Project title
Titolo del progetto

Role of tissue and radiological biomarkers in pathogenesis and clinical evolution of major interstitial lung diseases

Project description
Descrizione del progetto

The term “interstitial lung diseases” (ILDs) includes a wide spectrum of heterogeneous entities with different prognosis, as well as treatment options. Although historically thought as rare diseases, latest epidemiologic data show an increasing trend of incidence and prevalence worldwide, reflecting both changes in environmental exposure and higher awareness of these entities among clinicians. The last decade has, indeed, witnessed huge progresses in pathogenic comprehension that has led to broaden diagnostic and therapeutic landscape of ILDs. Nowadays, two oral anti-fibrotic drugs, pirfenidone and nintedanib, are available in the market for the treatment of idiopathic pulmonary fibrosis (IPF), the most prevalent and severe form, as they have been shown to reduce functional decline in these patients in large high-quality randomized trials. However, no curative treatments yet exist and the average survival from diagnosis is between 3 and 5 years.

The differential diagnosis between major fibrosing ILDs (i.e. IPF, idiopathic nonspecific interstitial pneumonia, connective tissue disease-associated ILD, sarcoidosis, hypersensitivity pneumonitis, asbestosis) is often a challenging process and requires an integrated multidisciplinary approach involving pulmonologists, radiologists, pathologists and rheumathologists. The diagnostic work-up of ILDs, indeed, includes medical history, physical examination, serologic and functional tests, high-resolution computed tomography (HRCT), bronchoalveolar lavage, and, in case of still inconclusive results, a lung tissue sample.

There is a significant heterogeneity among individual patients in terms of clinical presentation at baseline and the subsequent evolution, both variable and largely unpredictable, likely reflecting different underlying pheno-endo types. In this context, reliable biomarkers are urgently needed to improve our insights in pathogenesis, and to help in making a differential diagnosis between IPF and other ILDs, in estimating prognosis and survival, in revealing the course of disease, and monitoring drug efficacy. During recent years, biomarkers from serum, plasma and broncho-alveolar lavage (BAL) have been the most extensively studied, while data from tissue biomarkers is limited by the lack of pathological samples. Surgical lung biopsy is currently considered as gold standard when a pathological sampling is required. However, SLB is characterized by appreciable costs and risks, with a mortality rate of 2–4% within 90 days, even higher in patients with an underlying histological pattern of usual interstitial pneumonia (UIP). Moreover, many subjects are not eligible because of a combination of advanced stage, age, comorbidities, respiratory failure, and pulmonary hypertension. More recently, transbronchial lung criobiopsy (TBLC), a bronchoscopic sampling technique, has been proposed as a valuable less invasive, diagnostic tool. The growing amount of data supporting risk-benefit profile of TBLC in this context has led to its routine adoption as alternative tool to obtain lung tissue in selected interventional pulmonology centers worldwide, including our center. We have recently reported and published our experience on diagnostic yield (DY) and safety of TBLC in diagnostic work-up of ILDs. Overall, DY was excellent (88%) and adverse events included pneumothorax and mild-moderate bleeding, confirming pooled data from literature. Such a safer sampling offers us also the chance to increase our insights on disease mechanism of action, due the higher availability of lung tissue suitable for biomarker assessment. Learning from oncology, the most appropriate source to link biological phenomena to pathogenetic mechanisms and to develop targeted drugs are areas directly damaged from the disease. The latest data on main mechanisms of action of lung fibrogenic process focus on three major steps: 1) alveolar injury; 2) cellular origins of myofibroblasts; 3) role of stem/progenitor cells. Although the exact mechanisms involved in initiation and progression of lung fibrosis are not yet fully known, according to the current paradigm, lung fibrogenesis results from an aberrant wound healing response, involving dysregulated tissue repair and remodelling. The injured and hyperplastic alveolar epithelia containing dysfunctional type II alveolar epithelial cells are able to release pro-fibrotic factors, leading to migration, proliferation of fibroblasts, differentiation into myofibroblasts and production of extracellular matrix (ECM). There are 3 possible sources of myofibroblasts: (1) resident fibroblast proliferation and differentiation under the action of transforming growth factor-β (TGF-β); (2) epithelial-mesenchymal transition (EMT); (3) bone

