The Minapild Study Project : New tools to identify causes and prevent Interstitial Lung Diseases

M Catinon[1], JF Mornex[2], V Cottin[2], E Roux[1], F Thivolet[3], S Marchand-Adam[4], S Jouneau[5], M Kerjouan[5], A Sale[5], D Israel-Biet[6], JM Vergnon[7], N Freymond[8], S Cofta[9], J Müller-Quernheim[10], G Fouilloux[1], V Massardier[1], AM Trunfio-Sfarghiu[11], Y Song[12], A Auroux[13], M Veltkamp[14], M Vincent[1]

1: Minapath Development, Social Business Society, Villeurbanne, France; 2: Department of pneumology and rare diseases center, Hôpital Louis Pradel CHU HCL University of Lyon, Lyon1 University, INRA, UMR754, Lyon, France; 3: Department of Cytology and Pathology, Pôle Est, Hospices Civils de Lyon, Bron, France; 4: Pneumonology department CHU Tours, France; 5: Pneumonology department CHU Rennes, France; 6: Pneumology department, Paris Descartes University, AP-HP, European Hospital Georges Pompidou, Paris, France; 7: Department of Pneumology and Thoracic Oncology CHU Saint Etienne, France; 8: Pneumology department CHU Lyon Sud, HCL Lyon, France; 9: Pneumology Department Universitary Hospital Poznan, Poland; 10: Pneumology Department Universitary Hospital Fribourg, Germany; 11: Univ Lyon, INSA-Lyon, CNRS UMR5259, LaMCoS, F-69621, Villeurbanne, France; 12: Pneumology and occupational and clinical toxicology Beijing, China; 13: Ircelyon CNRS Villeurbanne, France; 14: Pneumology Department ILD Center of excellence St Antonius Hospital Nieuwegein, The Netherlands

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I. Aims and rational

1) Among Interstitial Lung Diseases (ILD) there is a confusion between the identification of a nosological entity (sarcoidosis, idiopathic fibrosis, connective diseases associated ILD…) and an etiology.
2) This confusion lead to a vicious circle of ignorance and prevents any efficient investigation of potential occupational or environmental causal factors. The boundaries between pneumoconiosis and sarcoidosis and idiopathic fibrosis are not clear [1]. Concerning ILDs with connective disease there is a known link between scleroderma, polyarthritis, lupus and exposure to silica or other inorganic particles.
3) If there is questionnaires for identifying asbestos exposure no such procedure are available for patients with ILDs.
4) In situ mineralogical analysis can contribute to identify abnormal level of some types of particles with Scanning Electron Microscopy coupled with Energy Dispersive X-ray (SEM/EDX), compared with our reference (4050 particles coming from 52 lung biopsies of 32 subjects autopsied at the Forensic Institute of Lyon).
5) Complementary with the SEM-EDX which can't identify nanoparticles, multielemental analysis can provide quantitative information by Inductively Coupled Mass Spectrometry (ICP-MS) or in situ by Laser Induced Breakdown Spectroscopy (LIBS).
6) Some patients can have a hypesensitivity to some mineral particles which can be measure by Lymphocyte Proliferation Test (LPT).

Our study aims at measuring the mineral exposome in 300 retrospective cases of ILD, and at evaluating the possible causal relationship between exposome and ILDs heretofore diagnosed as idiopathic.

II. Methods

1. Approach to the Minapild study

a. First step (Fig.1)

Our technology is conceived to function as a diagnostic service for clinical settings and players involved in diagnosis (Fig 1). The service begins with the processing of a biopsy sample into a paraffin block for preservation and conditioning to undergo our histological and software analysis. Minapath performs the analysis in three phases: i.) the patient is called to carry out the **45-minute questionnaire** to determine the source of the exposure; ii.) A software analysis is performed with **SEM-EDX** on the sample to identify the location and chemical composition of the mineral deposits; iii.) An **ICP-MS** analysis is performed to quantify the element composition of each mineral; iv.) a **report** is sent back to the clinicians enclosing the characterization of the mineral deposit present.

