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Nano to micron-sized particles detection in patient lungs and its pathological significance.

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Abstract

Mineralogical analyses of clinical samples have been proved useful to identify causal relationship between exposure to airborne particles and pulmonary diseases. However, this type of analyses only considers the micron-sized fraction of the particles, neglecting the specific impact of submicron/nano-sized particles which have been otherwise shown to be reactive and able to induce biological effects. To fill this gap, we previously developed an innovative protocol to isolate micron-sized particles from submicron/nano-sized particles contained in 100 broncho-alveolar lavages (BAL) and bronchial washes (BW) from patients who suffered from interstitial lung diseases (ILD) (NanoPI, ClinicalTrials.gov Identifier: NCT02549248). We then determined qualitatively and quantitatively the metal load in each of these fractions. The aim of the present paper is to investigate correlations between these mineralogical analyses and clinical data. The mean concentration of submicron silica particles was found to be significantly higher in BAL and BW from patients suffering from sarcoidosis than in patients suffering from other ILD (501 *vs* 246 ng/mL BAL and 564 *vs* 292 ng/mL BW respectively). This finding suggests a potential role of submicron silica particles in the etiology of sarcoidosis and highlights the usefulness of comprehensive mineralogical analyses to get new insights in the role of inhaled biopersistent particles in lung diseases.

Key-words

Submicron particles; Silica; Sarcoidosis; Etiology; Mineralogical analysis of broncho-alveolar lavages.

Introduction

Mineralogical analyses of clinical samples using electron or optical microscopy have been proven useful to identify a causal relationship between exposure to inhaled microparticles and pulmonary diseases ¹. Thus, the quantification of biopersistent particles can provide new insights in the role of inhaled particles in the etiology or development of some respiratory diseases ². It is perfectly exemplified by asbestosis where the assessment of asbestos bodies in patient lung tissues or in broncho-alveolar lavages (BAL) has allowed the definition of threshold values specific of diseases ³. However, this type of mineralogical analyses only considers the micron-sized fraction of the particles, neglecting the specific impact of nanoparticles and submicron particles which have been otherwise shown to be reactive and with the ability to induce biological effects. This is partly explained by the technical challenges associated to these analyses ⁴. To fill this gap, we specifically developed an innovative experimental protocol to isolate micron-sized particles from submicron/nano-sized particles, in order to separately analyze these fractions ^{5,6}. To illustrate the pathological relevance of mineralogical analyses, we investigated relationships between metal loads in patient BAL and clinical data with a particular emphasis on sarcoidosis ⁶.

Sarcoidosis is a multisystem inflammatory disease of undetermined etiology ⁷⁻¹⁰. It is characterized by the formation of granulomas in various organs, mainly the lungs and the lymphatic system ^{7,9,10}. Although the exact causative agent of sarcoidosis is still unknown, it is commonly acknowledged that this disease results from an exaggerated immune response to one or more unidentified antigens in individuals who are genetically susceptible ^{7,9}. The antigens have been proposed to be either of bacterial origin (especially mycobacteria and propionibacteria) or environmental factors, particularly if individuals are exposed to organic and inorganic particulates able to trigger inflammation ^{9,11}. The assumption that sarcoidosis is an antigen-driven disease is further supported by the fact that other granulomatous disorders

induced by known antigens, such as berylliosis (chronic beryllium disease), exhibit very similar pathological and clinical features ^{12,13}. Similarly, talc pneumoconiosis can mimic and thus be confused with sarcoidosis ^{14–16}. Progress in the diagnosis and prevention of sarcoidosis is expected from the identification of the exact causes of this disease ^{9,10}.

The contribution of inorganic particulates to sarcoidosis has long been suggested. In particular, nanoparticles are increasingly suspected to trigger granuloma formation ^{11,17–20}. A relationship between exposure to inhaled nanoparticles and long-term negative effects in humans has been suggested by Song *et al.* ^{21,22}. They reported the case of seven women who developed important lung symptoms after being exposed for several months to airborne polyacrylate nanoparticles at their workplace. Most importantly, nanoparticles of the same raw materials the workers were exposed to in the plant (particularly silica nanoparticles and nano- and microscale silicates) were found in the patients' tissue and effusions. However, the conclusion that silica nanoparticles were responsible for the pathological findings is only hypothetical as the patients had been exposed to other toxic substances; still, the study drew attention to silica nanoparticles as potential contributors to a lung disease.

In this context, we performed an exploratory clinical study where we qualitatively and quantitatively assessed the metal load in 100 BAL from patients who suffered from interstitial lung diseases (ILD) and were in need of this invasive procedure for clinical purposes ⁶. The aim of the present paper is to determine if correlations can be found between metal loads and clinical data, allowing getting new insights in the etiology of some respiratory diseases. To the best of our knowledge, it is the first time such comprehensive mineralogical analyses are performed and correlated with clinical data.

Materials and Methods

Patients

During 2 years, 100 patients from the Chest diseases and thoracic oncology Department of the University Hospital of Saint-Etienne were included in a prospective, monocentric and exploratory study (NanoPI, ClinicalTrials.gov Identifier: NCT02549248). Patients presented a clinical image of diffuse ILD and needed a bronchoscopy associated with a BAL (*i.e.*, this procedure was not performed on purpose for this study). All patients were informed about this study and had to give their written consent to participate. Our protocol was in accordance with ethical principles defined by the World Medical Association declaration of Helsinki and subsequent amendments and was approved by an ethics committee (Comité de Protection des Personnes, Sud-Est I) as well as by the French agency regulating biomedical research (Agence Nationale de Sécurité du Médicament et des produits de santé, ANSM).

Criteria for the inclusion in the study were: i) patients with an interstitial lung disease assessed on clinical signs and CT scan, requiring a flexible bronchoscopy associated with a BAL (for instance for patients suffering from sarcoidosis, idiopathic pulmonary fibrosis, hypersensitivity pneumonitis, infectious or cancerous interstitial diseases and interstitial diseases caused by drug reactions), ii) patients older than 18, iii) patients who had given their voluntary, informed and written consent, iv) patients having a social insurance or beneficiary (mandatory for any French clinical study). Exclusion criteria were: i) patients who had not given their consent, ii) when flexible bronchoscopy or BAL was not possible, iii) patients under legal protection or pregnant women, iv) patients with contagious disease (*e.g.*, HIV infection, tuberculosis, viral hepatitis) for safety reasons.

Broncho-alveolar lavages

BAL were performed following regular practice in the Chest diseases and thoracic oncology Department of the University Hospital of Saint-Etienne. Briefly, 50 mL of a warmed saline solution were injected in the selected area of lung and were slowly aspirated, the collected

sample constituting the bronchial wash (BW). Then, 2 to 4 additional 50 mL of warmed saline solution were injected and aspirated successively, the collected sample representing the BAL strictly speaking. All samples were collected into plastic tubes without any fixative agent. They were immediately transferred to the Histology-Cytology Department for standard cytological analysis. After this analysis, if there was still enough volume of sample 5 mL of BW and 20 mL of BAL were dedicated to the present study. An equivalent volume of sodium hypochlorite was added to the samples that were then stored at 4°C until use.

