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Sarcoidosis, inorganic dust exposure and content of bronchoalveolar lavage fluid: the MINASARC pilot study

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Abstract. Inhalation of mineral dust was suggested to contribute to sarcoidosis. We compared the mineral exposome of 20 sarcoidosis and 20 matched healthy subjects. Bronchoalveolar lavage (BAL) samples were treated by digestion-filtration and analyzed by transmission electron microscopy. The chemical composition of inorganic particles was determined by energy-dispersive X-ray (EDX) spectroscopy. Dust exposure was also assessed by a specific questionnaire. Eight sarcoidosis patients and five healthy volunteers had a high dust load in their BAL. No significant difference was observed between the overall inorganic particle load of each group while a significant higher load for steel was observed in sarcoidosis patients (p=0.029). Moreover, the building activity subscore was significantly higher in sarcoidosis patients (p=0.018). These results suggest that building work could be a risk factor for sarcoidosis which could be considered at least in some cases as a granulomatosis caused by airborne inorganic dust. The questionnaire should be validated in larger studies. *(Sarcoidosis Vasc Diffuse Lung Dis 2018; 35: 327-332)*

Key words: sarcoidosis, dust exposure, bronchoalveolar lavage

Introduction

Several epidemiological studies (1,2) and editorials (3,4) suggested that the inhalation of mineral dust may contribute to sarcoidosis. Along with beryllium exposure, the 1999 statement of international experts on sarcoidosis mentioned aluminum, talc and zirconium as possible causes, but did not mention crystalline silica (5). Moreover, several studies (6,7) and the recent mini-epidemic among World Trade Center cleanup workers (8,9) suggest that the exposure to mineral dust can indeed cause sarcoidosis. Finally, a number of granulomatosis cases (with symptoms similar to those of sarcoidosis) have been attributed to exposure to metals, namely beryllium

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(10), titanium (11,12), aluminum (13), and steel (14).

In order to investigate the putative link between sarcoidosis and inorganic dusts, we conducted a pilot multicenter case-control study on 20 patients with sarcoidosis matched with 20 healthy volunteers on sex, age, and tobacco use.

Methods

Sarcoidosis patients

Twenty patients with biopsy-proven granulomas were recruited from four centers. Inclusion criteria included: age 20-50 years, suspected sarcoidosis stage I-IV with no cause identified by basic questioning, and successful extraction of bronchoalveolar lavage (BAL) fluid using standard procedures.

Healthy subjects

Healthy subjects were recruited by Dermscan (Lyon, France). Endoscopies and BALs were performed in the endoscopy unit of the Saint-Joseph Saint-Luc hospital (Lyon, France). Each sarcoidosis subject was matched with a healthy volunteer by age (±10 years), sex, and smoking status. Pregnant women were excluded as were subjects with a psychiatric pathology.

Mineralogical analysis of BAL fluid

BAL samples were extracted following the procedure currently recommended in French hospitals as previously described (15). The mineralogical analysis was performed by transmission electron microscopy (TEM, Jeol 1400 EX, operated at 120 kV) on a device equipped with a CCD camera (Gatan Orius 600) and an X-ray emission detector (Jeol JED-2300). Samples were randomly assigned to one of two mineralogists specialized in environmental exposure. Nature of the mineral and metal particles was determined by X-ray emission spectroscopy. Exogenous compounds were counted and expressed as a number of particles per milliliter of BAL fluid. The sensitivity limit of this approach is 434/mL corresponding to the observation of a single particle by TEM.

The dust exposure questionnaire (Online resource 1)

The questionnaire was developed based on previous surveys of occupational exposure and carcinogenic risks (15,16). It quantifies and classifies a subject's mineral exposome. The categories account for professional (occupation, task, work sector etc.) and non-professional activities and for cumulative duration of exposure and the presence and effectiveness of any (individual or collective) protection device against dust.

All subjects were interviewed by telephone and by the same investigator (CCa). Answers to each question were scored from 0 to 5. The greater the number of exposures and their duration were and the more deficient the protection, the higher the score was (Figure 1).

In situ optical mineralogical analysis

Three expert pathologists (FT, MK, JFB) examined histological slides for each subject by optical microscopy and noted the presence or not of opaque (under natural light) or birefringent (under polarized light) particles in or outside the granulomas.

Statistical analysis

For quantitative variables, differences between groups were tested using non-parametric Mann-Whitney test. For qualitative variables, a Fisher exact test was used. A logistic regression analysis was conducted to identify factors potentially associated with presence of sarcoidosis. Results are presented as odds ratios (OR) together with their 95% confidence intervals (95%CI). A p value <0.05 was considered as statistically significant. All analyses were performed using R (R Foundation for Statistical Computing, Vienna, Austria).

