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Mäkelä, Kati

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Original Contribution

Inorganic particulate matter in the lung tissue of idiopathic pulmonary fibrosis patients reflects population density and fine particle levels

Kati Mäkelä[a,*], Hely Ollila[a], Eva Sutinen[a], Vesa Vuorinen[b], Emilia Peltola[b], Riitta Kaarteenaho[c], Marjukka Myllärniemi[a]

[a] Individualized Drug Therapy Research Program, Faculty of Medicine, University of Helsinki and Helsinki University Hospital, Helsinki, Finland
[b] Department of Electrical Engineering and Automation, School of Electrical Engineering, Aalto University, Espoo, Finland
[c] Research Unit of Internal Medicine, University of Oulu and Medical Research Center Oulu, Oulu University Hospital, Oulu, Finland

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ABSTRACT

Idiopathic pulmonary fibrosis (IPF) is a chronic lung disease with a dismal prognosis and an unknown etiology. Inorganic dust is a known risk factor, and air pollution seems to affect disease progression. We aimed to investigate inorganic particulate matter in IPF lung tissue samples. Using polarizing light microscopy, we examined coal dust pigment and inorganic particulate matter in 73 lung tissue samples from the FinnishIPF registry. We scored the amount of coal dust pigment and particulate matter from 0 to 5. Using energy dispersive spectrometry with a scanning electron microscope, we conducted an elemental analysis of six IPF lung tissue samples. We compared the results to the registry data, and to the population density and air quality data. To compare categorical data, we used Fisher's exact test; we estimated the survival of the patients with Kaplan-Meier curves. We found inorganic particulate matter in all samples in varying amounts. Samples from the southern regions of Finland, where population density and fine particle levels are high, more often had particulate matter scores from 3 to 5 than samples from the northern regions (31/50, 62.0% vs. 7/23, 30.4%, $p = 0.02$). The highest particulate matter scores of 4 and 5 ($n = 15$) associated with a known exposure to inorganic dust ($p = 0.004$). An association between particulate matter in the lung tissue of IPF patients and exposure to air pollution may exist.

1. Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic lung disease in which lung tissue is replaced by fibrosis, leading to poor ventilation function and eventually to death without lung transplantation. Despite active research in the field, the etiology and pathogenesis of IPF remain partly obscure. In addition to infections and immunological mechanisms, exposures to environmental factors have been suggested [1]. In case-control studies, exposure to metal dust has correlated with an increased risk for IPF [2-6]. Other occupational factors like stone cutting or polishing, working in metallurgical or steel industry, wood dust, farming, livestock, vegetable or animal dust, and hairdressing have also been associated with IPF [4,6,7].

Inorganic dust can be visualized as birefringent particulate matter (PM) in lung tissues using polarizing light microscopy. The amount of inorganic PM examined by both polarizing light microscopy and scanning electron microscopy (SEM) has been greater in lung tissue samples of IPF than in samples of other types of lung diseases [8]. High concentrations of silicon (Si) and aluminum (Al) have been detected in the lung tissue of IPF patients [8,9] and also in the pulmonary lymph nodes of IPF patients compared with controls [10].

Long-term exposure to nitrogen oxides in traffic pollution has been associated with a higher prevalence of radiological interstitial lung abnormalities [11]. Chronic exposure to nitrogen dioxide (NO_2) in traffic pollution has been shown to increase the incidence of IPF [12]. Increased air pollution concentrations in the six preceding weeks have been associated with acute exacerbations of IPF [13,14]. In a prospective cohort study of IPF patients, lower mean lung function values were linked to high exposure to particulate matter $< 10\,\mu m$ in diameter (PM_{10}), fine particles (particulate matter $< 2.5\,\mu m$ in diameter, $PM_{2.5}$), and NO_2, but no association with changes in lung function values was detected [15]. In a retrospective analysis of 135 IPF patients, however, PM_{10} levels were associated with the rate of decline in forced vital capacity (FVC) [16]. Increased exposure to $PM_{2.5}$ was associated with

* Corresponding author at: Transplantation Laboratory, Pulmonary Diseases, B410b, Haartman Institute, Haartmaninkatu 3, FI-00290 Helsinki, Finland.
E-mail addresses: kati.makela@helsinki.fi (K. Mäkelä), hely.ollila@tuni.fi (H. Ollila), eva.sutinen@helsinki.fi (E. Sutinen), vesa.vuorinen@aalto.fi (V. Vuorinen), emilia.peltola@aalto.fi (E. Peltola), riitta.kaarteenaho@oulu.fi (R. Kaarteenaho), marjukka.myllarniemi@helsinki.fi (M. Myllärniemi).