Sample pre-treatment and analysis

BAL and BW samples underwent a well-characterized and validated size fractionation protocol to separate microparticles ($> 1 \mu\text{m}$) from submicron particles (ranging from 100 nm to $1 \mu\text{m}$) and nanoparticles and ions (particles $< 100 \text{ nm}$). The method and its application have been described in detail in our previous publications ^{5,6}. A summary of the protocol is provided as Supplementary information. All fractions were analyzed using both dynamic light scattering (DLS) method (Nanosizer®, Malvern Instrument) and inductively coupled plasma atomic emission spectroscopy (ICP-AES, Jobin-Yvon JY138 Ultrac). DLS allowed verifying that size fractionation was successful. ICP-AES allowed determining for each metal the quantity of matter expressed in parts-per-billion (ppb), *i.e.*, as ng/mL. We focused our investigation on the following metals: aluminum (Al), beryllium (Be), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), nickel (Ni), silicon (Si), titanium (Ti), tungsten (W), zinc (Zn) and zirconium (Zr). To assess potential contamination from the particles present in the environment, the materials and solutions used, we used “blank” samples consisting of saline solution aspirated through the bronchoscope and placed in the same tubes as clinical samples, a filtered sodium hypochlorite solution (*i.e.* the solution in which the LBA were conditioned) was then added (volume to volume) and blank samples were processed and analyzed exactly

the same way as clinical samples were. Samples of interest were also observed using scanning electron microscopy coupled to an EDX detector (Energy-dispersive X-ray spectroscopy), as previously described ⁶.

Comparison to clinical data

We classified patients in two groups: patients suffering from sarcoidosis and patients suffering from other types of ILD. Details on the cohort are reported in Table 1. The diagnosis was obtained on biopsies showing bronchial or node non-necrotic granulomatous reaction and a typical CT scan pattern of peri-broncho-vascular micro-nodules. 13 patients presented a stage 2 sarcoidosis and 1 a stage 3. The clinical features of patients suffering from sarcoidosis are summarized in Table 2.

Results

We previously established a size fractionation protocol to separate microparticles from submicron particles and nanoparticles in patient pulmonary lavages and assessed the metal load in these fractions ^{5,6}. In the present paper we explored how these physico-chemical results corresponded to clinical data. Specifically, we compared the metal load in BAL and BW samples from patients suffering from sarcoidosis and patients suffering from other ILD. Comprehensive results are reported in Supplementary information. As shown in Table 3, only submicron Si particles (both in BW and BAL), Si microparticles in BAL, and Al microparticles in BW seemed to have a differing presence in the two groups. However, only the difference in submicron Si particles reached statistically significant levels between the two groups ($p < 0.05$, as calculated with a Mann-Whitney test).

Figure 1 reports the detailed comparison for submicron Si particles concentration in BW and BAL in patients suffering either from sarcoidosis or other types of ILD.

Two assumptions could be made to explain the different medians between the sarcoidosis and other ILD groups. This difference could be due to either i) a different submicron Si particles concentration or ii) a different prevalence of patients with detectable submicron Si particles. To try to shed light on this aspect, we only considered pulmonary lavage fluids of which the Si concentration in the submicron particle fraction was higher than the limit of detection (LoD). We then determined the frequency of patients exhibiting a Si load higher than the LoD and we calculated the mean Si concentration in submicron particle fraction considering only these patients (see Table 4). From the results, we can confirm the second hypothesis.

The size and the chemical nature of the extracted particles were verified using scanning electron microscopy coupled to an EDX analysis. Figure 2 is an illustration of submicron Si particles found in the BW from patient n°12. It is representative of what was observed in all patients suffering from sarcoidosis and exhibiting a Si concentration higher than the LoD in their submicron particle fraction as assessed with the ICP analysis. Conversely, we also verified with this technique that actually no submicron Si particles were found in the 2 patients suffering from sarcoidosis who exhibited a null Si submicron particles concentration as assessed with the ICP analysis.

Discussion

Mineralogical analyses of patient pulmonary lavages that underwent a size-fractionation protocol allowed us to detect a significantly higher concentration of submicron Si particles in patients suffering from sarcoidosis than in patients suffering from another ILD (Table 3 and Figure 1). Although this finding has to be confirmed with further analysis of larger cohorts, it suggests a potential role of these particles in this disease. Of note, this correlation was observed only for particles in the submicron size range but not for bigger (*i.e.* micron-sized) particles. It confirms the observations from our previous study where we had not found any

clear correlation between the metal content of microparticle, submicron particle, and nanoparticle fractions of broncho-alveolar lavages ⁶. Thus, this finding further stresses the need to separately analyze particle populations of different sizes in order to achieve a more comprehensive mineralogical analysis.

The other corollary of this finding is that it evidences the impact of the particle size on the pathogenesis of a disease. The toxicity of silica particles, especially crystalline silica, is not new and is well-documented ²³. Please note that strictly speaking we assessed the silicon content of BAL and BW and further physico-chemical characterizations are necessary to better define the exact nature of the silicon-based particles identified. However, we can reasonably assume that due to its ubiquitous nature silica is the component we are mainly exposed to. A wide variety of diseases can be induced after silica inhalation ranging from acute lung inflammation to silicosis, but also lung fibrosis, cancer, emphysema, pulmonary tuberculosis, or autoimmune diseases, such as scleroderma (systemic sclerosis), rheumatoid arthritis, chronic renal disease, and lupus ^{23–26}. Yet so far, little attention has been paid to the specific impact of the particle size on human health effects associated with silica exposure. In the context of mineralogical analyses, this can be mainly explained by the technical challenges one has to face in order to separately analyze differentially sized particle populations, as we have already demonstrated ^{2,4}. However, despite some inconsistencies, *in vitro* and *in vivo* studies brought interesting data on the toxicity of nano-sized silica ²⁵. Nanoparticles are characterized by specific physico-chemical features, very distinct from those of their bulk form. They confer on them enhanced properties for many applications. More particularly, they are characterized by an increased surface-to-volume ratio and a higher surface reactivity ²⁵. It seems therefore logical that they could trigger different adverse effects than larger particles ^{20,25}. In addition, due to their small size they can translocate across barriers of the body (such as the air-blood barrier), enter the circulation, and interact with

various organs ²⁵. As an example, a recent study from Kreyling *et al.* ²⁷ investigated the deposition patterns of inhaled 20 nm gold nanoparticles in rats and their biokinetics. They observed that about 30% of the nanoparticles were deposited on airway epithelia and rapidly cleared by mucociliary clearance. About 80% of the nanoparticles deposited in the alveoli was relocated from the epithelium into the interstitium within 24h. Re-entrainment within macrophages back to the lung epithelium and to the larynx and gastro-intestinal tract occurred and were responsible for nanoparticle clearance. Translocation across the air-blood-barrier led to persistent retention in secondary organs and tissues such as liver, soft tissue, spleen, etc.

However, translocation, as well as the biological effects induced by the particles, do not depend only on the particle size. Indeed, many factors may play a role such as the shape, surface chemistry, surface charge, etc. ^{28,29}. Furthermore, for a same material, a 5 nm, a 20 nm or a 80 nm particle (all considered as nanoparticles) can exhibit very different biological behavior and thus trigger very different biological responses. Therefore, the relationship between translocation, particle size and pathogenicity is not as simple as that and it requires further studies to better understand if indeed (and if necessary why) nanoparticles are less important for the pathogenesis of sarcoidosis than submicron particles.

