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Sarcoidosis and inorganic dust exposure in the MINASARC (Mineralo-Nano-SARCoidosis) study

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I. Introduction

It has been suggested that sarcoidosis could be associated with exposure to inorganic particles (Newman, 2012; Vincent, 2015). Mineral exposome may be studied by a specific questionnaire (SQ) throughout the lifetime, both in professional and extraprofessional contexts and by mineralogical analysis (MA) performed on broncho-alveolar lavages (BAL). MINASARC study is a prospective case-control study measuring mineral exposome by SQ and MA of BAL by Transmission Electron Microscopy (TEM) in 20 sarcoidosis cases (SarC) compared to 20 healthy volunteers (HV). Our objective is to compare MA and SQ results between SarC and HV and study the correlation between MA and SQ.

II. Material and methods

Population studied

Every SarC is matched to a HV by age (10-year age brackets), sex and smoking habits (non-smoker or less than 5 packs/year (PY); between 5 and 10 PY; over 10 PY).

Criteria for inclusion and exclusion of HV

Inclusion: age 20-50; absence of respiratory pathology; simple spirometry within normal limits. FEV1>85% in theory; normal pulmonary radiography; absence of cardiac pathology; protected by health insurance regime; having signed an informed consent template; hepatitis B, C and HIV pathology <0.
Exclusion: Pregnant woman; active cardio-respiratory pathology; psychiatric pathology; refusing the terms of the informed consent.

Criteria for inclusion and exclusion of SarC

Inclusion: suspected sarcoidosis stages 1-4; age 20-50; having signed an informed consent template; being submitted to an endoscopic examination with BAL; without probable causal factor already identified during the usual questioning; accepting to complete a professional and environmental questionnaire once sarcoidosis is confirmed.
Exclusion: refusing the terms of the informed consent; refusing to complete a professional and environmental questionnaire; non-completion or failure of BAL.

Mineralogical analysis (MA)

The MA of the BAL is implemented with a transmission electron microscope (TEM Jeol 1400 EX, 120kV) with a CCD camera (Gatan Orius 600) and an X-ray detector (Jeol JED-2300).

The samples from HV and SarC are blindly examined after a digestion-filtration preparation. The analysis is performed on 100 contiguous particles observed consistently over the tiles located in the center of the grid. The X-ray emission analysis allows to determine the nature of the mineral particles and classify them according to various exogenous mineral families such as: Silica ; Aluminosilicates; Ti compounds ; Cr compounds ; Cr Oxides ; Al compounds ; Fe Oxides ; Talc ; Steel ; S Compounds. The results are expressed in number of particles per mL of BAL. A comparison of the two groups is conducted from the total number of particles observed by TEM and the particle content in the each particle family.

Specific questionnaire (SQ)

SarC and HV are subjected to a questionnaire about their "whole life" exposure to inorganic dusts. A scoring is proposed for all professional and non-professional activities, in order to quantify and summarize the cumulative exposure duration in life and the existence (or absence) of protection against dust as well as its effectiveness perceived by the respondent. For each question, the score can range from 0 to 5. The more numerous the situations of exposure are, the longer the cumulative exposure duration, the faultier the protections, the higher the score is. The detail of the score calculation algorithm is shown in the poster titled: "The MINASARC study: A case-control study measuring mineral exposome in sarcoidosis" (poster PA397). Each type of activity can be summarized by a subscore which is a part of the global one. For instance, a building activity score (BAS) can be computed by adding the points from questions related to construction activities, both in occupational and non-occupational contexts.

Statistical analysis

The overall dust rates and the rates for each particle class were compared between SarC and HV. In order to normalize the distribution of variables of interest, we realize the logarithmic transformation of the measured values. When data follow a normal distribution, the statistical test used is the Student t test for paired data. Otherwise, we use the Wilcoxon signed-rank test.

III. Results

Table 1 summarizes the characteristics of both SarC and HV stating the patient identification (IP), his/her age, sex, smoking status, country of birth, ongoing occupation, stage of the disease and the sampling type on which a sarcoid-like granuloma has been identified.