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an accelerated rate of increase in oxygen use in the six-minute walk test (6MWT) [16]. An elevated risk of mortality in IPF patients by increased long-term cumulative concentrations of PM_{10} and $PM_{2.5}$ was reported [14].

Expanding epidemiological evidence suggests that air pollution negatively affects IPF, however, the histological evidence is limited. We hypothesized that inorganic PM may exist in the lung tissue samples of IPF patients. Therefore, we aimed to determine the amount of birefringent inorganic PM in lung tissue samples from the FinnishIPF registry [17]. For this observational histological study, we developed a semiquantitative scoring method using polarizing light microscopy. We compared the results of the analysis with patients' demographics, location of residence, smoking status, occupational and exposure history, clinical data, and survival. Furthermore, we investigated the elemental composition of inorganic PM in the lung tissue samples of IPF patients, by energy dispersive spectrometry (EDS) with a field emission scanning electron microscope (FESEM).

2. Materials and methods

2.1. Patient collection

The study population was collected from the FinnishIPF registry [17] for the polarizing light microscopy analysis. The FinnishIPF registry is a prospective, clinical cohort study of Finnish IPF patients diagnosed by the 2011 ATS/ERS/JRS/ALAT criteria [1] and approved by the Ethics Committees of the Finnish University Hospitals of Helsinki, Turku, Tampere, Kuopio, and Oulu. All patients provided written informed consent. The registry was screened for patients with available lung tissue sample before January 2017, resulting in 73 IPF patient samples, of which 63 (86.3%) were surgical lung biopsies (SLB), six (8.2%) were explant samples, and four (5.5%) were autopsy samples. Sixty samples had been previously analyzed histologically [18]. The characteristics of the study population in the polarizing light microscopy analysis are shown in Table 1. For the FESEM-EDS analysis, 14 IPF samples were collected from the FinnishIPF registry [17]. IPF samples were different from those used in the polarizing light microscopy analysis.

2.2. Data collection

The results of IPF patients were compared with the FinnishIPF registry data [17], which included gender, birth, diagnosis, transplantation, and death date, location of residence, university hospital district, smoking status, pack-years, occupational and exposure history, body mass index (BMI), spirometry, diffusion capacity, blood count, arterial gas, 6MWT, and bronchoalveolar lavage fluid (BALF) values. The registry data, including occupational and exposure data, were collected from medical records. Occupational data were not available for all patients, and no recorded exposures were considered as no known exposures. The occupational data were categorized as organic dust exposure-related occupations, inorganic dust, chemical, or diesel exposure-related occupations, and occupations of no expected occupational exposure. The exposure data were categorized as no known exposure, organic dust exposure, and inorganic dust exposure. Occupational data and known exposures to inhalable dusts are shown in Table 1.