The results of our advanced mineralogical analyses led us to propose a potential role of submicron silica particles in the pathogenesis of sarcoidosis. This assumption makes sense if we consider the clinical manifestations of the disease. Indeed, although it can affect any organ, more than 90% of cases involve the lungs which are affected first, arguing in favor of an inhaled antigen ¹⁰. As a matter of fact, inhaled antigens first deposit in the lungs where they can locally trigger granulomas. Then, due to their small size, nanoparticles and submicron-sized particles are able to cross the alveolo-capillary barrier, reach the blood and/or lymph circulation and be distributed to different distant organs where they can also induce granulomas ^{17,20,30}. The specific size of these particles could also explain why

submicron silica particles have not been involved so far. Indeed, to the best of our knowledge, very few studies focus on the biomonitoring of inhaled nanoparticles in human biological samples ² and when they do, the mineralogical analysis is performed on whole samples, *i.e.* without separating the bigger particles from the smaller ones. As mentioned before, it results in a partial view and the specific effects of the submicron particles cannot be adequately assessed ⁶.

Furthermore, our claim that submicron silica particles could be a causative agent of sarcoidosis, or at least an adjuvant signal, is consistent with many data reported in the literature. Indeed, correlations have been established between sarcoidosis and both environmental and occupational exposure, especially metal industry and intense agriculture through exposure to insecticides or to microbial bioaerosols in work environments with mold/mildew exposures ^{8,11,13,31}. Regarding this issue, of particular interest is the study from Deubelbeiss *et al.* ¹³ which aimed at investigating the incidence, prevalence and regional distribution of sarcoidosis in Switzerland with respect to environmental exposures. It clearly demonstrated that the regional workforce in the metal industry, water supply, air transport factories and the area of potato production, artificial meadows (grassland) and bread grains were positively associated with the frequency of sarcoidosis. Positive correlations between sarcoidosis and exposure to dust are also unfortunately illustrated by the studies on the World Trade Center responders who exhibit elevated rates of sarcoidosis, higher than in the rest of the population ^{8,11}. Newman and Newman ¹¹ proposed a slightly different contribution of inorganic particulates to the sarcoidosis pathogenesis. They suggested that exposure to inorganic particles promotes the inflammatory context necessary for the development of an excessive granulomatous immune response to a number of different antigens. Anyway, the role of fine particulates in this pathology is experiencing a growing interest. And our finding brings an additional argument in favor of an environmental factor.

Regarding the mean age of the patients (Table 1), we observed that it was lower in the sarcoidosis group (where submicron Si particles concentration was higher) than in the other ILD group (where Si particles concentration was lower), suggesting that Si deposition in the lungs is not necessarily related to the patient age. This observation strengthens our hypothesis that submicron Si particles may be involved in the onset or development of sarcoidosis. Similarly, the smoking status doesn't seem to play a key role as it is similar between the two groups: 14% and 12% for sarcoidosis and other ILD respectively.

When all patients were considered (Table 3 and Figure 1), we found different median and mean submicron silica particle concentrations between patients suffering from sarcoidosis and patients suffering from other ILD. But when we considered only the patients exhibiting a positive submicron silica particle concentration (*i.e.*, concentrations higher than the LoD, Table 4) we observed no statistically significant difference. However, the frequency of the submicron silica particle presence was much higher in patients suffering from sarcoidosis (Table 4). It is consistent with the fact that a genetic susceptibility is necessary to develop the disease, and it could explain why out of two individuals exhibiting a similar submicron silica particle concentration, only one could develop the disease. Still, we must be very cautious in the interpretation of this finding and we should keep in mind that a higher concentration of submicron silica particles in pulmonary lavages from patients suffering from sarcoidosis is not an evidence of causality. It just suggests that the lung biopersistence of these submicron silica particles may be involved, one way or another, in the pathogenesis of this disease. To develop sarcoidosis three conditions must be met: exposure to one or more antigens, a favorable genetic background, and a hyper-reactive immune system ^{7,9,10}. Further studies are necessary to investigate more deeply the potential role of inhaled, submicron silica particles in sarcoidosis. Indeed, this work is an observatory study and from our finding we can now focus on this disease and these specific particles and carry out further physico-chemical

characterizations to sharpen our conclusions and better define some features such as the origin or the crystalline/amorphous nature of the silica particles identified. It should be especially interesting to combine advanced mineralogical analyses to *in vitro* / *in vivo* studies so as to precisely determine if these particles are able to trigger biological effects and by which mechanisms as previously suggested ^{2,17}.

Conclusion

We suggest that inhaled, submicron silica particles could have a role in the development of sarcoidosis and present an additional argument in favor of an environmental factor involved in the etiology of this disease. This specific case underscores the relevance of mineralogical analyses including the micro to nano-sized fractions towards a better understanding of the development of some respiratory diseases. It opens new perspectives in the clinical nanotoxicology field.

Conflicts of interest

There are no conflicts of interest to declare.

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Figure captions

Figure 1 – Submicron Si particles concentration in BW and BAL of patients suffering either from sarcoidosis or another type of ILD. The median (in bold), minimal and maximal values, as well as the first and third quartiles are indicated. The number of patients from each group is reported in parentheses (please note that due to technical reasons 7 BW and 6 BAL samples could not be analyzed).

Figure 2 – Submicron Si particles detected in the BW from patient n°12 using scanning electron microscope coupled to an EDX detector.

Figure 1

Submicron Si particles concentration (ppb)