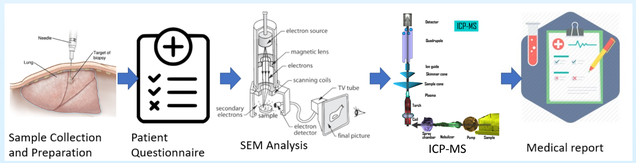

Figure 1: First step to the assessment of the mineral exposome in the Minapild study

b. Second step (Fig.2)

Only if step 1 is insufficient to establish a causal relationship between inorganic dust exposure and disease, patients with a notification of suspected causality are summoned for a blood sampling which will be analyzed by **LPT**, and by **LIBS** for the biopsy (Fig.2). The priority elements to be identified are: beryllium, silicon, aluminum, iron, chromium, nickel, cobalt, cerium, titanium, zirconium, tin, zinc, tungsten and vanadium.

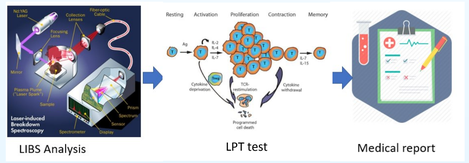

Figure 2: Second step to the assessment of the mineral exposome in the Minapild study

2. Analysis of histological section in SEM (Fig.3)

A first HES stained histological section is observed under an optical microscope (ZEISS Axio scope A.1). A second 5 µm thick unstained section is deposited on a carbon disc with a diameter of 25 mm. This section is intended for observation by scanning electron microscopy (JEOL JSM-6010PLUS / LV) equipped with an Oxford X-Max micro-analyzer 50 mm^2.

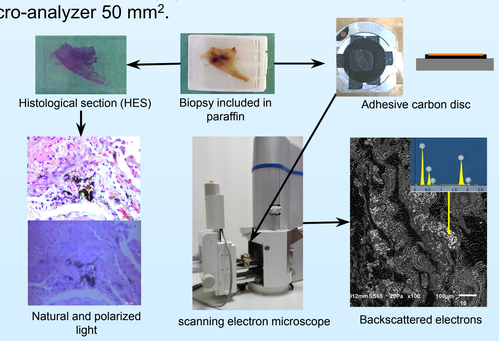

Figure 3: *In situ* mineralogical analysis in optical and electron microscopy from a paraffin-embedded biopsy with microanalysis.

We have studied more than 4000 particles from 53 lung biopsies of 32 subjects autopsied at the Forensic Institute of Lyon, whose cause of death was not lung disease and whose families provided informed consent. With SEM-EDX analysis have allowed us to establish a control distribution of particles types for patients with no known lung disease (Figure 4).

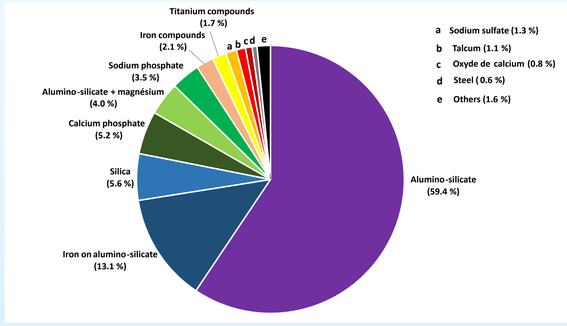

Figure 4: *SEM-EDX in situ analysis of 53 blocks of pulmonary parenchyma from forensic institute of Lyon (4050 particles analyzed).*

3. Other mineralogical analysis (ICP-MS and LIBS)

SEM can't identify beryllium and nanoparticles. Multielemental analysis with ICP-MS and LIBS allow to identify elements overload (exept silicium for ICP-MS).

Each specimen patient will be compared to samples from the forensic institute. For ICP-MS in µg per dry gram of tissue and for LIBS in number of pixels per mm^2 of histological section.

III. Expected Results

The study will evaluate the proportion of cases that can be related to a mineral particle exposure according with the fact that each clinical investigator follow notification given by Minapath medico-scientific council. Causal relationship will be established with the help of Hill's criteria [2].

IV. Conclusions

The MINAPILD study based on a systematic in depth questionnaire and mineralogical analysis may alter the paradigm of approach to the diagnosis of ILDs, with potential impact of such process in many lung samples (bronchoalveolar lavage, bronchial and transbronchial biopsy and lung biopsy).

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•**Références** : [1] Vincent M et al., Am J Ind Med 2015;58:S31-S38. [2] Hill AB. The environment and disease: Association or causation? Proc R Soc Med. 1965;58:295-300.