2.3. Polarizing light microscopy analysis

Originally, the idea was to screen for birefringent PM from IPF samples. We noticed that PM co-localized often with coal dust pigment, hence, we decided to analyze also the coal dust pigment in the samples. Every section stained with hematoxylin and eosin (HE) of each patient was firstly examined, after which one representative section from each case was selected for light microscopy analysis for the evaluation of coal dust pigment and birefringent PM. A semiquantitative, non-validated scoring method for observing the coal dust pigment and PM was developed. The scoring was conducted blinded to the origin of the sample or registry data. For observing the coal dust pigment, the samples were screened with a light microscope (Olympus BX51, Olympus Corporation, Tokyo, Japan) using 100× magnification (UIS2 Series objective, field number FN 22, numerical aperture NA 0.25, resolution 1.30 μm). The amount of coal dust pigment was graded from 0 to 5, also taking into account the size of coal dust accumulations, the distribution of the accumulations, and the approximated size of the sample (Table 2). To assign the inorganic PM score, the sections were screened thoroughly under polarizing light with 200× magnification (UIS2 Series objective, FN 22, NA 0.40, resolution 0.84 μm), and when necessary, 400× magnification (UIS2 Series objective, FN22, NA 0.65, resolution 0.52 μm) was used. The general shape, size, color, and distribution of the birefringent PM were noted. PM in the intra-alveolar spaces was noted only inside macrophages, otherwise birefringent PM outside tissues was considered as a potential artefact and did not count towards the score. The amount of birefringent particles was evaluated on a scale from 0 to 5 (Table 3). The most important factors affecting the score were the amount and density of PM and the approximated size of the total sample. As a tool for evaluating the amount of inorganic PM, particles were counted as clusters (Table 3).

Table 1
Characteristics of 73 IPF patients whose lung tissue samples were analyzed with polarizing light microscopy.

	Mean ± SD or %	n
Age at diagnosis (years)	61.7 ± 10.4	73
Age at sample collection (years)	62.3 ± 10.2	73
Age at death (years)	70.1 ± 8.1	37
Age at transplantation (years)	56.3 ± 8.6	16
Lung transplant recipients (%)	21.9	16
Deaths (%)	50.7	37
Follow-up time (months)	67.3 ± 40.3	73
Sex		
Male (%)	69.9	51
Female (%)	30.1	22
Smoking at diagnosis		
Never (%)	37.0	27
Ex-smoker (%)	47.9	35
Current smoker (%)	15.1	11
Pack-years of smoking	21.8 ± 12.5	41
BMI (kg/m^2)	28.9 ± 4.9	68
FVC%	76.2 ± 16.7	68
DLCO%	56.4 ± 15.9	68
6MWT (m)	425.3 ± 165.5	18
Occupational data available	91.8	67
Organic dust exposure-related		10
Inorganic dust, chemical, or diesel exposure-related		16
No exposure		41
Exposure to organic dust	15.1	11
Cotton dust		2
Flour		1
Farming		7
Wood dust		1
Exposure to inorganic dust	23.3	17
Asbestos		6
Cement dust		1
Diesel exhaust fumes		1
Glass dust		2
Metal dust		3
Sand dust		1
Stone dust		3

BMI, body mass index; FVC%, forced vital capacity, % predicted; DLCO%, diffusing capacity for carbon monoxide, % predicted; 6MWT, six-minute walk test. The values are from the time of diagnosis. For follow-up time, death or lung transplantation was used as an end-point event. Follow-up time for patients having no end-points was defined as the time interval between IPF diagnosis date and March 5, 2018. Six-minute walk test (6MWT) was performed without extra oxygen.

Table 2
The coal dust pigment scores using light microscopy at 100× magnification.

Score	Small coal dust accumulations	Medium coal dust accumulations	Large coal dust accumulations	Areas without coal dust accumulations	Distribution of coal dust
0	0	0	0	100%	No coal dust
1	0–4	0	0	80–99%	Very little coal dust accumulations around the sample
2	5–39	0–4	0	60–79%	Even or focally distributed
3	40–59	5–9	0–4	40–59%	Quite evenly distributed
4	60–79	10–14	5–9	20–39%	Even or many focal clusters
5	80 or more	15 or more	10 or more	0–19%	Almost in every field or huge clusters focally