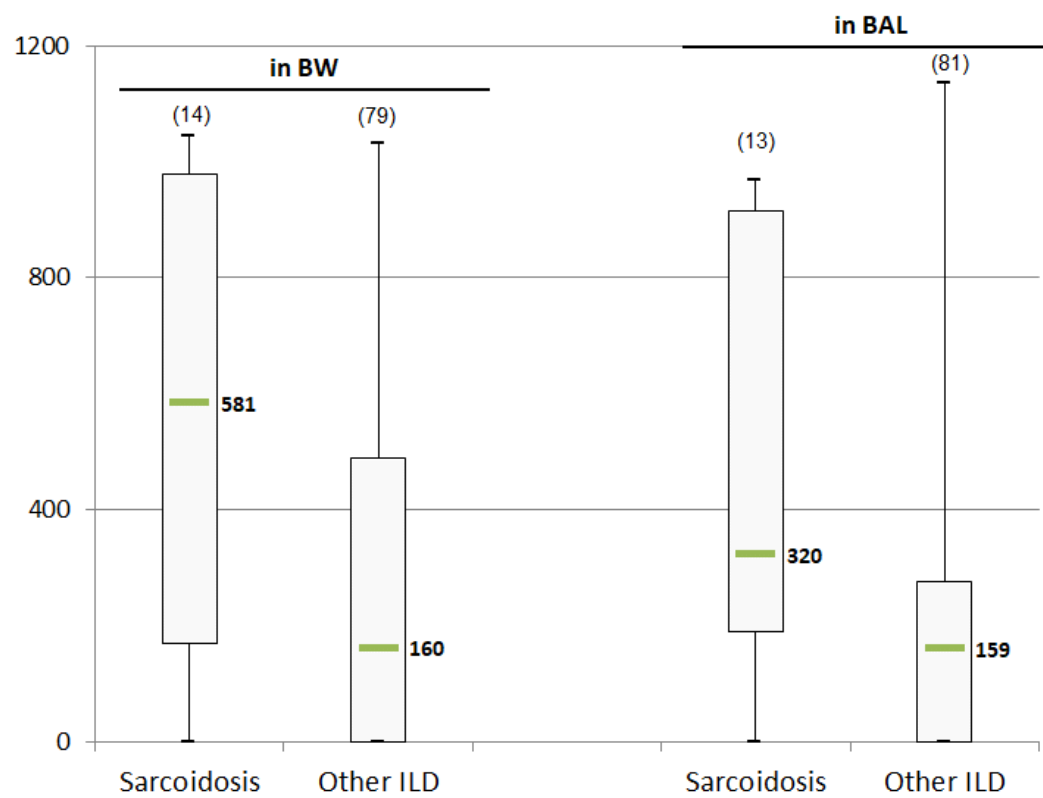


Figure 2

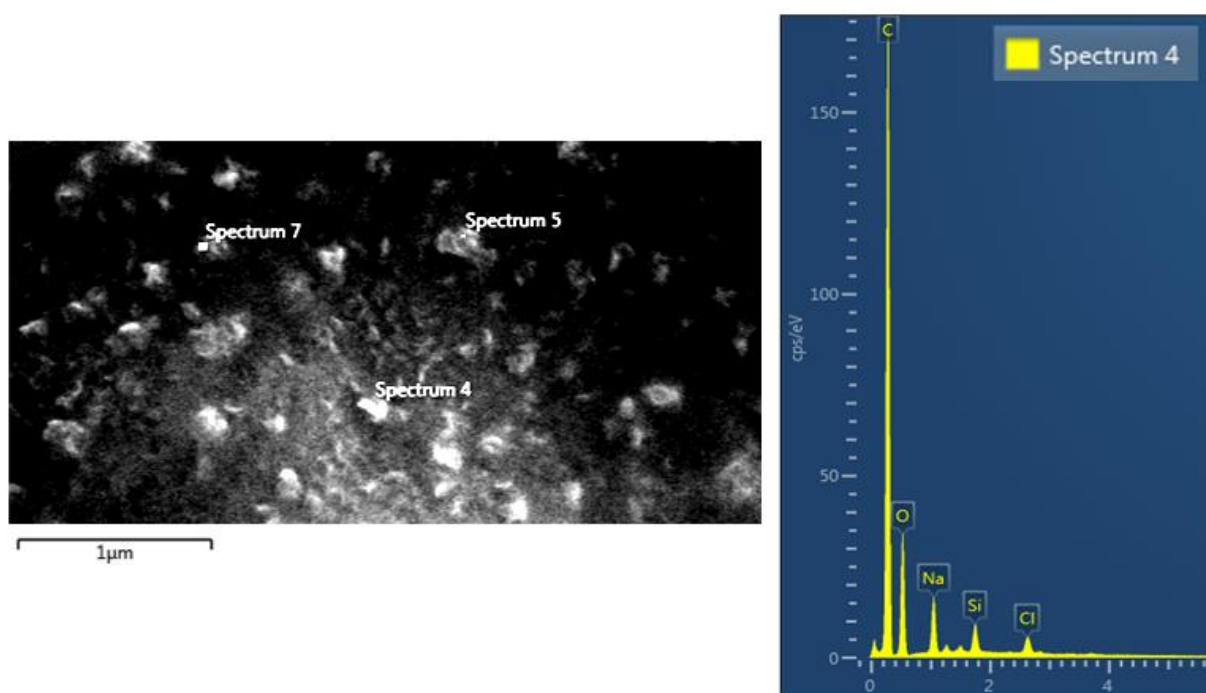


Table 1 – Description of the cohort.

Disease	Number of patients	Mean age [min-max]	Sex ratio (M:F)	Smokers (former smokers)
Sarcoidosis	14	53 [28-85]	6:8	14% (7%)
Other ILD:	86	72 [27-91]	62:24	12% (42%)
Idiopathic nonspecific interstitial pneumonia	11			
Idiopathic pulmonary fibrosis	9			
Drug related ILD	15			
Infectious ILD	12			
Hypersensitivity pneumonitis	7			
Auto-immune pneumonitis	5			
Lymphangitis carcinomatosa /Neoplasia	4			
Desquamative interstitial pneumonia	3			
Pneumoconiosis	2			
Pulmonary veno-occlusive disease	2			
Silicosis	1			
Microscopic polyangiitis	1			
Granulomatosis with polyangiitis (Wegener's granulomatosis)	1			
Left heart failure	1			
Lipoid pneumonia	1			
Bronchiolitis obliterans	1			
Antisynthetase syndrome	2			
Others	8			

Table 2 – Clinical features of the patients suffering from sarcoidosis. Please note that it was impossible to perform the BAL for patient n°2, only BW was obtained for this patient. All patients are Caucasian except patient n°11 who is black. GI: non necrotic granulomatous inflammation, MDD: multi-disciplinary discussion, EBUS: Endo-bronchial ultra-sound, TBNA: transbronchial needle aspiration, Neg: negative. * indicates the cases of multi-organ sarcoidosis.

Patient	Gender	Age	Smoker	Occupation(s)	% lymphocytes in BAL	CT scan	Diagnostic tools
1	F	59	No	Seamstress, industrial cleaning (wood), housekeeping	58	Stage II	GI on bronchial biopsies and EBUS TBNA
2	F	69	No	Farming	-	Stage II	GI on lung biopsies, mediastinal nodes and bone marrow*
3	F	64	No	Seamstress, housekeeping, childminder, library assistant	86	Stage II	GI on mediastinal lymph nodes (mediastinoscopy)
4	M	79	No	Serviceman, druggist, accountant	83	Stage III	CD4 alveolitis, ACE elevated, lung biopsies refused, diagnosis validated by MDD
5	F	44	No	Animator (retirement home)	30	Stage II	GI on bronchial biopsies and EBUS TBNA (4R,7, 11R), Lofgren syndrome
6	F	44	No	Industrial cleaning	15	Stage II	Neg. bronchial biopsies, ACE elevated, typical CT scan, initial Lofgren syndrome
7	F	69	No	Secretary, police	50	Stage II	GI on mediastinoscopy + ENT lesions*
8	M	48	Yes	Drilling (stone quarry), machine operator, public works	7	Stage II	ACE elevated, specific uveitis, diagnosis validated by MDD*
9	F	52	No	Homecare assistant	33	Stage II	Neg. EBUS and mediastinoscopy, diagnosis validated by MDD
10	M	37	No	Masonry, Serviceman (Paris firefighter)	32	Stage II	GI on bronchial biopsies
11	M	43	No	Electrician, agro-food company, metallurgy, automobile industry	34	Stage II	GI on transbronchial biopsies and conventional TBNA, sytemic involvement: ENT, skin, liver*
12	F	27	No	Controller (train), music teacher	32	Stage II	GI on bronchial biopsies
13	M	25	No	Electrician, salesman	39	Stage II	GI on bronchial biopsies, initial Lofgren syndrome
14	M	24	Yes	Plasterer, painter, masonry, mover, mechanic industry, serviceman, greengrocer	14	Stage II	GI on bronchial biopsies

Table 3 – Comparison of the median and mean concentration of Si and Al microparticles and submicron particles in BW and BAL of patients suffering either from sarcoidosis or another type of ILD. p calculated with a Mann-Whitney test. Differences are considered significant when $p < 0.05$ and are indicated in bold.

	Median		Mean	
	Sarcoidosis	Other ILD	Sarcoidosis	Other ILD
Submicron Si particles concentration in BW (ppb)	581.5	159.8	564.2 ± 442.7	292 ± 385.2
	p = 0.017			
Submicron Si particles concentration in BAL (ppb)	320.4	159.3	501.2 ± 399.4	246.2 ± 342.1
	p = 0.022			
Si microparticles concentration in BAL (ppb)	0	0	247.4 ± 279.5	92.3 ± 198.5
	p = 0.242			
Al microparticles concentration in BW (ppb)	186	0	151.8 ± 121.9	109.2 ± 191.2
	p = 0.254			

Table 4 – Mean Si concentration in the submicron particle fraction extracted from pulmonary lavage fluids of patients at levels higher than the limit of detection (LoD). The number of patients exhibiting Si concentration higher than the LoD is also indicated. A Mann-Whitney test was performed to investigate statistically significant differences of Si concentration between the sarcoidosis and other ILD groups; ns means not significant.