Table 1: summarize of SarC and HV included in MINASARC study. PI: patient identify; Smo Sta: Smoking Status (0:< 5 PY; 1: 5 to 10 PY; 2: > 10PY); Stage: radiologic stage of the disease; EBUS: Endobronchial Ultrasound; BB: bronchial biopsy; SAGB: salivary gland biopsy; LB: liver biopsy; SB: skin biopsy; IEB: inner ear biopsy.

PI	Age	Sex	Native country	Smo Sta	Current occupation	Stage	Sampling type	PI	Age	Sex	Native country	Smo Sta	Current occupation
SARCOIDOSIS PATIENTS								HEALTHY VOLUNTEERS					
1	41	F	Algeria	0	Unemployed	1	EBUS	21	47	F	France	0	Auxiliary staff of school
2	37	M	Tunisia	0	Transport business manager	2	EBUS	22	33	M	France	0	Pollster
3	29	M	France	0	Doctor	2	EBUS	23	38	F	France	0	Housewife
4	40	F	Algeria	0	Housewife	1	BB	24	41	M	France	1	Shop high tech assistant
5	39	M	France	0	Baker	2	BB	25	22	F	France	0	Medicine student
6	40	M	France	0	Salaried public relation	2	EBUS	26	36	F	France	0	Beautician
7	46	M	France	2	House painter	4	BB and SAGB	27	38	M	Italy	0	Speaker on the radio
8	38	M	Cameroon	0	Couturier	2	BB, SAGB and LB	28	23	F	France	0	Photographer
9	29	F	France	0	Salaried of public service	1	EBUS	29	44	M	France	2	Unemployed
10	35	M	Morocco	0	Salaried in insurance	2	BB and SAGB	30	22	M	France	0	Salaried in public sector housing
11	25	M	France	0	Salesman building material	2	EBUS	31	40	H	France	0	Unemployed
12	39	M	Angola	0	Salaried fire safety	4	BB and SB	32	32	F	France	0	Teacher
13	36	M	Iraq	0	Building electrician	2	BB	33	33	M	France	0	Teacher
14	48	M	Portugal	1	Mason	1	BB and SAGB	34	20	M	France	0	Safety agent
15	26	M	France	1	Mason	2	BB	35	33	M	France	0	Police force
16	40	M	United States	0	Unemployed	1	EBUS	36	33	M	France	0	Truck driver
17	29	F	France	0	Beautician manicurist	1	EBUS	37	29	M	France	1	Salesman builing material
18	34	F	France	0	Salaried public relation	3	BB and IEB	38	34	M	France	0	Police force
19	40	F	Portugal	0	Interior designer	2	BB	39	37	M	France	0	Police force
20	41	M	United States	0	Salaried marketing	3	BB	40	36	M	Tunisia	0	Truck driver

For quantitative analysis of the score of SQ, the Wilcoxon signed-rank test shows that SarC have a significantly higher score than HV ($p=0.036$).

A ROC curve on the dust scores of the questionnaire gives us a value of 29. This value is the optimum distance compared to the chance to the state "high score/sick", the method therefore admits a sensitivity of 85 % and a specificity of 50%.

Figure 1 shows the values of dust loads in number of particles per mL of BAL on the 19 couples included in the study (one HV having smoked just before BAL has been discarded). The red dots correspond, for each class of particles, to the highest values of dust loads having the same order of magnitude (1 Log range). Patients with one or more high values have a dust load considered as "high." According to this classification rule, for each class, the ranks of the patients selected as having high values are: Aluminosilicates: 1st ; Silica: 1st, 2nd, 3rd ; FeO: 1st ; Ti Compounds: 1st, 2nd, 3rd ; TiO: 1st and 2nd ; Steel: 1st ; CrO: 1st ; Cr Compounds: 1st et 2nd ; Al Compounds: 1st et 2nd ; S Compounds: 1st.