2.4. Scanning electron microscopy analysis

Paraffin-embedded lung tissue samples with a width of 2.5 μm were cut with a microtome (Leica RM2255; Leica Biosystems Nussloch GmbH, Nussloch, Germany). Samples were fixed overnight at +37 °C and then stained with HE. All samples were screened with polarizing light microscopy, and six IPF samples were chosen for FESEM-EDS analysis because of their abundant birefringent PM. Selected samples were cut to a width of 10 μm on a titanium (Ti) plate and then fixed overnight at +37 °C and dried with hexamethyldisilazane (HMDS Cas 999-97-3). For localizing the particles, the samples were investigated with a material microscope and filmed (Nikon LV-DIA Base microscope; Nikon Instruments, Tokyo, Japan) with a motorized XY staging system (OptiScan III; Prior Scientific, Rockland, MA, USA) connected to a DS-Fi1 digital camera (Nikon Instruments, Tokyo, Japan) to locate the particles (Supplemental material, Fig. A.1). The samples were then coated with a carbon (C) layer to improve conductivity. The samples were investigated with FESEM (JSM-6335F, JEOL, Tokyo, Japan), and particles found were analyzed with EDS. The elemental composition, the size of the particles, the distribution of different-sized particles, shapes, and surface structures were recorded. All samples had abundant particles, and for further elemental analysis, 10–24 particles per sample were chosen. Ti and C were excluded from elemental analysis, as the samples were on a Ti plate and tissues were carbonaceous.

2.5. Statistical methods

We used IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA). Kolmogorov-Smirnov and Shapiro-Wilk tests were used for determining whether data were normally distributed. Fisher's exact test was used to compare categorical data. For correlations, Spearman's correlation was used. For continuous measurements, in the comparison between two groups, *t*-test or Mann-Whitney *U* test were used depending on whether data were normally distributed. In the comparison between more than two groups, one-way ANOVA or Kruskal-Wallis test were used depending on whether the data were normally distributed. In survival analysis, Kaplan-Meier method was used, and the significance was evaluated with the log-rank test. Survival was defined as the time between IPF diagnosis and death or lung transplantation, which served as end-point events. The follow-up time for the patients having no end-point events was defined as the time between IPF diagnosis date and March 5, 2018. Two-sided *p*-values equal or below 0.05 were considered significant.

3. Results

3.1. Coal dust pigment and inorganic particulate matter

Coal dust pigment and birefringent PM were observed in all samples. Birefringent PM localized mainly with coal dust pigment, and the inorganic PM score was associated with the coal dust pigment score in all samples ($R = 0.75$, $p < 0.001$, Supplemental material, Table A.1). We detected particles of varying shapes, sizes, and colors (Fig. 1). Both weakly birefringent, small, triangular PM typical for silica (silicon dioxide, SiO_2) and strongly birefringent, large, needle-shaped or platy PM typical for silicates were detected. PM was localized either focally in clusters in larger numbers or widely distributed as single particles. It localized both near the bronchovascular bundles and in the fibrotic interstitium. PM existed rarely in the intra-alveolar macrophages.

3.2. Inorganic particulate matter, population density, and particulate matter levels

Samples from the southern regions of Finland, i.e. university hospital districts of Helsinki, Turku, and Tampere, had more often PM scores from 3 to 5 than samples from the northern regions of Finland, comprising the university hospital districts of Kuopio and Oulu (31/50, 62.0%, vs. 7/23, 30.4%, $p = 0.02$, Supplemental material, Table A.2). According to Statistics Finland's databases [19], on January 1, 2018, the population density in the southern university hospital districts was 46 people/km^2. The population density in the northern hospital district area was 7 people/km^2. According to the measurements of the Finnish Meteorological Institute in 2016 [20], the annual mean $PM_{2.5}$ levels in southern university hospital districts were higher compared to northern ones (6.0 ± 1.2 μg/m^3 vs. 4.4 ± 1.7 μg/m^3, $p = 0.002$), however, no such difference was noted regarding to the annual mean PM_{10} levels (12.3 ± 3.2 μg/m3 vs. 11.6 ± 5.1 μg/m3, $p = 0.6$).

Table 3
The birefringent inorganic particulate matter scores using polarizing light microscopy at 200× magnification.

