Lung tissue light microscopy analysis of inorganic fibrous and non fibrous minerals on 29 control patients obtained from Lyon's Forensic Institute

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INTRODUCTION

Microscopic techniques for analyzing asbestos fibers in lung tissue have provided information in the understanding of asbestos-related diseases, and about mineral dust in lung tissue. Nevertheless differences in sampling, preparation and counting techniques impose on laboratories to define control population and establish reference values for the methods that they use. These reference values, which can be used to define whether the observed particle or fiber concentration indicates abnormal retention of minerals particles, participate in estimation the probability that the disease case in question can be attributed to past mineral exposure. Guidelines for mineral analysis in biological samples recommend to define control populations for each laboratory with values obtained with the same techniques and procedures than for cases [1]. The aim of this study is to evaluate inorganic fibrous and non fibrous minerals in lung of reference population acquiring from subjects who were suicide or accident victims.

POPULATION AND METHODS

218 autopsies have been realised by the Forensic Institute of Lyon between February and June 2006. Subjects were studied according to following criteria.

Exclusion criteria : putrid corpse, evident pulmonary disease on clinical aspect or histological study, prosecutor refusal, drowning accident *Inclusion criteria* : family agreement

Minimal questionnaire about past exposure, occupation, addiction to smoking and residence were presented to families. We studied 29 samples from lower lobe by optical microscopy (table 1).

Description		n = 29
Range age	2 - 93 y (mean : 48,9 y)	
Sexe		
	Male	17
	Female	12
Addiction to smoking		
	Smokers	7
	Occasional smokers	6
	No smokers	13
	No informed	3
Resident		
	Town	20
	Village	7
	No informed	2

Table 1

Preparation samples : Lower lobe specimens were digested by sodium hypochlorite and collected on cellulose esters filters (pore size: 0.45µm), dried and fixed on glass slides by fusion in acetone vapors. Two samples were prepared for each specimen : one for which carbon particles were taken away for an easier asbestos bodies counting, and another for a dust evaluation.

Light microscope : magnification x400, transmitted light and phase contrast.

Counting : asbestos bodies (AB, pseudo-AB included), uncoated fibers (UF) longer than 15 mm, ferruginous bodies (FB) on opaque fiber (FBOF), FB on opaque particle (FBOP) and FB on transparent particle (FBNP) with the largest diameter of the particles greater than 15 µm (fig 1).

Results are expressed in g^{-1} of dry lung.

Dusty level : evaluated by software ImageJ [2]. Particles smallest than 2 pixels are ignored.

Feret's diameter (µm) was reported. Results are expressed in g^{-1} of dry lung. (Fig 2).

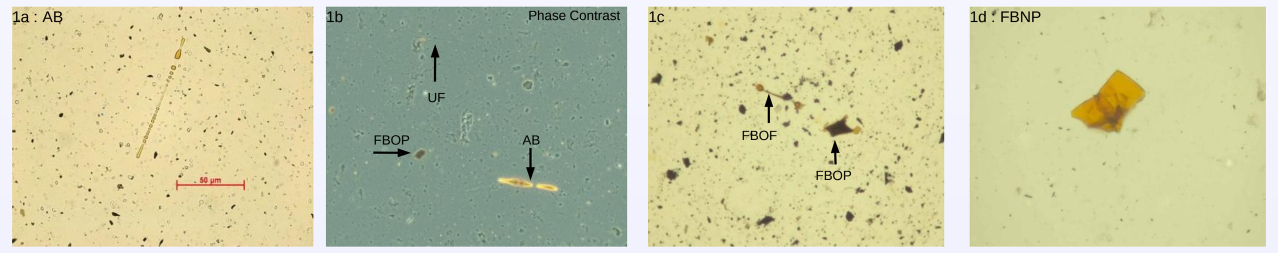

Figure 1 (x 400)