Ethical approval

This study was approved by the Research Ethics Committee of Lyon (approval No. 2013-050 B). All study participants provided written informed consent.

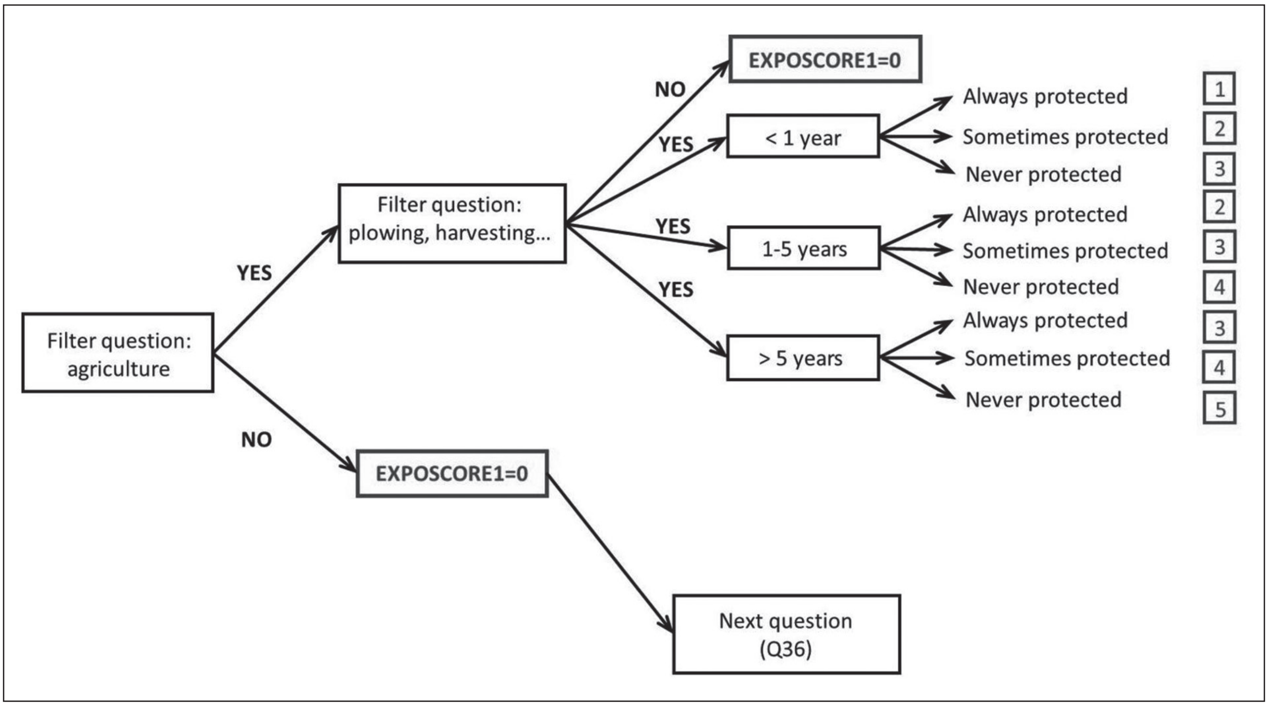

Fig. 1. Flow chart showing how the dust questionnaire was scored

Results

Study population

Both groups consisted of 6 women and 14 men and mean age was 36 years (Table 1). Forty-five percent of sarcoidosis patients were of Western Europe origin *vs* 95% of healthy volunteers (p=0.009)

Mineral load

There was no significant difference between both groups in terms of mineral load, either overall, or considering each particle type separately, except a possible higher steel load among sarcoidosis patients (p=0.029; Table 1 and Figure 2) and a trend for higher chromium compound level (p=0.075). Three patients had high silica load (311,760, 125,000 and 218,736 particles per mL of BAL fluid, respectively). Using a multivariable logistic regression analysis, the overall dust load was not associated with sarcoidosis after controlling for age, gender and smoking status (OR=3.78, 95%CI [0.29-50.0], p=0.313).

Dust exposure

The questionnaire analysis showed that the median dust exposure score of sarcoidosis patients (29.5 range [5-118]) was higher than that of healthy subjects (15.5 [0-75]) although this difference was not considered significant (p=0.062). The questionnaire score was not associated with the overall dust load (p=0.122) but was associated with the level of titanium compound (p=0.046) and to a lesser extent to the level of iron oxide (p=0.056). A trend was also observed with the silica level (p=0.083).