Score	Amount of single particles	Particle clusters	Stardust clusters	Areas without particles	Distribution of particles
0	0	0	0	100%	No particles
1	0–49	0–4	0	80–99%	Sparse
2	50–99	5–9	0	60–79%	Sparse
3	100–149, lots of single particles	10–14, can be less if lots of single particles	0	40–59%	Single particles evenly or focally in clusters
4	150–199, lots of single particles	15–19	0–4	20–39%	Single particles evenly and focally in clusters
5	200 or more, lots of single particles	20 or more	5 or more	0–19%	Almost in every field

Particle cluster, three or more particles near each other in the same field; stardust cluster, a cluster containing at least ten particles and covering at least half a field.

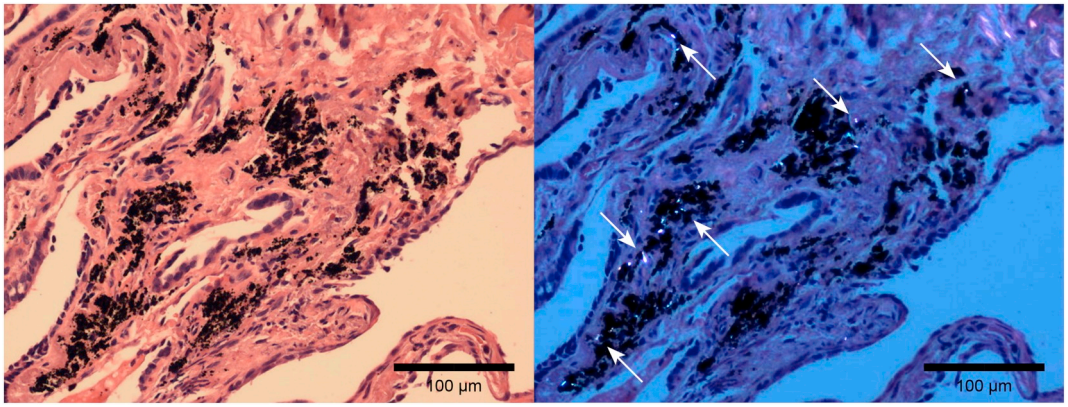

Fig. 1. Birefringent inorganic particles in polarizing light microscopy. White arrows point to birefringent inorganic particles.

3.3. Inorganic particulate matter and clinical data

Most of the patients with a sample of the highest PM scores of 4 and 5 had a known history of exposure to inorganic dust (8/15, 53.3%, vs. 9/58, 13.5%, $p = 0.004$). The highest PM scores of 4 and 5 associated with male gender ($p = 0.03$). For patients with a smoking history, pack-years of smoking were inversely correlated with the PM score (R = -0.401, $p = 0.01$, $n = 41$). Most of the patients with a sample of a PM high score of 4 and 5 were ex-smokers (score from 4 to 5, 11/15, 73.3% vs. score from 0 to 3, 24/58, 41.4%), but none of them were current smokers ($p = 0.048$). PM scores were not associated with age, occupational history, BALF, BMI, blood count, arterial gas, spirometry, diffusion capacity, or 6MWT values at the time of diagnosis, sample collection, transplantation, or death in the samples of IPF patients.

3.4. Inorganic particulate matter, occupational history, and survival

During the follow-up of 73 IPF patients 37 patients (50.7%) died. Sixteen patients (21.9%) underwent lung transplantation, and two of them died after transplantation. There was no statistically significant difference between the median survival times of IPF patients according to the PM score ($N = 73$, 82 months, 95% confidence interval, CI, 64–100 months, $p = 0.08$). The median survival times for patients with a history of organic dust exposure-related occupation ($n = 10$, 28 months, 95% CI 5–50 months) was the shortest compared to patients with a history of inorganic dust, chemical, or diesel exposure-related occupation ($n = 16$, 103 months, 95% CI 32–173 months) or patients with a history of occupation of no expected occupational exposure ($n = 41$, 87 months, 95% CI 72–101 months, Fig. 2, $p < 0.001$). The amount of events and censored patients were unequal, as all patients with a history of organic dust exposure-related occupation died or had lung transplantation.