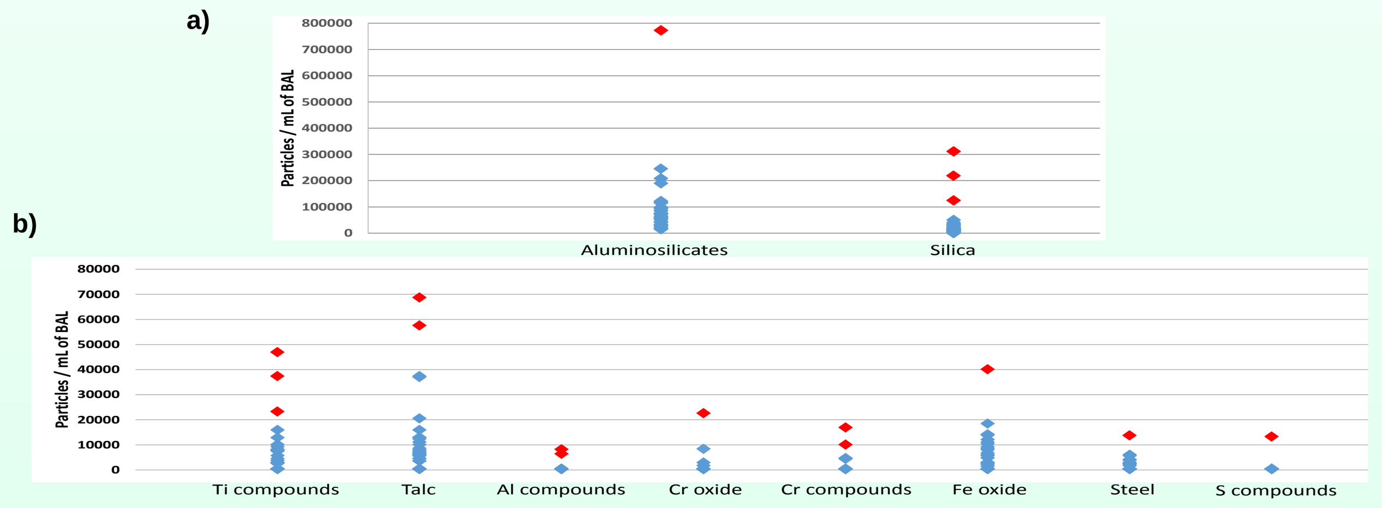

Figure 1: particles rate (particles per mL of BAL analyzed with TEM) for each particle class: aluminosilicate et silica (a) ; Ti compounds; Ti oxide; Al compounds; Cr oxide; Cr compounds; Fe oxide; steel; S compounds (b). The red points corresponds to higher values used for determining a suspect MA status.

For 19 analyzable pairs, there is no significant difference between the SarC and HV groups in terms of overall mineral load (Wilcoxon: $p=0.702$) and for the different particle class (Student: $p=0.726$ and 0.634 for aluminosilicate and silica; Wilcoxon: $p=0.343$; 0.451; 0.092; 1; 0.181 and 1 for titanium, iron, steel, chromium, aluminum and talc).

Table 2 shows the comparison of the MA data on inorganic particles rates analyzed in TEM in SarC and matched to HV. The colored boxes correspond, on the one hand, to the questionnaire scores exceeding 29 and, on the other hand, the mineralogical analyses showing high dust rate with the accuracy mention of the nature of the particles.

The 13 subjects with a suspect MA ("high" dust loads) have a questionnaire score significantly higher than subjects with a normal MA (Mann and Whitney test $p=0.0007$). This result shows that SQ and MA are well correlated.

Table 2: summarize of SQ and MA in SarC and HV. Each pair is on the same line. PI: patient identify; QS: questionnaire score ; TIP TEM : total inorganic particles observed in TEM (nb/mL/LBA) ; MA : mineraligical alalysis status.

