24	88,44 ± 30,76	//	87,50 ± 20,53	//	//	//	//	//	//	//
FVC % del pred T36	89,71 ± 16,81	//	101,33 ± 18,58	//	//	//	//	105,56 ± 21,75	//	//
FEV1 % del pred T0	96,50 ± 19,75	56,44 ± 11,7	95,33 ± 18,45	100,20 ± 21,15	84,18 ± 28,20	97,8 ± 30,55	101,63 ± 34,89	105,11 ± 10,33	67,17 ± 43	100,67 ± 13,28
FEV1 % del pred T6	97,25 ± 17,26	//	93,50 ± 29,05	90 ± 31,61	//	104,33 ± 19,60	11,50 ± 20,86	94 ± 8,04	91,50 ± 61,52	89,00 ± 15,13
FEV1 % del pred T12	90,20 ± 20,08	//	90,20 ± 21,89	103 ± 22,63	//	96 ± 39,67	93,25 ± 12,50	103,83 ± 18,33	//	//
FEV1 % del pred T24	91,56 ± 25,17	//	82,75 ± 23,04	//	//	//	//	//	//	//
FEV1 % del pred T36	91 ± 19,23	//	93 ± 4,58	//	//	//	//	104,88 ± 22,51	//	//
DLCO % del pred T0	57,10 ± 18,03	33,5 ± 7,25	70,50 ± 12,52	56 ± 27,42	58,20 ± 23,13	55 ± 9,56	50,14 ± 8,75	61 ± 16,4	48,33 ± 37,85	64,33 ± 25,11
DLCO % del pred T6	57 ± 14,48	//	66,5 ± 7,72	72,75 ± 12,26	//	57 ± 6,08	62 ± 7,07	67 ± 13,33	//	56,33 ± 27,47
DLCO % del pred T12	60,67 ± 24,75	//	80,33 ± 25,11	77,5 ± 6,36	//	55,33 ± 4,73	63,50 ± 36,06	61,43 ± 14,16	//	//
DLCO % del pred T24	47 ± 21,74	//	67,25 ± 12,18	//	//	//	//	//	//	//
DLCO % del pred T36	50,29 ± 17,92	//	65,67 ± 15,14	//	//	//	//	64,71 ± 24,48	//	//
TLC % del pred T0	71,80 ± 15,69	61,9 ± 6,96	//	57,67 ± 14,57	126,82 ± 21,01	//	73,25 ± 16,92	93 ± 7	//	//
TLC % del pred T6	61,40 ± 19,05	//	//	59,50 ± 28,99	//	//	74 ± 23,61	//	//	//
TLC % del pred T12	//	//	//	95,5 ± 19,09	//	70 ± 43,49	//	//	//	//
TLC % del pred T24	//	//	//	//	//	//	//	//	//	//
TLC % del pred T36	65,50 ± 13,18	//	//	//	//	//	//	96 ± 14,14	//	//

Table9: Functional worsening in the study population at T0 (time at diagnosis and starting treatment), T6 (6 months after diagnosis and starting treatment), T12 (12 months after diagnosis and starting treatment), T24 (24 months after diagnosis and starting treatment), T36 (36 months after diagnosis and starting treatment).

	SAA (mcg/ml)
IPF	**61,64 ± 8,52**
AE-IPF	**39,76 ± 11,81**
Sarco	**40,27 ± 21,68**
HP	**40,08 ± 12,31**
PLCH+LAM	**46,12 ± 18,32**
NSIP	**43,23 ± 11,48**
UIP non IPF	**28,91 ± 16,81**
SSc-ILD	**37,74 ± 9,35**
Emphysema	**28,71 ± 12,07**
Other-ILDs	**19,40 ± 16,43**
HC	**4,68 ± 2,39**

Table10: SAA serum levels in the study population.

ROC of IPF vs	AUC	P values	Cut-off	Sensitivity %	Specificity %
UIP-nonIPF	98.1	0.0003	46.73	75	92.31
fHP	94.7	<0.0001	45.28	68.97	100
NSIP	92.3	0.0038	56.83	100	69.23
SARCOIDOSIS	79.31	0.0026	54.36	72.41	84.62
OTHER ILD	98.5	0.0019	49.73	100	92.31
EMPHYSEMA	98.5	0.0019	51	100	92.31
SSc-ILD	97.6	<0.0001	45.21	81.25	100
PLCH	74.8	0.0397	48.86	72.73	92.31
AE-IPF	90.4	0.0024	48.84	87.5	92.31
ROC of HC vs					
IPF	100	<0.0001	27.95	100	100
UIP-nonIPF	98.53	0.0001	8.84	94.12	100
fHP	97.4	<0.0001	16.36	100	96.55
NSIP	100	0.0004	17.72	100	100
SARCOIDOSIS	93.3	<0.0001	5.9	82.35	93.1
Other ILD	94.12	0.0033	5.7	76.47	100
Emphysema	100	0.0009	14.63	100	100
SSc-ILD	100	<0.0001	16.98	100	100
LAM	100	0.0009	20.26	100	100
PLCH	100	<0.0001	14.45	100	100
AE-IPF	100	<0.0001	20.29	100	100
ROC of other ILD vs					
fHP	82.76	0.0209	21.28	90	96.55
NSIP	86.7	0.0446	22.64	80	100

Table11: ROC-curve analysis according to SAA serum levels in the study population.

	Eosinophils x10^3/mcrl	Monocytes x10^3/mcrl
IPF	0,09 ± 0,10	0,71 ± 0,13
AE-IPF	0,07 ± 0,11	0,64 ± 0,30
Sarco	0,18 ± 0,12	0,55 ± 0,21
HP	0,21 ± 0,14	0,61 ± 0,27
PLCH+LAM	0,15 ± 0,08	0,77 ± 0,31
NSIP	0,25 ± 0,21	1,00 ± 0,28
UIP non IPF	0,03 ± 0,04	0,05 ± 0,01
SSc-ILD	0,03 ± 0,02	0,06 ± 0,01
Emphysema	0,01 ± 0,01	0,05 ± 0,01
Other-ILDs	0,02 ± 0,01	0,04 ± 0,02

Table12: Eosinophils and monocytes blood-count at the time of SAA sampling in the study population.