marrow-derived cells (BMPCs). Intrapulmonary fibroblasts increase the expression of collagen genes and mesenchymal proteins, such as vimentin and α-smooth muscle actin (α-SMA), through Wnt/β-catenin signaling and take part in fibrosis development. The formation of fibroblast foci (FF), specific aggregates of actively proliferating fibroblasts, myofibroblasts and ECM proteins, is the key feature reflecting sites of active ongoing fibrogenesis and has been associated with disease activity and a more rapid disease progression in IPF patients. The role of mesenchymal stem cells (MSCs) in this contex is controversial, as preliminary data showed that, on one hand, MSCs differentiate into type II AECs and ameliorate pulmonary fibrosis through canonical Wnt pathway (GSK-3β and β-catenin) and noncanonical Wnt pathway (JNK and PKC). On the other hand, MSCs differentiate into fibroblasts and promote pulmonary fibrosis by activating Wnt/β- catenin signaling.

As previously reported, due to the limited availability of lung tissue so far, data on diagnostic and prognostic role of these major actors of ILDs pathogenesis are scanty. However, in our Pulmonary Unit, a tertiary referral center for diagnosis and management of ILDs, the recent adoption of cryobiopsy has led to the collection of a large amount of tissue suitable for this analyses. In particular, more than 200 patients per year (prevalent estimate) are seen in our weekly dedicated outpatient care. Approximately the half of these are new diagnoses, of whom a significant proportion requires pathological assessment and undergoes cryobiopsy as part of diagnostic work-up of ILDs. Afterwards, patients are seen in the clinic approximately every 3 months and clinical and functional data are collected in dedicated databases. Therefore, the main objective of the present project is to investigate pathogenetic and prognostic role of a number of tissue biomarkers, deemed as expression of leading biologic processes involved in fibrogenesis, by means of a qualitatively and quantitatively assessment of these in lung samples obtained by cryobiopsy. In detail, these tissue biomarkers include tenascin C, matrix metalloproteinase-7 (MMP-7), polymorphism in the promoter of mucin-5 subtype B (MUC5B), Wnt/β-catenin, EBV protein, FF and “sandwich foci”, bronchiolar epithelial proliferation index. Moreover, we aim to investigate the role of MSCs. MSCs will be isolated from cryobiopsy derived from controls and IPF patients. Isolated cells will be characterized according to the criteria defined by Dominici, such as immunophenotype, differentiative potential and gene expression. After stemness definition, cells will be induced towards AECs differentiation, looking for the key mechanisms and for potential differences between MSCs derived from controls and from IPF patients. In addition, since the paracrine effect of MSCs has been demonstrated to participate to the development of other pathologies, the expression/secretion of specific soluble factors known to participate to IFP onset will be analyzed

Imaging, as well, is a key step in the evaluation of patients with fibrosing ILDs and high resolution computed tomography (HRCT) is the cornerstone in this context. Several studies have reported on diagnostic and prognostic significance of selected features, but limited data exist on relationship with histological patterns. Moreover, more recently, further radiological features have been identified, such as diffuse pulmonary ossification (DPO) and pleuroparenchymal fibroelastosis (PPFE) , but their diagnostic and prognostic role has yet to be established. Therefore, in the present project, we aim also to evaluate prevalence of selected radiological patterns and their relationship with pathological processes and clinical data.

In detail, in the first part of the project, the study population will be retrospectively derived from our database according to predefined eligibility criteria and it will be used a derivation cohort. In the second part, the most relevant biomarkers will be further validated and assessed in a prospective fashion in a validation cohort. Patients diagnosed with one of major fibrosing ILDs (IPF, idiopathic nonspecific interstitial pneumonia, connective tissue disease-associated ILD, sarcoidosis, hypersensitivity pneumonitis) with complete diagnostic work-up, including cryobiopsy, and a subsequent clinical follow-up (visit, pulmonary function tests, vital status), will be included. Radiological assessment of selected biomarkers (i.e.ground glass extension, PPFE, DPO) will be performed by a radiologist. through a semiquantitative evaluation. Pathological biomarkers will be analyzed through semi-quantitative evaluation (FF, “sandwich foci”, osseous metaplasia, elastosis component) and immunohistochemical assessment. In details, immunohistochemical analysis will include expression of tenascin C, MMP-7, MUC5B, Wnt/β-catenin, bronchiolar epithelial proliferation ki-67 index, anti-EBV protein. The same

evaluation will be performed in lung samples of control subjects (histologically defined normal lung sections from subjects who underwent lobectomy for cancer) to detect significant difference in biomarker expression levels. MSCs will be evaluated in a prospective fashion.