SARCOIDOSIS PATIENTS				HEALTHY VOLUNTEERS			
PI	QS	TIP TEM /ml	MA	MA	TIP TEM /ml	QS	PI
1	18	119 000	normal	normal	96 000	15	21
2	7	163 000	normal	Suspect (3rd Ti compo)	122 000	69	40
3	12	85 200	normal	normal	70 200	18	30
4	5	92 000	normal	suspect (2nd TiO)	206 000	32	26
5	41	64 500	normal	normal	131 000	0	36
6	38	73 700	suspect (1st Cr compo)	normal	260 000	15	39
7	118	71 700	suspect (1st FeO)	normal	75 500	34	29
8	48	142 000	normal	normal	57 200	9	35
9	21	102 000	normal	normal	114 000	12	25
10	12	60 100	normal	normal	161 000	7	33
11	73	1 247 000	suspect (1st silica and SiAl, 2nd Ti compo)	suspect (1st CrO and Al compo)	206 000	71	34
12	14	153 000	suspect (1st steel and TiO)	normal	122 000	16	22
13	23	463 000	suspect (3rd silica)	normal	92 700	9	27
14	76	88 200	normal	uninterpretable	uninterpretable	6	24
15	60	509 000	suspect (2nd silica)	suspect (2nd Al compo)	161 000	75	37
16	53	144 000	suspect (2nd Cr compo)	suspect (1st Ti compo)	224 000	31	38
17	32	266 000	suspect (1st Na and Ca sulfide)	normal	113 000	20	28
18	27	202 000	normal	normal	99 800	6	23
19	73	43 300	normal	normal	150 000	23	32
20	23	69 300	normal	normal	116 000	14	31

In **Tables 3a and 3b**, we observe that in the SarC group, 5 patients have an occupation in building activity and 5 others have carried out such an activity in the past including 3 more 5 years. Fifteen among the 20 SarC have carried out construction and demolition activities outside in extra-professional contexts. In the HV group, 1 subject is active in the building sector, and 5 used to be so (3 of them for more than 1 year). Seven have had non occupational demolition and construction activities for less than 1 year. The BAS is significantly higher in SarC group ($p=0{,}01824$; Wilcoxon signed-rank test).

Table 3: native country, questionnaire score (QS), current occupation, old and occupational building activity and MA on the HV (a) and SarC (b). PI : patient identify; QS: questionnaire score; BAS: building activity score; MA: mineraligica alalysis statue.

a)

PI	Native country	QS	Current occupation	Old building activity	Non occupational building activity	BAS	MA
21	France	15	Auxiliary staff of school	no	no	0	normal
22	France	16	Pollster	no	no	0	normal
23	France	6	Housewife	no	no	0	normal
24	France	6	Shop high tech assistant	no	no	0	uninterprétable
25	France	12	Medicine student	no	no	0	normal
26	France	32	Beautician	no	no	0	suspect (2nd TiO)
27	Italy	9	Speaker on the radio	no	less than 1 year	0	normal
28	France	20	Photographer	no	no	0	normal
29	France	34	Unemployed	no	less than 1 year	12	normal
30	France	18	Salaried in public sector housing	less than 1 year	less than 1 year	3	normal
31	France	14	Unemployed	less than 1 year	no	3	normal
32	France	23	Teacher	no	no	21	normal
33	France	7	Teacher	no	no	0	normal
34	France	71	Safety agent	1 to 5 years	less than 1 year	0	suspect (1st CrO and Al compo)
35	France	9	Police force	no	no	0	normal
36	France	0	Truck driver	no	no	0	normal
37	France	75	Salesman builing material	1 to 5 years	less than 1 year	25	suspect (2nd Al compo)
38	France	31	Police force	no	less than 1 year	4	suspect (1st Ti compo)
39	France	15	Police force	no	no	0	normal
40	Tunisia	69	Truck driver	1 to 5 years	less than 1 year	27	suspect (3rd Ti compo)

b)

