Original Study

A biobank for Interstitial Lung Diseases according to the European Network "eurIPF" and "BBMRI"

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SUMMARY. The creation of a Biobank for Interstitial Lung Diseases (ILDs), according to the standards set by the European Idiopathic Pulmonary Fibrosis Registry (eurIPFreg) and Biobanking and Biomolecular Research Infrastructure (BBMRI) is a major step in the understanding of these highly complex diseases. Particular emphasis is placed on Idiopathic Interstitial Pneumonias and especially Idiopathic Pulmonary Fibrosis (IPF), a chronic and progressive fibrotic interstitial pneumonia, with an average life span of 3 years. Our aim is to present a methodological guide for the development of a biobank for ILDs. *Pneumon 2014, 27(3):209-213.*

INTRODUCTION

Diffuse parenchymal lung diseases

Diffuse parenchymal lung diseases (DPLDs) represent a broad category of pneumonological diseases (>200 morbid conditions) characterized by aberrant extracellular matrix deposition in the lung interstitium and airway spaces of the distal lobes[1]. Although in the above categorization of diseases some acute conditions are also included (e.g. hypersensitivity pneumonia, acute interstitial pneumonia), most interstitial lung diseases manifest with symptoms of chronic disease, leading to progressive decline in respiratory function[2].

Clinical classification separates DPLDs into those exhibiting an acute, subacute, and chronic course. DPLDs with chronic course are divided into those of known etiology (e.g. due to environmental or pharmaceutical agents), those with systemic manifestations, and those affecting the lung without systemic manifestations, such as Idiopathic Interstitial Pneumonias (IIPs) (Figure 1). The IIPs are a special category of DPLDs in which the etiological agent is unknown. This makes their semiology extremely difficult. Therefore a combination of imaging, clinical and laboratory findings are required before a clear differential diagnosis is attained[3].

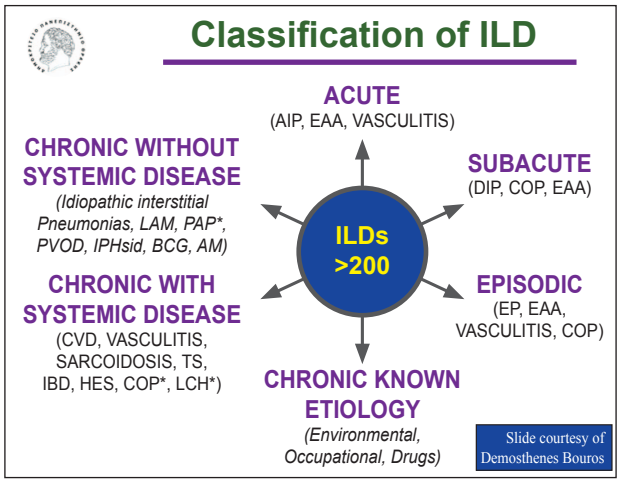

FIGURE 1. Classification of Interstitial Lung Diseases according to their clinical presentation. *Abbreviations:* LAM: Lymphangioleiomyomatosis, PVOD: Pulmonary Veno-Occlusive Disease, PAP: Pulmonary Alveolar Proteinosis, IPHsid: Idiopathic Pulmonary Hemosiderosis, CVD: Collagen-Vascular Disease, IBD: Inflammatory Bowel Disease, HES: Hypereosinophilic Syndrome, COP: Cryptogenic Organizing Pneumonia, LCH: Langerhans' Cell Histiocytosis, AIP: Acute Interstitial Pneumonia, EAA: Extrinsic Allergic Alveolitis, DIP: Desquamative Interstitial Pneumonia, EP: Eosinophilic Pneumonia. *= may have systemic involvement.

Most recently the American Thoracic Society and the European Respiratory Society (ATS/ERS) reported an updated classification of IIPs. The latter includes six major and two rare disease entities (Table 1)[4].

The major IIPs are categorized into chronic fibrotic (IPF and NSIP), smoking-related (RB-ILD and DIP) and acute or subacute (COP and AIP). The presence of comorbidities, including combined pulmonary fibrosis and emphysema, CPFE), often renders their classification into a specific category extremely difficult, while it further complicates clinical therapeutic regimens[5]. The establishment of an accurate diagnostic algorithm for IIPs is a major priority, equally important to the discovery of novel diagnostic methods capable of detecting serious complications (e.g. fibrosis) in the earliest stages of disease propagation[6].

Rapid research development in the field of molecular biomarkers holds a great promise for the near future. These biomarkers have high diagnostic and prognostic value and their incorporation into clinical and laboratory practice is expected to open new scientific horizons in the battle against these debilitating diseases[7]. The creation of a biobank for ILDs is a cutting-edge innovation in this direction. A task that demands concerted effort and international cooperation of medical centers and research institutions alike.