Occupation and mineralogical results

The three patients with high silica load were all construction workers. One healthy volunteer (1/20, 5%) and five sarcoidosis patients (25%) worked in the construction industry. In addition to these five sarcoidosis patients, four others had done so in the past and 16 in total had performed construction and demolition tasks informally. Among healthy subjects, one currently worked in construction and seven oth-

Table 1. Subjects' characteristics and dust load. Data are given as median (range), or n (%) unless otherwise stated

	Sarcoidosis patients (n=20)	Healthy volunteers (n=20)	P
Age, years median (min-max)	38.5 (25-48)	33.5 (20-47)	0.132
Male gender	14 (70)	14 (70)	1
Region of origin			0.009
Europe	11* (55)	19 † (95)	
Northern Africa	4 (20)	1 (5)	
Sub-Saharan Africa	2 (10)	0 (0)	
USA	2 (10)	0 (0)	
Middle East (Iraq)	1 (5)	0 (0)	
Smoking status (pack-years)			1
0-5	17 (85)	17 (85)	
5-10	2 (10)	2 (10)	
>10	1 (5)	1 (5)	
Sarcoidosis stage			-
I	6 (30)	-	
II	10 (50)	-	
III	2 (10)	-	
IV	2 (10)	-	
Building activity subscore	6.5 (0-40)	0 (0-27)	0.018
Overall dust load (particles/mL)	101,611 (43,347-1,247,038)	121,742 (57,213-260,417)	0.589
Dust exposure score	29.5 (5-118)	15.5 (0-75)	0.062
Specific dust load			
Aluminosilicate	56,437 (15,058-773,164)	63,889 (29,751-190,104)	0.530
Silica	15,655 (434-311,760)	12,839 (4,213-32,661)	0.328
Titanium compound	434 (434-37,411)	2,881 (434-46,967)	0.343
Titanium oxide	6,097 (434-68,743)	6,042 (434-57,613)	0.802
Iron oxide	434 (434-40,155)	3021 (434-18,519)	0.418
Steel	434 (434-13,749)	434 (434-3,841)	0.029
Chromium compound	434 (434-16,944)	434 (434-434)	0.075
Chromium oxide	434 (434-2,947)	434 (434-22,634)	0.913
Aluminium compound	434 (434-434)	434 (434-8,230)	0.152
Talc	434 (434-7,113)	434 (434-6,173)	0.970

** France 9, Portugal 2; † France 18, Italy 1;*

ers had previously held activities in this sector. The median building activity sub-score, built from construction-related questions, was significantly higher for sarcoidosis patients (6.5 [0-40]) than for healthy subjects (0 [0-27], p=0.018).

In situ mineralogical analysis by optical microscopy

In the majority of cases (16/20), the pathologist's diagnosis matched the one suggested by the pulmonologist. Only one patient had his current occupation (construction worker) mentioned in the medical file. There was no mention of analysis under polarized light in any of the pathologists' reports. However, a re-examination of the histological slides under polarized light revealed anisotropic particles for 13 subjects in the tissue harboring granuloma but only one within the granuloma.

Discussion

Our study did not highlight any significant difference in the overall dust load between sarcoidosis patients and healthy volunteers. However, higher steel load and a trend for a higher level of chromium compounds were observed in sarcoidosis patients. Moreover, all three subjects with highest silica levels had a sarcoidosis diagnosis. Although airborne mineral dust exposure may be involved in the etiol-