	BW		BAL	
	Sarcoidosis	Other ILD	Sarcoidosis	Other ILD
Mean Si submicron particles concentration \pm SD (ppb)	658.28 \pm 405.11	549.28 \pm 370.83	529.37 \pm 363.35	463.86 \pm 345.75
	ns		ns	
Prevalence of patients with detectable Si submicron particles	12/14 86%	42/79 53%	11/13 85%	43/81 53%

Supplementary information

1. Details on the sample pre-treatment for the separation of the micro, sub-micro and nano/ion fractions

The process developed for particle size fractionation consists of a 2 step-centrifugation. The first centrifugation was short and allowed to separate the fraction containing microparticles from the fraction containing sub-micro and nano-sized particles and potentially soluble metals (ions). The second centrifugation, longer than the first one, allowed separating sub-microparticles from nanoparticles and ions.

Step 1: isolation of the “micro” fraction – Samples were vortexed and sonicated using a Branson sonicator and Cup Horn (70%, 2 min at room temperature). 1 mL of sample was transferred into a 2 mL Protein LoBind tube (Eppendorf®). 0.5 mL of an extraction cushion consisting of a glycerol 75%v/v, NaOH 0.001 M 25%v/v solution was added to the samples. Because of their difference of density, the sample and the glycerol solution did not mixed. The tubes were centrifuged 10 min at 0°C, 1600g (4000 rpm) (Thermo Fisher Scientific Heraeus megafuge 16R). Tubes were then punctured at the level of the 0.25 mL mark with a needle (22G x 1^{1/2} Terumo®) mounted on a 2 mL syringe and the supernatant was aspirated and transferred into a new Protein LoBind tube, this part contained the sub-microparticles, nanoparticles and ions (≈ 1.25 mL). The pellet containing the microparticles was resuspended with 0.75 mL of NaOH 0.001 M and was transferred into a new Protein LoBind tube and stored at 4°C until analysis.

Step 2: separation of the “sub-micro” fraction from nano and ions – 0.5 mL of an extraction cushion (glycerol 75%v/v, NaOH 0.001 M 25%v/v) was added to the tube containing the sub-microparticles, nanoparticles and ions fractions obtained from step 1. Tubes were centrifuged during 3h30 at 0°C, 2500 g (5000 rpm). Tubes were then punctured at the 0.5 mL mark with a

needle (22G x 1^{1/2} Terumo[®]) mounted on a 2 mL syringe and the supernatant was slowly aspirated and transferred into a new Protein LoBind tube, this part contained nanoparticles and ions. The pellet containing the sub-microparticles was resuspended with 0.6 mL of NaOH 0.001 M and was transferred into a new Protein LoBind tube and stored at 4°C until analysis. To assess the background noise a “blank” sample consisting of distilled water underwent the same protocol.

2. Comprehensive results of the mineralogical analyses

In Tables S1 to S12 are reported the mean concentrations observed for each element in the micron, submicron and nano/ion fractions for BW and BAL.

Table S1 - Mean Al concentration \pm standard deviation (ppb).

Disease	Number of patients	BW			BAL		
		Micro particles	Submicron particles	Nanoparticles/Ion	Micro particles	Submicron particles	Nanoparticles/Ion
Sarcoidosis	14	152 \pm 122	18 \pm 26	55 \pm 132	0	11 \pm 11	3 \pm 4
Other ILD:	86	109 \pm 191	8 \pm 17	28 \pm 101	38 \pm 129	5 \pm 9	2 \pm 3
Idiopathic nonspecific interstitial pneumonia	11	274 \pm 433	24 \pm 37	127 \pm 249	0	7 \pm 10	2 \pm 3
Idiopathic pulmonary fibrosis	9	23 \pm 68	0	2 \pm 3	51 \pm 101	0	2 \pm 3
Drug related ILD	15	103 \pm 134	8 \pm 17	24 \pm 79	80 \pm 118	5 \pm 8	2 \pm 3
Infectious ILD	12	150 \pm 144	12 \pm 16	27 \pm 81	0	11 \pm 11	2 \pm 3
Hypersensitivity pneumonitis	7	148 \pm 139	11 \pm 9	15 \pm 31	0	10 \pm 10	2 \pm 3
Auto-immune pneumonitis	5	0	0	3 \pm 3	196 \pm 437	0	0
Lymphangitis carcinomatosa /Neoplasia	4	137 \pm 137	11 \pm 11	54 \pm 80	58 \pm 100	10 \pm 10	5 \pm 3
Desquamative interstitial pneumonia	3	58 \pm 101	0	2 \pm 4	0	0	0
Pneumoconiosis	2	0	0	3 \pm 4	0	0	6 \pm 0
Pulmonary Pulmonary veno-occlusive disease	2	0	0	0	0	0	0
Silicosis	1	290	19	7	0	25	0
Microscopic polyangiitis	1	0	0	0	0	0	0
Granulomatosis with polyangiitis (Wegener's granulomatosis)	1	0	0	0	0	0	7
Left heart failure	1	0	0	0	0	0	0
Lipoid pneumonia	1	0	0	0	0	0	0
Bronchiolitis obliterans	1	0	0	0	0	0	0
Antisynthetase syndrome	2	235 \pm 68	11 \pm 16	3 \pm 4	0	10 \pm 14	0
Others	8	62 \pm 124	0	1 \pm 3	58 \pm 116	2 \pm 4	1 \pm 3

Table S2 - Mean Be concentration \pm standard deviation (ppb).

Disease	Number of patients	BW			BAL		
		Micro particles	Submicron particles	Nanoparticles/Ion	Micro particles	Submicron particles	Nanoparticles/Ion
Sarcoidosis	14	0	0 \pm 1	0	0	0	10 \pm 35
Other ILD:	86	0	0 \pm 1	0 \pm 1	0	0	6 \pm 26
Idiopathic nonspecific interstitial pneumonia	11	0	0	0	0	0	0
Idiopathic pulmonary fibrosis	9	0	0 \pm 1	0	0	0	0
Drug related ILD	15	0	0	0 \pm 1	0	0 \pm 1	17 \pm 45
Infectious ILD	12	0	0 \pm 1	0 \pm 1	0	0	0
Hypersensitivity pneumonitis	7	0	1 \pm 2	0	0	0	32 \pm 54
Auto-immune pneumonitis	5	0	1 \pm 1	0	0	0	0
Lymphangitis carcinomatosa /Neoplasia	4	0	2 \pm 2	2 \pm 4	0	0	8 \pm 14
Desquamative interstitial pneumonia	3	0	0	0	0	0	0
Pneumoconiosis	2	0	0	0	0	0	0
Pulmonary veno-occlusive disease	2	0	0	0	0	0	0
Silicosis	1	0	0	0	0	0	0
Microscopic polyangiitis	1	0	0	0	0	0	0
Granulomatosis with polyangiitis (Wegener's granulomatosis)	1	0	0	0	0	0	0
Left heart failure	1	0	0	0	0	0	0
Lipoid pneumonia	1	0	0	0	0	0	0
Bronchiolitis obliterans	1	0	0	0	0	0	0
Antisynthetase syndrome	2	0	0	0	0	0	0
Others	8	0	0	0	0	0	0

Table S3 - Mean Co concentration \pm standard deviation (ppb).