PI	Native country	QS	Current occupation	Old building activity	Non occupational building activity	BAS	MA
1	Algeria	18	Unemployed	no	less than 1 year	0	normal
2	Tunisia	7	Transport busuness manager	no	no	0	normal
3	France	12	Doctor	no	no	0	normal
4	Algeria	5	Housewife	no	no	0	normal
5	France	41	Baker	no	less than 1 year	6	normal
6	France	38	Salaried public relation	no	less than 1 year	7	suspect (1st CrO)
7	France	118	House painter	more than 5 years	more than 5 years	40	suspecte(1st FeO)
8	Cameroon	48	Couturier	no	1 to 5 years	4	normal
9	France	21	Salaried of public service	1 to 5 years	no	4	normal
10	Morocco	12	Salaried in insurance	no	less than 1 year	9	normal
11	France	73	Salesman building material	1 to 5 years	less than 1 year	19	suspect (1st crystalline silica)
12	Angola	14	Salaried fire safety	less than 1 year	less than 1 year	0	suspect (1st steel and TiO)
13	Iraq	23	Building electrician	1 to 5 years	less than 1 year	10	suspect (3rd silica and crystalline silica)
14	Portugal	76	Mason	more than 5 years	1 to 5 years	25	normal
15	France	60	Mason	more than 5 years	1 to 5 years	27	suspect (2nd silica and crystalline silica)
16	United States	53	Unemployed	less than 1 year	1 to 5 years	16	suspect (2nd Cr compo)
17	France	32	Beautician manicurist	no	less than 1 year	4	suspect (1st Na and Ca sulfide)
18	France	27	Salaried public relation	no	no	0	normal
19	Portugal	73	Interior designer	less than 1 year	1 to 5 years	17	normal
20	United States	23	Salaried marketing	no	1 to 5 years	7	normal

IV. Discussion and conclusion

•About AM: Bio-persistence of non-fibrous mineral particles was demonstrated (Pairon *et al*. 1994). The BAL's dilutions are moderated and have not an important influence in particle level. We assess that TEM without automatic analysis is too time consuming to analyze 100 particles. Scanning Electron Microscopy (SEM) with automated analysis has to be evaluated and SEM analyses *in situ* on tissues in paraffin blocks will complete our study. Finally, filter with 0.4µm pores allows us to observe only agglomerated nanoparticles. Indeed, the isolated nanoparticles pass through the filter.

•Building activities are overrepresented in SarC group in MINASARC study. Exposures to silica are generally underestimated in these activities. A study of 80 building workers equipped with individual sensors shows that 64.5% of them have an unusually high silica exposure (Rappaport *et al*. 2003).

•A cognitive problem has been generated by the truncation in the definition of silicosis at the Johannesburg conference (1930). The 1930 definition of silicosis was restricted to the presence of a silicotic nodule and discarded dust-granulomatosis cases from the diagnosis of an early pneumoconiosis (Vincent *et al*. 2015). The decrease in mining activities also lead hygienists and physicians to overlook mineral dust exposure. The risk of dust-induced granulomatosis is therefore underestimated by pulmonologists. There is no sufficient medical examination about dust exposure and the informations generally given to pathologists are scarce (in our study, one of five building activities is mentioned to pathologist and pathologists never mentioned polarized light observation).

•Genetic background, hypersensitivity and exposure ways: if sarcoidosis is not related to airborne exposure to inorganic particles, other contamination ways have to be considered: oral absorption, cutaneomucous application, as for podoconiosis (Ayele et al. 2012) and finally, particles issued from wear prosthesis (Péoc'h *et al*. 1996).

In conclusion, the results of the MINASARC study show the relevance of measuring dust exposure in patients with sarcoidosis. For 8 patients, the MA allows to suspect a role of inhaled inorganic particles as a trigger of the disease. For the other 12 patients, the negativity of the MA could be explained by non-airborne and/or nanoparticle exposures. The podoconiosis, previously described as idiopathic elephantiasis is a good example of a pathology linked to inorganic dusts and genetical hypersensitivity background. So, within the whole epidemiological diversity of the disease, the environmental factors do not always play the same part. Finally, to test this physiopathological hypothesis on sarcoidosis, larger prospective studies are needed. We think that a mineralogical analysis in the lymphatic system which is the common path to all inorganic dusts could be of great interest. Sarcoidosis being considered as an exclusion diagnosis, the completion of the SQ, of a MA and hypersensitivity tests for identified minerals by MA could be suggested for patients being diagnosed with a granulomatous disease.

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