Correlation analyses between tissue biomarker expression levels, radiological biomarkers and clinical endpoints (i.e. baseline severity of the diseases, functional progression/decline, overall survival etc), will be evaluated.

This project will be conducted in a Teaching Hosptital that is a referral center for these pathologies, with a large proportion of patients coming from other regions and it will involve a working group of qualified researchers with established experience in different fields who already collaborate in daily clinical practice for diagnosis and management of ILDs.

Interdisciplinarity Project

Interdisciplinarietà del progetto

ILDs may primitively involve lung parenchyma or be expression of systemic pathologies, such as connective tissue disorders and sarcoidosis, that target different anatomical districts, including lungs. The differential diagnosis between the major fibrosing ILDs, indeed, include idiopathic interstitial pneumonia, connective tissue disease-associated ILD, sarcoidosis, hypersensitivity pneumonitis, and asbestosis. Notwithstanding such entities are hugely heterogeneous in terms of etiologies, clinical manifestations and prognosis, they share a list of pathogenic and clinical features.
The multidisciplinary approach involving pulmonologists, radiologists, pathologists and rheumatologists, is, indeed, mandatory for a proper clinical management as well as for an exhaustive research activity.

Objectives

Obiettivi

The main objectives of the present project are:

-To investigate the role of selected tissue biomarkers and MSCs in pathogenesis of major ILDs through a qualitative and quantitative assessment of these, comparing expression levels in lung tissue of cases obtained by lung crybiopsy and in histologically normal lung samples.

-To investigate the role of selected radiological biomarkers in pathogenesis and clinical evolution of major ILDs through a qualitative and quantitative assessment of these in HRTC images.

-To explore correlations between selected pathological and radiological features.

-To assess clinical impact on diagnosis, prognosis and overall survival of most relevant biomarkers

-To confirm the role of cryobiopsy in the diagnostic work-up of ILDs.

Expected Impact

Risultati attesi

Despite recent advance in pathogenic comprehension and clinical management of major ILDs, the exact mechanisms of actions implicated in pathogenesis are still largely unknown and no curative treatments are available for most of these. This is partly due to historically limited access to lung tissue for biological studies, as the standard sampling procedure, surgical lung biopsy, is characterized by unfavorable risk-benefit profile. In the last two years, the adoption of cryobiopsy, an innovative less invasive tool to obtain lung tissue, has changed the landscape of diagnostic work-up, offering also clinicians the chance to directly identify and characterize pathogenetic processes as wells as the leading biomarkers involved. However, this technique is routinely carried out in few centers worldwide, and, thus, data in this context are still limited. In our center, around 80 cryobiopsies per year are currently performed, representing one of the largest cohort of patients worldwide suitable for pathological analyses.

The present project is expected to improve knowledge in pathogenesis of major ILDs, and to provide relevant information on clinical impact of selected pathological features. Moreover, it will be firstly possible to correlate selected pathological and radiological elements, and to characterize different pheno-endotypes. A more exhaustive comprehension of underlying mechanisms of action is eventually expected to provide useful data to develop targeted drugs

in order to modify natural history and to improve survival of patients diagnosed with these entities.

New equipment to be shared

Condivisione delle attrezzature da acquisire

To assess expression levels of pathological biomarkers, a list of monoclonal antibodies (tenascin C, MMP-7, MUC5B, Wnt/β-catenin, bronchiolar epithelial proliferation ki-67 index, anti-EBV protein) will be purchased and shared with other departments

Keywords

Parole chiave

interstitial lung diseases, cryobiopsy, pathogenesis, biomarkers, mesenchymal stem cells