Disease	Number of patients	BW			BAL		
		Micro particles	Submicron particles	Nanoparticles/Ion	Micro particles	Submicron particles	Nanoparticles/Ion
Sarcoidosis	14	0	1 \pm 1	1 \pm 2	0	0 \pm 1	0
Other ILD:	86	0 \pm 3	1 \pm 2	0 \pm 1	0	0 \pm 1	0
Idiopathic nonspecific interstitial pneumonia	11	0	1 \pm 2	0	0	0	0
Idiopathic pulmonary fibrosis	9	0	0 \pm 1	0	0	1 \pm 1	0
Drug related ILD	15	1 \pm 2	1 \pm 2	0 \pm 1	0	0 \pm 1	0 \pm 1
Infectious ILD	12	0	1 \pm 2	1 \pm 1	0	1 \pm 1	0
Hypersensitivity pneumonitis	7	0	1 \pm 3	0 \pm 1	0	0 \pm 1	0
Auto-immune pneumonitis	5	0	1 \pm 1	0	0	0	0
Lymphangitis carcinomatosa /Neoplasia	4	6 \pm 10	0 \pm 1	2 \pm 3	0	1 \pm 1	0
Desquamative interstitial pneumonia	3	0	0 \pm 1	0	0	0	0
Pneumoconiosis	2	0	0	0	0	1 \pm 1	0
Pulmonary veno-occlusive disease	2	0	0	0	0	0	0
Silicosis	1	0	0	0	0	0	0
Microscopic polyangiitis	1	0	0	0	0	0	0
Granulomatosis with polyangiitis (Wegener's granulomatosis)	1	0	0	0	0	0	0
Left heart failure	1	0	0	0	0	0	0
Lipoid pneumonia	1	0	1	0	0	0	0
Bronchiolitis obliterans	1	0	0	0	0	0	0
Antisynthetase syndrome	2	0	1 \pm 1	0	0	0	0
Others	8	0	0 \pm 1	0	0	0 \pm 1	0

Table S4 - Mean Cr concentration \pm standard deviation (ppb).

Disease	Number of patients	BW			BAL		
		Micro particles	Submicron particles	Nanoparticles/Ion	Micro particles	Submicron particles	Nanoparticles/Ion
Sarcoidosis	14	1 \pm 1	15 \pm 5	13 \pm 2	0 \pm 1	12 \pm 1	13 \pm 1
Other ILD:	86	0	13 \pm 2	13 \pm 3	0 \pm 1	12 \pm 1	12 \pm 2
Idiopathic nonspecific interstitial pneumonia	11	0	13 \pm 1	13 \pm 2	0	13 \pm 0	13 \pm 1
Idiopathic pulmonary fibrosis	9	0	13 \pm 3	11 \pm 4	0	12 \pm 1	13 \pm 0
Drug related ILD	15	0 \pm 1	13 \pm 1	13 \pm 1	0	12 \pm 2	12 \pm 1
Infectious ILD	12	0	13 \pm 3	13 \pm 3	0	12 \pm 1	13 \pm 1
Hypersensitivity pneumonitis	7	0	14 \pm 4	13 \pm 2	0	11 \pm 2	11 \pm 5
Auto-immune pneumonitis	5	0 \pm 1	13 \pm 1	13 \pm 1	0	12 \pm 1	12 \pm 1
Lymphangitis carcinomatosa /Neoplasia	4	0	15 \pm 2	17 \pm 6	0	12 \pm 0	13 \pm 1
Desquamative interstitial pneumonia	3	0	12 \pm 1	13 \pm 0	0	12 \pm 1	12 \pm 1
Pneumoconiosis	2	0	11 \pm 1	13 \pm 0	0	13 \pm 0	14 \pm 1
Pulmonary veno-occlusive disease	2	0	13 \pm 0	12 \pm 0	0	13 \pm 0	12 \pm 1
Silicosis	1	0	12	11	0	13	14
Microscopic polyangiitis	1	0	10	14	0	13	14
Granulomatosis with polyangiitis (Wegener's granulomatosis)	1	0	11	11	0	12	13
Left heart failure	1	0	10	12	0	12	13
Lipoid pneumonia	1	0	12	13	0	11	12
Bronchiolitis obliterans	1	0	13	12	5	13	13
Antisynthetase syndrome	2	0	13 \pm 1	12 \pm 1	0	13	13
Others	8	0	12 \pm 1	13 \pm 1	0	13 \pm 0	12 \pm 1

Table S5 - Mean Cu concentration \pm standard deviation (ppb).

Disease	Number of patients	BW			BAL		
		Micro particles	Submicron particles	Nanoparticles/Ion	Micro particles	Submicron particles	Nanoparticles/Ion
Sarcoidosis	14	7 \pm 14	83 \pm 151	9 \pm 14	2 \pm 8	3 \pm 7	0
Other ILD:	86	3 \pm 8	13 \pm 51	3 \pm 9	8 \pm 16	3 \pm 7	0 \pm 2
Idiopathic nonspecific interstitial pneumonia	11	4 \pm 12	9 \pm 12	7 \pm 13	12 \pm 17	4 \pm 9	0
Idiopathic pulmonary fibrosis	9	0	0	0	13 \pm 20	0	0
Drug related ILD	15	5 \pm 13	5 \pm 10	4 \pm 9	5 \pm 15	1 \pm 5	0
Infectious ILD	12	0	7 \pm 10	3 \pm 8	12 \pm 20	6 \pm 9	1 \pm 5
Hypersensitivity pneumonitis	7	7 \pm 12	61 \pm 147	6 \pm 12	7 \pm 11	2 \pm 7	0
Auto-immune pneumonitis	5	1 \pm 2	0	0	10 \pm 13	0	0
Lymphangitis carcinomatosa /Neoplasia	4	1 \pm 3	74 \pm 88	14 \pm 14	0	5 \pm 9	0
Desquamative interstitial pneumonia	3	0	0	0	0	0	0
Pneumoconiosis	2	0	0	0	0	0	0
Pulmonary veno-occlusive disease	2	0	0	0	13 \pm 19	0	0
Silicosis	1	0	19	0	0	17	0
Microscopic polyangiitis	1	0	0	0	28	0	0
Granulomatosis with polyangiitis (Wegener's granulomatosis)	1	0	0	0	0	0	0
Left heart failure	1	0	0	0	53	0	0
Lipoid pneumonia	1	0	0	0	0	0	0
Bronchiolitis obliterans	1	0	0	0	0	0	0
Antisynthetase syndrome	2	0	9 \pm 12	8 \pm 11	0	12 \pm 16	0
Others	8	7 \pm 15	0	0	0	0	0

Table S6 - Mean Fe concentration \pm standard deviation (ppb).