European Idiopathic Pulmonary Fibrosis Registry "eurIPFreg"

The European Network for Idiopathic Pulmonary Fibrosis (eurIPFnet), that involves the participation of 13 European countries, is a major multi-national effort to create a registry and a biobank of patients with pulmonary fibrosis, based at the University of Giessen (Germany)[8]. The registry hosts a web-based database that allows clinical cases of ILDs to be registered by authorized users. The main focus is on IPF, which is the most serious form of IIPs and secondarily on other diffuse parenchymal lung diseases. The collection of data takes place on a digital platform in the form of a questionnaire that investigates critical elements of the patient's history, in order to better describe its pathological profile, as well as to assess potential risk factors (e.g. personal and/or occupational exposure).

The biobank where biological material is collected (blood, plasma/serum, bronchoalveolar lavage, exhaled

TABLE 1. Classification of Idiopathic Interstitial Pneumonias (IIPs).

Idiopathic Interstitial Pneumonias (IIPs)			
Major		**Rare**	
Idiopathic Pulmonary Fibrosis	(IPF)	Idiopathic lymphoid interstitial pneumonia	(LIP)
Idiopathic Non-specific Interstitial Pneumonia	(iNSIP)	Idiopathic pleuroparenchymal fibroelastosis	(PPFE)
Respiratory bronchiolitis–interstitial lung disease	(RB-ILD)	Unclassifiable idiopathic interstitial pneumonias	
Desquamative interstitial pneumonia	(DIP)		
Cryptogenic organizing pneumonia	(COP)		
Acute interstitial pneumonia	(AIP)		

Source: An Official American Thoracic Society/European Respiratory Society Statement: Update of the International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias (2013).[4]

condensate and lung biopsy) and operates within the network has set a target to exploit the individual (genetic) information through high-throughput analytical tehnologies[9]. Patients enrolled in the study have to sign an informed consent form and the relevant data are encoded by the process of pseudo-anonymization to fully protect privacy.

The aim of the project is to better understand the mechanisms of IPF pathogenesis and to reveal biomarkers that meet diagnostic criteria, as well as to discover new regulatory factors for future therapeutic approaches.

Biobanking and Biomolecular Resources Research Infrastructure "BBMRI"

Biobanking and Biomolecular Resources Research Infrastructure (BBMRI) is one of the first projects of the European Strategy Forum for Research Infrastructures set to establish a European biobank initiative. During the last three years, BBMRI has grown into a large consortium of more than 225 related organizations (mainly biobanks) from 30 countries, making it one of the largest infrastructure projects for research and innovation in Europe[10].

The objective of this initiative is the creation of a large database through the collection of biological material from European populations and the advancement of high quality biomedical research resulting from the processing of these data with cutting-edge methods and protocols. The project is realized with the allocation of research infrastructures in most, if not all, of the participating Member States. The coordination unit is based in Graz, Austria, where all national member states refer to. Through the standardization and harmonization of all participating bodies a significant synergy of actions is achieved that results in large gains in statistical power and scale economy. This collaboration promotes scientific excellence at the European level and multiplies the effectiveness of research in life sciences, with specific emphasis on biomedicine.

Biobank for Interstitial Lung Diseases - Pulmonary Clinic, Democritus University of Thrace

The ILD biobank of the Pulmonary Clinic in the Democritus University of Thrace was approved by the Scientific Council of the University Hospital of Evros. The project was funded by the NSRF 2007-2013 Cooperation Program (CO-09-12-680) entitled "Functional Genomics in the pathogenesis of pulmonary fibrosis. Discovery of pathogenic mechanisms and new therapeutic targets" with Professor Demosthenes Bouros as the Scientific Coordinator. The specimens are stored in a specially designed room with controlled temperature and secure access (Building of Preclinical Studies, Department of Pharmacology, Democritus University of Thrace) (Figure 2).

MATERIALS AND METHODS

Informed Consent

Patients who contribute in the ILD biobank by donating biological material give their informed consent. All

FIGURE 2. Biobank for interstitial lung diseases – Department of pneumonology, Democritus University of Thrace.

necessary guarantees for the protection of personal data are provided through the process of anonymization. The objectives of the study are fully disclosed and the potential benefits that might accrue to the participant and other patients are presented. It is emphasized that participation is completely voluntary and that in no way affects the medical care and/or relationship with the physician. The right of withdrawal from the study at any given point is provided. The consent form is also signed and filed up by the attending physician.

Questionnaire

The patient questionnaire records critical biometric and demographic data (e.g. age, sex, weight, height, place of residence etc). A series of questions assessing the general health status of the patient such as symptoms (dyspnea, chronic cough, duration), smoking history, coexisting morbidities, medications, family history and possible exposure to risk factors in the living and working environment are recorded.

Standard Operating Procedures (SOPs)

Obtaining and maintaining biological material (blood, plasma/serum, bronchoalveolar lavage, exhaled condensate and biopsy) is performed by standard procedures (SOPs) for which specific protocols have been prepared. Specifically, about 20 mL of venous blood are collected, from which serum and plasma are isolated. Samples are separated in aliquots of 200μL and stored in a deep freezer (-80°C), complemented with digital data loggers for continuous temperature monitoring. Approximately 30 mL bronchoalveolar lavage fluid is collected bronchoscopically, followed by counting the number and type of cells and centrifugation to obtain a supernatant. Exhaled breath condensate (EBL) is collected with a special device in quantities of 1-2 mL and stored as above[11]. Finally, lung biopsies are collected and ground sections (~0.5 cm^3) are frozen.