3.5. Elemental composition of inorganic particulate matter

An example of particles analyzed in FESEM is shown in Fig. 3. For the elemental compositions of the particles observed in FESEM-EDS analysis, see Table 4. The most common elements in the measuring points were Al, Si, and potassium (K). Moreover, particles consisted of calcium (Ca), sodium (Na), iron (Fe), magnesium (Mg), chromium (Cr), sulfur (S), and copper (Cu). Particles either consisted of two to three elements or contained several elements. Samples mainly contained one kind of elemental composition in all measuring points, but in one sample of a female patient with no known dust exposure ten different compositions existed. No element was associated with smoking status or known exposure.

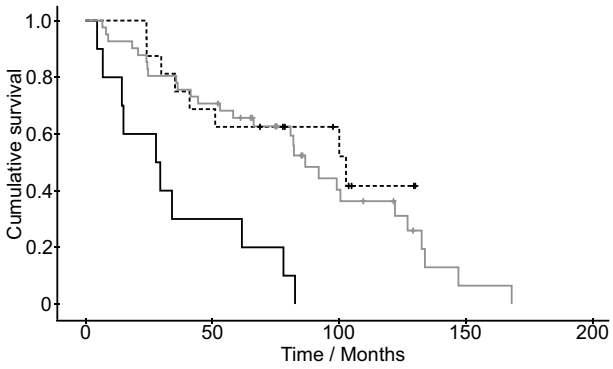

Fig. 2. Kaplan-Meier curve for survival comparison between idiopathic pulmonary fibrosis patients with a history of organic dust exposure-related occupation (black line, $n = 10$), patients with a history of inorganic dust, chemical, or diesel exposure-related occupation (black dotted line, $n = 16$), and patients with a history of occupation of no expected occupational exposure (gray line, $n = 41$, $p < 0.001$).

4. Discussion

We evaluated inorganic PM and coal dust pigment of 73 lung tissue samples of IPF patients. We used a semiquantitative scoring method with polarizing light microscopy. Furthermore, we analyzed the elemental compositions of inorganic particles in six IPF samples with FESEM-EDS. Birefringent PM existed in all samples in varying amounts. Inorganic PM scores were higher in the samples of patients from the southern Finnish university hospital districts than in those from northern districts. Possible explanations for the finding are higher population density and $PM_{2.5}$ levels in the southern university hospital districts compared to northern districts. The highest PM scores were associated with a known inorganic dust exposure. A history of organic dust exposure-related occupation seemed to be associated with a poor prognosis.

4.1. Inorganic particulate matter and polarizing light microscopy

In routine pathology, polarizing light microscopy is a viable method for detecting inorganic PM, such as SiO_2 and silicates, which mainly originate from environmental crustal materials. SiO_2 and silicates in the lungs range from under 0.1 µm to 5 µm [21]. When two autopsy samples of patients with known exposure to environmental dust were evaluated in polarizing light microscopy and in SEM-EDS, silica particles with diameter below 1 µm were detected with both methods [22]. As we screened the samples for PM with polarizing light microscopy using 200× magnification with the highest resolution of 0.84 µm, some of the fine particles were missed. In two previous observational studies

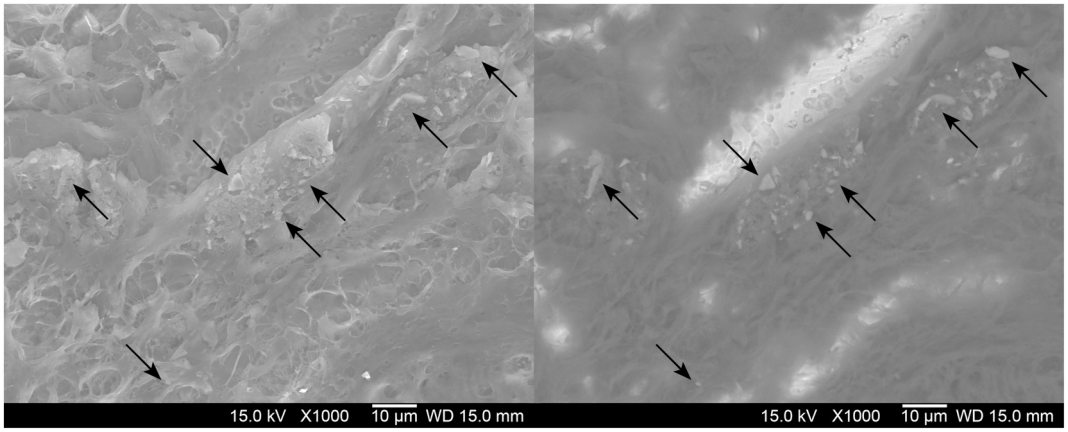

Fig. 3. Particles in a field emission scanning electron microscope shown in secondary electron and backscattered electron images. Black arrows point to particles.