Disease	Number of patients	BW			BAL		
		Micro particles	Submicron particles	Nanoparticles/Ion	Micro particles	Submicron particles	Nanoparticles/Ion
Sarcoidosis	14	48 \pm 52	26 \pm 19	177 \pm 196	18 \pm 34	4 \pm 6	5 \pm 7
Other ILD:	86	32 \pm 109	21 \pm 69	62 \pm 211	42 \pm 80	14 \pm 23	22 \pm 82
Idiopathic nonspecific interstitial pneumonia	11	8 \pm 24	13 \pm 5	29 \pm 18	36 \pm 56	14 \pm 20	10 \pm 11
Idiopathic pulmonary fibrosis	9	20 \pm 41	8 \pm 6	18 \pm 12	22 \pm 44	11 \pm 17	5 \pm 6
Drug related ILD	15	68 \pm 230	12 \pm 9	23 \pm 18	18 \pm 37	9 \pm 6	10 \pm 7
Infectious ILD	12	30 \pm 43	12 \pm 7	30 \pm 25	28 \pm 39	10 \pm 8	14 \pm 21
Hypersensitivity pneumonitis	7	11 \pm 29	7 \pm 7	9 \pm 10	24 \pm 41	5 \pm 8	4 \pm 7
Auto-immune pneumonitis	5	14 \pm 31	5 \pm 7	3 \pm 7	77 \pm 125	7 \pm 10	5 \pm 7
Lymphangitis carcinomatosa /Neoplasia	4	15 \pm 25	15 \pm 4	54 \pm 23	19 \pm 34	12 \pm 7	7 \pm 7
Desquamative interstitial pneumonia	3	0	4 \pm 7	7 \pm 6	0	10 \pm 1	13 \pm 2
Pneumoconiosis	2	0	7 \pm 9	15 \pm 22	33 \pm 47	11 \pm 16	13 \pm 18
Pulmonary veno-occlusive disease	2	70 \pm 99	27 \pm 22	6 \pm 8	266 \pm 248	46 \pm 44	15 \pm 0
Silicosis	1	158	615	1745	73	15	16
Microscopic polyangiitis	1	69	53	383	105	56	489
Granulomatosis with polyangiitis (Wegener's granulomatosis)	1	275	89	360	377	131	562
Left heart failure	1	70	70	50	122	115	17
Lipoid pneumonia	1	0	0	0	0	0	0
Bronchiolitis obliterans	1	0	0	0	200	0	0
Antisynthetase syndrome	2	0	18 \pm 25	114 \pm 162	38 \pm 54	9 \pm 13	0
Others	8	0	17 \pm 22	147 \pm 259	18 \pm 36	6 \pm 7	6 \pm 7

Table S7 - Mean Ni concentration \pm standard deviation (ppb).

Disease	Number of patients	BW			BAL		
		Micro particles	Submicron particles	Nanoparticles/Ion	Micro particles	Submicron particles	Nanoparticles/Ion
Sarcoidosis	14	3 \pm 8	9 \pm 18	0	0	0	0
Other ILD:	86	3 \pm 7	1 \pm 6	0	0	0	0
Idiopathic nonspecific interstitial pneumonia	11	2 \pm 7	0	0	0	0	0
Idiopathic pulmonary fibrosis	9	2 \pm 6	0	0	0	0	0
Drug related ILD	15	3 \pm 8	0	0	0	0	0
Infectious ILD	12	2 \pm 6	0	0	0	0	0
Hypersensitivity pneumonitis	7	3 \pm 8	6 \pm 16	0	0	0	0
Auto-immune pneumonitis	5	0 \pm 1	0	0	0	0	0
Lymphangitis carcinomatosa /Neoplasia	4	1 \pm 1	9 \pm 15	0	0	0	0
Desquamative interstitial pneumonia	3	9 \pm 15	0	0	0	0	0
Pneumoconiosis	2	0	0	0	0	0	0
Pulmonary veno-occlusive disease	2	0	0	0	0	0	0
Silicosis	1	0	0	0	0	0	0
Microscopic polyangiitis	1	0	0	0	0	0	0
Granulomatosis with polyangiitis (Wegener's granulomatosis)	1	17	0	0	0	0	0
Left heart failure	1	0	0	0	0	0	0
Lipoid pneumonia	1	0	0	0	0	0	0
Bronchiolitis obliterans	1	22	0	0	0	0	0
Antisynthetase syndrome	2	0	0	0	0	0	0
Others	8	0	0	0	0	0	0

Table S8 - Mean Si concentration \pm standard deviation (ppb).

Disease	Number of patients	BW			BAL		
		Micro particles	Submicron particles	Nanoparticles/Ion	Micro particles	Submicron particles	Nanoparticles/Ion
Sarcoidosis	14	279 \pm 226	564 \pm 443	0	247 \pm 280	501 \pm 399	0
Other ILD:	86	205 \pm 257	292 \pm 385	10 \pm 92	92 \pm 199	246 \pm 342	0
Idiopathic nonspecific interstitial pneumonia	11	168 \pm 165	457 \pm 489	0	143 \pm 250	347 \pm 411	0
Idiopathic pulmonary fibrosis	9	101 \pm 151	90 \pm 111	0	0	59 \pm 88	0
Drug related ILD	15	240 \pm 381	211 \pm 361	54 \pm 210	39 \pm 151	153 \pm 304	0
Infectious ILD	12	227 \pm 191	487 \pm 464	0	186 \pm 259	426 \pm 413	0
Hypersensitivity pneumonitis	7	261 \pm 244	499 \pm 439	0	155 \pm 265	382 \pm 408	0
Auto-immune pneumonitis	5	337 \pm 312	127 \pm 116	0	46 \pm 103	141 \pm 130	0
Lymphangitis carcinomatosa /Neoplasia	4	427 \pm 379	478 \pm 478	0	246 \pm 247	435 \pm 439	0
Desquamative interstitial pneumonia	3	181 \pm 158	144 \pm 125	0	0	138 \pm 119	0
Pneumoconiosis	2	180 \pm 254	126 \pm 178	0	0	110 \pm 156	0
Pulmonary veno-occlusive disease	2	0	0	0	0	0	0
Silicosis	1	387	953	0	516	953	0
Microscopic polyangiitis	1	298	231	0	0	205	0
Granulomatosis with polyangiitis (Wegener's granulomatosis)	1	0	166	0	0	153	0
Left heart failure	1	0	153	0	0	160	0
Lipoid pneumonia	1	317	199	0	0	209	0
Bronchiolitis obliterans	1	0	0	0	0	0	0
Antisynthetase syndrome	2	177 \pm 250	493 \pm 697	0	228 \pm 322	465 \pm 657	0
Others	8	52 \pm 104	51 \pm 102	0	0	67 \pm 133	0

Table S9 - Mean Ti concentration \pm standard deviation (ppb).

Disease	Number of patients	BW			BAL		
		Micro particles	Submicron particles	Nanoparticles/Ion	Micro particles	Submicron particles	Nanoparticles/Ion
Sarcoidosis	14	0	1 \pm 2	8 \pm 19	0	0	0 \pm 1
Other ILD:	86	3 \pm 27	1 \pm 2	4 \pm 11	0 \pm 2	0 \pm 1	1 \pm 1
Idiopathic nonspecific interstitial pneumonia	11	26 \pm 79	2 \pm 4	13 \pm 25	0	0	1 \pm 1
Idiopathic pulmonary fibrosis	9	0	1 \pm 1	1 \pm 2	0	1 \pm 2	1 \pm 1
Drug related ILD	15	0	1 \pm 2	5 \pm 13	0	0	1 \pm 1
Infectious ILD	12	0 \pm 1	1 \pm 2	3 \pm 8	0	0	0
Hypersensitivity pneumonitis	7	0	1 \pm 1	2 \pm 5	0	0	0
Auto-immune pneumonitis	5	0	1 \pm 2	1 \pm 2	0	0	0 \pm 1
Lymphangitis carcinomatosa /Neoplasia	4	0 \pm 1	1 \pm 2	6 \pm 7	0	0	1 \pm 1
Desquamative interstitial pneumonia	3	0	0	1 \pm 1	0	0	1 \pm 1
Pneumoconiosis	2	0	0	0	9 \pm 13	0	1 \pm 1
Pulmonary veno-occlusive disease	2	0	0	1 \pm 2	0	0	1 \pm 2
Silicosis	1	0	0	0	0	0	0
Microscopic polyangiitis	1	0	0	2	0	0	0
Granulomatosis with polyangiitis (Wegener's granulomatosis)	1	0	0	0	0	0	0
Left heart failure	1	0	0	0	0	0	0
Lipoid pneumonia	1	0	0	0	0	0	0
Bronchiolitis obliterans	1	0	0	0	0	0	0
Antisynthetase syndrome	2	0	0	1 \pm 1	0	0	0
Others	8	0	1 \pm 2	1 \pm 2	0	1 \pm 1	1 \pm 1

Table S10 - Mean W concentration \pm standard deviation (ppb).