Database

Access to the database is through the website of the Department of Pneumonology, of the Democritus University of Thrace (www.pneumonalex.gr). Authorization is required for data entry. The administrator enables the user to create a profile (username and password) upon request. The database includes demographic/biometric data, clinical and laboratory findings (e.g. spirometry, 6-minute walk test, results of blood, biochemical and immunological analyses, high-resolution computed tomography as DICOM file and comorbidities), medication and other patient characteristics.

Clinical and laboratory findings

So far, 167 samples from patients with interstitial lung diseases (ILDs) and 189 controls have been collected. These include 51.5% cases of IPF, 12.1% NSIP and 4.2% other idiopathic interstitial pneumonias (e.g. DIP, LIP, COP). The remaining 30% are extrinsic allergic alveolitis, systemic diseases (e.g. sarcoidosis, Wegener's etc) and other interstitial lung diseases (e.g. eosinophilic pneumonia, alveolar proteinosis etc).

Furthermore, a systematic monitoring of patients (follow-up) that includes records of clinical progression and median survival time, as well as response to various treatment regimens is carefully carried out.

CONCLUSION

The establishment of a biobank for ILDs and especially for IIPs, provides an important opportunity for translational research and may shed further light on the complex interactions between genetic and epigenetic (environmental) factors genetic and epigenetic (environmental) factors behind these pathologies.

Through BBMRI, the European Strategy Forum on Research Infrastructures (ESFRI) has developed and coordinated a pan-European action in the field of biomedical research that aims to create a network of cooperation between biobanks, in order to accelerate scientific progress and innovation in the coming years[12].

This network brings together European biobanks, translational research laboratories and bioinformatics centers, so that the interaction between researchers and existing databases is actively promoted[13]. Networking ensures high flow of information and of biological material between various centers. This requires laboratories to adopt common standards for the collection and storage of samples, as well as for data management[14].

Associated biobanks should facilitate such exchanges, by providing the appropriate infrastructure and know-how for the successful implementation of multicenter research in the near future. Public and private institutions (Universities, Research Centers and Hospitals) associated with these services should provide all methodological tools to facilitate this process both in terms of flow data and of biological material, alike.

From our experience the emergence of new biomarkers for IPF is a promising prospect. These biomarkers could be used prognostically in patients with IPF, as regards disease progression and survival. Moreover, investigations that are currently in progress are expected to answer some very basic questions about the pathophysiological mechanisms that characterize the natural history of this debilitating disease.

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REFERENCES

1. Bouros D. Idiopathic interstitial pneumonias: Classification revision. Pneumon 2010;23:359-62.
2. Bouros D. Guidelines for the management of respiratory diseases: From the international guidelines to the hellenic reality. Pneumon 2012;25:Suppl 1.
3. Antoniou KM, Daniil Z, Polychronopoulos V, Papiris S, Bouros D. Pneumon 2012;25(Suppl 1):110-2.
4. Travis WD, Costabel U, Hansell DM, et al; ATS/ERS Committee on Idiopathic Interstitial Pneumonias. 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 2013;188:733-48.
5. Bouros D. Current therapy of idiopathic pulmonary fibrosis: Primum non noccere! Pneumon 2012;25:259-61.
6. Bouros D. Idiopathic pulmonary fibrosis: The dawn of a new era. Pneumon 2014;27:117-9.
7. Bouros D, Tzouvelekis A. Idiopathic pulmonary fibrosis: on the move. Lancet Respir Med 2014;2:17-9.
8. Guenther A; European IPF Network. The European IPF Network: towards better care for a dreadful disease. Eur Respir J 2011;37:747-8.
9. Yuille M, van Ommen GJ, Bréchot C, et al. Biobanking for Europe. Brief Bioinform 2008;9:14-24.
10. Holub P, Greplova K, Knoflickova D, Nenutil R, Valik D. The biobanking research infrastructure BBMRI_CZ: a critical tool to enhance translational cancer research. Klin Onkol 2012;25 (Suppl 2):2S78-81.
11. Kubáň P, Foret F. Exhaled breath condensate: determination of non-volatile compounds and their potential for clinical diagnosis and monitoring. A review. Anal Chim Acta 2013;805:1-18.
12. Calzolari A, Valerio A, Capone F, et al. The European Research Infrastructures of the ESFRI Roadmap in Biological and Medical Sciences: status and perspectives. Ann Ist Super Sanita 2014;50:178-85.
13. Asslaber M, Zatloukal K. Biobanks: transnational, European and global networks. Brief Funct Genomic Proteomic 2007;6:193-201.
14. Raghu G, Collard HR, Egan JJ, et al; ATS/ERS/JRS/ALAT Committee on Idiopathic Pulmonary Fibrosis. 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.