of forensic autopsy samples of relatively young patients without known respiratory disease, inorganic dust in polarizing light microscopy was associated with histological fibrotic alterations [23,24]. Collagen in the fibrotic alterations is also birefringent under polarizing light [25], which might disturb the detection of PM in fibrotic lung tissue. Artefactual birefringent PM is also possible [26], however, we aimed to avoid including artefactual PM in the score.

Polarizing light microscopy studies focusing on a possible connection between inorganic PM and IPF are scarce. In a study of 22 IPF patients who had undergone lung transplantation, dust deposits existed in 18% of open lung biopsies and in 9% of explant samples [27]. A case-control study reported that birefringent PM existed in greater amounts in the samples of IPF patients ($n = 15$) than in the samples of patients with other lung diseases ($n = 20$) [8]. The inorganic PM was counted in ten fields per sample [8] whereas we analyzed whole slides. Birefringent PM co-localized often with coal dust accumulations [8], which is consistent with our finding. Exposure history was not associated with the visible inorganic PM in lung tissue in the study by Tsuchiya et al. [8] or in a study of smokers' fibrosis [21]. In our study, however, the small amount of patients with the samples of the highest PM scores had more often known exposure history to inorganic dust. The highest PM scores were associated with male gender and ex-smoker status, which has not, to our knowledge, been investigated before in IPF patients. No current smokers existed in this subcohort, and pack-years of smoking had an inverse correlation to PM scores, suggesting a possibility that high amounts of PM do not originate from tobacco smoke.

4.2. Elemental composition of inorganic particulate matter

Si, Al, Fe, and Mg can be found in lung scars both in samples of otherwise healthy patients and in IPF patient samples by electron microscopy combined with an electron microprobe microanalyzer [28]. Using an electron microscope combined with electron microdiffraction, Si, Al, Fe, Ti, and C have been found in lung tissue samples of patients without known exposure other than air pollution [29]. In more recent studies, SEM-EDS has been widely used for counting and recognizing particles, as it can reach a resolution of less than one nanometer. In a case-control study evaluating 34 forensic autopsy lung samples, Al and Si have been associated with smoking [30]. In a descriptive study of 11 autopsy samples of healthy non-smoking individuals without occupational exposure from an area of low PM levels, the main minerals in the lung parenchyma were SiO_2, clay mineral kaolin ($Al_2Si_2O_5(OH)_4$), and mica group silicates, but also metals, feldspar silicates, and clay mineral talc ($Mg_3Si_4O_{10}(OH)_2$) were detected [31]. In a case-control study of 18 open lung biopsies of smokers and non-smokers, the particles were mainly composed of aluminum silicate, SiO_2, Ti, and talc, and fibrosis was associated with aluminum silicate, SiO_2, and Ti concentrations [21].

We found that Si, Al, and K existed in all samples, however, our sample size in FESEM-EDS analysis was very small ($N = 6$) and we had no control group. In previous case-control studies of IPF lung tissue samples ($n = 25$ and $n = 15$, respectively), the amounts of Si [9] and also Al [8] have been greater than in control samples ($n = 31$ and

Table 4
Elemental compositions observed in the particles of six IPF samples using energy dispersive spectrometry with a field emission scanning electron microscope. The age of the patients ranged from 39 to 64 years.