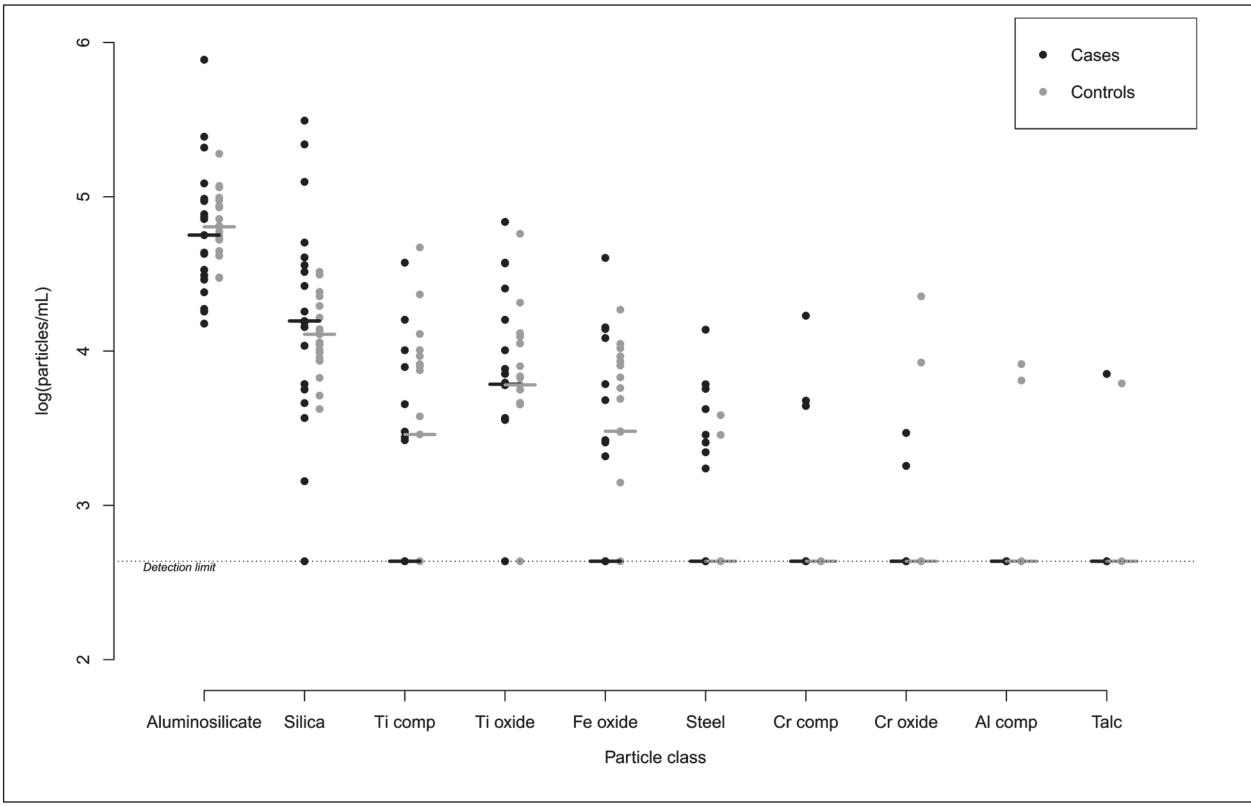

Fig. 2. Mineral content in sarcoidosis patients (black dots) and healthy volunteers (grey dots). Ti comp=titanium compound, Cr= chromium, Al=aluminium

ogy of several sarcoidosis cases in this study, absence of alveolar high overload dust could be explained in some cases by talc powder application (17). The questionnaire used in this study quantifies and classifies a subject's mineral exposome. Our results indicate that the dust exposure score calculated with this questionnaire is higher for sarcoidosis patients than for healthy participants, although the difference is slightly above significance (p=0.062). Since this questionnaire is used retrospectively with the knowledge of the presence/absence of the disease, a recall bias effect cannot be ruled out. However, the association between high questionnaire score and high level of titanium compound and to a lesser extent iron oxide and silica levels highlights the quality of this questionnaire and indicates that it could be useful as a surrogate marker of mineral exposure.

Interestingly, a majority (11/20) of sarcoidosis patients but only two healthy subjects were born outside France. A genetic cause seems unlikely because countries of origin are diverse (Table 1). These results may rather reflect previous poorer healthcare and/or living conditions and more exposure-prone occupations of immigrants in France.

In this study, the building activity sub-score was significantly higher for sarcoidosis patients than for healthy subjects and the three patients with the highest silica loads were sarcoidosis patients working in construction. According to Hill's criteria (18), the causal link between these patients' building activities and their granulomatous disease may be hypothesized.

The present study suggests that the risks associated with silica exposure in the construction sector are underestimated both in the workplace and by pulmonologists. Indeed, although five patients were professional construction workers and 11 others had worked in construction or demolition activities prior to the study, this information was only passed on to the pathologist in one case. For most patients, the pulmonologist only mentioned that sarcoidosis was suspected; silicosis was not considered as a possible diagnosis. Pathologists, unaware of the patient's possible exposure to silica, seldom search for birefringent particles under polarized light (the still recommended approach for detecting silica particles). As the boundaries between silicosis and other granuloma diseases remain poorly defined (19) and since analysis under polarized light is seldom performed, foreign body granulomatous reactions are thus often ascribed to sarcoidosis. However, the combination of questioning and mineralogical analysis would allow potential exposures to silica to be identified and would make the correct diagnosis easier to reach, especially for construction workers.

A clear limitation of this study is the sample size. However, although underpowered, this pilot study is unprecedented in its coupling of occupational and environmental questioning with the mineralogical analysis of BAL fluid. Construction workers are overrepresented among sarcoidosis patients and inhaled mineral dust could be involved in 8 of the 20 sarcoidosis cases. We recommend that patients presenting with a granulomatous disease should have their mineral exposome investigated by exhaustive questioning and mineralogical analysis. Systematic search of opaque or bi-refringent particles need to be mentioned by pathologists. Patients with high particle concentrations should be screened for hypersensitivity by skin or lymphocyte transformation tests. This approach should allow to better identify exposures and abnormal sensitivities, and to implement targeted protective measures.

These hypotheses need to be confirmed in larger samples of patients by combining mineralogical analysis and dust exposure questioning.

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