Disease	Number of patients	BW			BAL		
		Micro particles	Submicron particles	Nanoparticles/Ion	Micro particles	Submicron particles	Nanoparticles/Ion
Sarcoidosis	14	2 \pm 6	34 \pm 83	0	1 \pm 4	0	4 \pm 14
Other ILD:	86	3 \pm 11	5 \pm 25	0	6 \pm 8	0	23 \pm 117
Idiopathic nonspecific interstitial pneumonia	11	0	3 \pm 8	0	5 \pm 7	0	6 \pm 19
Idiopathic pulmonary fibrosis	9	3 \pm 10	0	0	4 \pm 8	0	2 \pm 7
Drug related ILD	15	6 \pm 17	16 \pm 55	0	8 \pm 10	0	77 \pm 226
Infectious ILD	12	5 \pm 14	0	0	4 \pm 7	0	52 \pm 171
Hypersensitivity pneumonitis	7	0	7 \pm 18	0	3 \pm 7	0	0
Auto-immune pneumonitis	5	0	8 \pm 19	0	6 \pm 8	0	0
Lymphangitis carcinomatosa /Neoplasia	4	4 \pm 7	9 \pm 15	0	0	0	0
Desquamative interstitial pneumonia	3	0	0	0	4 \pm 8	0	0
Pneumoconiosis	2	0	0	0	9 \pm 12	0	0
Pulmonary veno-occlusive disease	2	0	0	0	18 \pm 3	0	0
Silicosis	1	0	0	0	0	0	0
Microscopic polyangiitis	1	0	0	0	0	0	0
Granulomatosis with polyangiitis (Wegener's granulomatosis)	1	0	0	0	0	0	0
Left heart failure	1	0	0	0	15	0	0
Lipoid pneumonia	1	0	0	0	0	0	0
Bronchiolitis obliterans	1	0	0	0	16	0	0
Antisynthetase syndrome	2	0	0	0	9 \pm 13	0	0
Others	8	11 \pm 23	0	0	14 \pm 12	0	0

Table S11 - Mean Zn concentration \pm standard deviation (ppb).

Disease	Number of patients	BW			BAL		
		Micro particles	Submicron particles	Nanoparticles/Ion	Micro particles	Submicron particles	Nanoparticles/Ion
Sarcoidosis	14	10 \pm 6	66 \pm 127	19 \pm 16	11 \pm 2	5 \pm 8	4 \pm 5
Other ILD:	86	7 \pm 10	11 \pm 50	10 \pm 10	11 \pm 4	2 \pm 5	1 \pm 3
Idiopathic nonspecific interstitial pneumonia	11	7 \pm 7	8 \pm 14	16 \pm 14	11 \pm 3	4 \pm 8	2 \pm 4
Idiopathic pulmonary fibrosis	9	8 \pm 18	0	8 \pm 6	10 \pm 5	0	0
Drug related ILD	15	8 \pm 13	3 \pm 9	8 \pm 6	9 \pm 5	2 \pm 6	2 \pm 3
Infectious ILD	12	6 \pm 6	5 \pm 9	12 \pm 9	13 \pm 4	2 \pm 6	1 \pm 3
Hypersensitivity pneumonitis	7	9 \pm 4	47 \pm 115	11 \pm 13	11 \pm 3	2 \pm 6	1 \pm 4
Auto-immune pneumonitis	5	4 \pm 7	0	6 \pm 4	17 \pm 9	0	0
Lymphangitis carcinomatosa /Neoplasia	4	12 \pm 8	80 \pm 139	21 \pm 14	9 \pm 1	5 \pm 6	2 \pm 4
Desquamative interstitial pneumonia	3	6 \pm 5	0	3 \pm 6	10 \pm 1	0	0
Pneumoconiosis	2	8 \pm 11	0	3 \pm 4	12 \pm 3	0	0
Pulmonary veno-occlusive disease	2	0	0	0	12 \pm 1	0	0
Silicosis	1	13	15	33	11	0	0
Microscopic polyangiitis	1	9	0	13	17	0	8
Granulomatosis with polyangiitis (Wegener's granulomatosis)	1	0	0	8	12	0	0
Left heart failure	1	0	0	0	11	0	0
Lipoid pneumonia	1	0	0	0	11	0	0
Bronchiolitis obliterans	1	0	0	7	11	0	0
Antisynthetase syndrome	2	6 \pm 9	3 \pm 4	11 \pm 3	10	0	0
Others	8	8 \pm 15	0	10 \pm 7	9 \pm 6	0	0

Table S12 - Mean Zr concentration \pm standard deviation (ppb).

Disease	Number of patients	BW			BAL		
		Micro particles	Submicron particles	Nanoparticles/Ion	Micro particles	Submicron particles	Nanoparticles/Ion
Sarcoidosis	14	0 \pm 1	4 \pm 8	0	0	0	0
Other ILD:	86	1 \pm 7	2 \pm 5	2 \pm 12	0	1 \pm 4	2 \pm 12
Idiopathic nonspecific interstitial pneumonia	11	0	4 \pm 8	0	0	0	0
Idiopathic pulmonary fibrosis	9	0	1 \pm 1	1 \pm 2	0	2 \pm 5	0
Drug related ILD	15	0	2 \pm 4	3 \pm 9	0	1 \pm 1	8 \pm 27
Infectious ILD	12	5 \pm 18	2 \pm 6	1 \pm 3	0	0 \pm 1	1 \pm 3
Hypersensitivity pneumonitis	7	0	3 \pm 7	15 \pm 39	0	0	0
Auto-immune pneumonitis	5	2 \pm 2	2 \pm 5	1 \pm 3	0	1 \pm 1	0
Lymphangitis carcinomatosa /Neoplasia	4	0	10 \pm 11	0	0	10 \pm 15	0
Desquamative interstitial pneumonia	3	0	0	0	0	0	0
Pneumoconiosis	2	3 \pm 4	0	0	0	0	0
Pulmonary veno-occlusive disease	2	0	0	0	0	0	0
Silicosis	1	0	0	0	0	0	0
Microscopic polyangiitis	1	0	0	0	0	0	0
Granulomatosis with polyangiitis (Wegener's granulomatosis)	1	0	0	0	0	0	0
Left heart failure	1	0	0	0	0	0	0
Lipoid pneumonia	1	0	0	0	0	0	0
Bronchiolitis obliterans	1	0	0	0	0	0	0
Antisynthetase syndrome	2	0	0	0	0	0	0
Others	8	0	1 \pm 1	0	0	1 \pm 1	0