Sample	Sex	Smoking status	Profession	Exposure	Measuring points	Elemental composition
1	Male	Ex	Storage worker	Glass dust	19	Si-Al-K
2	Male	Ex	Mixing plant worker	Stone dust	21	Si-Al-K Si-Al-K-Fe Si-Al-Ca
3	Male	Never	Teacher	None	10	Si-Al-K
4	Male	Ex	Policeman	Cement dust	13	Si-Na-Mg-Al-K-Ca-Fe
5	Male	Ex	Project manager	None	17	Si-Na-Mg-Al-K-Ca-Fe
6	Female	Ex	Nurse	None	24	Si-Al Si-Al-K Si-Al-K-S Si-Na-Al Si-Al-Ca Si-Al-Cr Si-Na-Al-K-Ca Si-Na-Mg-Al-Ca-Fe Si-Na-Mg-Al-Ca-Cr S-Cu

Si, silicon; Al, aluminum; K, potassium; Fe, iron; Ca, calcium; Na, sodium; Mg, magnesium; S, sulfur; Cr, chromium; Cu, copper.

$n = 20$, respectively). Similar results were reported in a case-control study of autopsy pulmonary lymph nodes of 46 patients [10]. Crystalline SiO_2 is highly fibrogenic, but its effects can be altered by other minerals, such as silicates, which are quite weak fibrogenic minerals alone, and iron, as well as by coal [32]. The fibrogenicity of Al remains unclear, however, the development of pulmonary fibrosis for patients occupationally exposed to Al has been reported [33].

4.3. Inorganic particulate matter, population density, and particulate matter levels

Inorganic PM scores were higher in samples of patients from the southern Finnish university hospital districts than from the northern districts. In Finland, the population density and $PM_{2.5}$ levels are higher in the southern regions than in the northern regions. Our result is suggestive for an association between inorganic PM in the lung tissue, population density, and $PM_{2.5}$ levels, which can be thought to reflect greater exposure to air pollution from traffic and industry. The guideline of 25 $\mu g/m^3$ by the World Health Organization (WHO) for mean daily $PM_{2.5}$ concentration [34] is also exceeded episodically in southern Finland because of long-range transported pollutants [35]. We do not, however, have data of previous locations of residence of the registry patients, or the duration of the living in specific university hospital district area.

In the United Kingdom, increased mortality in IPF has been associated with living in the traditionally industrialized areas, a working history in a major engineering company, and birth in urban areas [36-38]. In a Brazilian case-control study, the amount of anthracosis and histological alterations have been greater in forensic autopsy lung samples of patients without chronic lung diseases from areas of high PM levels than in samples from areas of low PM levels [39]. Greater amounts of retained PM seen in electron microscopy and increased fibrotic alterations seen in light microscopy have been reported when autopsy lung samples from Mexico City, Mexico, a city of high PM levels, were compared with ones from Vancouver, Canada, a city of low PM levels [40,41].

4.4. Inorganic particulate matter, occupational history, and survival

Unlike the previous epidemiological finding of high PM levels increasing IPF mortality [14], our histological PM score did not associate with prognosis. A history of organic dust exposure-related occupation seemed to associate with worse prognosis compared to other occupations. In a cohort study of 1311 IPF patients, those with a history of wood, metal, sand, stone, diesel, or chemical exposure-related occupation had worse prognosis than IPF patients without occupational dust exposure [42]. Controversial to our finding, in a retrospective case-control study of IPF patients with known exposure to bird dust or mold had better survival than patients without known exposure to bird dust or mold; however, their survival was worse than for chronic hypersensitivity pneumonitis patients [43].

4.5. Conclusions

To our knowledge, no other study has quantitated inorganic PM in IPF samples using polarizing light microscopy in a sample size like ours. Using our novel although not yet validated method, we provided histological evidence of PM in IPF lungs. In this study, numerous confounding factors existed and thus our results are only suggestive. However, our study generates a hypothesis that a threshold level of exposure to air pollution may exist when inorganic PM starts to cumulate in fibrotic lungs. Examination of lung tissue with polarizing light microscopy may reveal new insights into the etiology and pathogenesis of IPF. Inorganic PM seen in the lungs probably reflects long-term exposure to increased air pollution levels, which could possibly modulate the formation of fibrosis.

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Declaration of interest

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Appendix A. Supplementary data

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