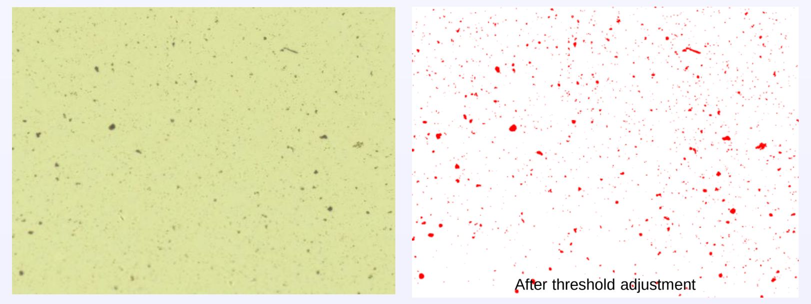

Figure 2 Particles photos (x400) and imaging analysis

RESULTS

1) Asbestos bodies and uncoated fibers

Table 2

Results/ g dry lung	AB	UF	FBOF	FBOP	FBNP
Number of subjects	29	29	29	29	29
Median	53	84	26	31	34
Geometric mean (GM)	67,9	112,6	27,3	50,7	50,6
Geometric Standard Deviation (GSD)	10,4	31	1,9	15	10,9
Min	15	20	8	13	13
Max	648	2 284	182	966	782
IC	24,7	41	9,9	18,5	18,4

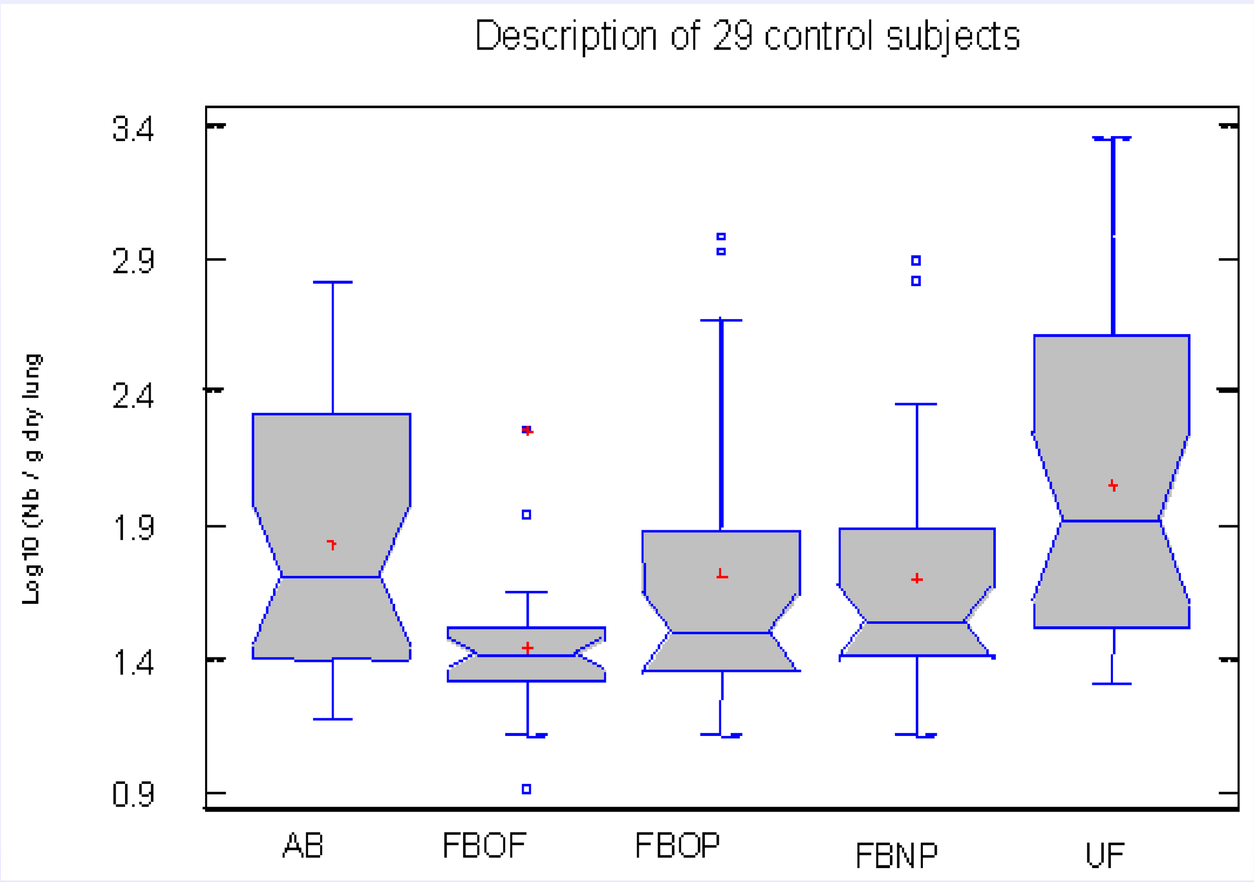

Figure 3

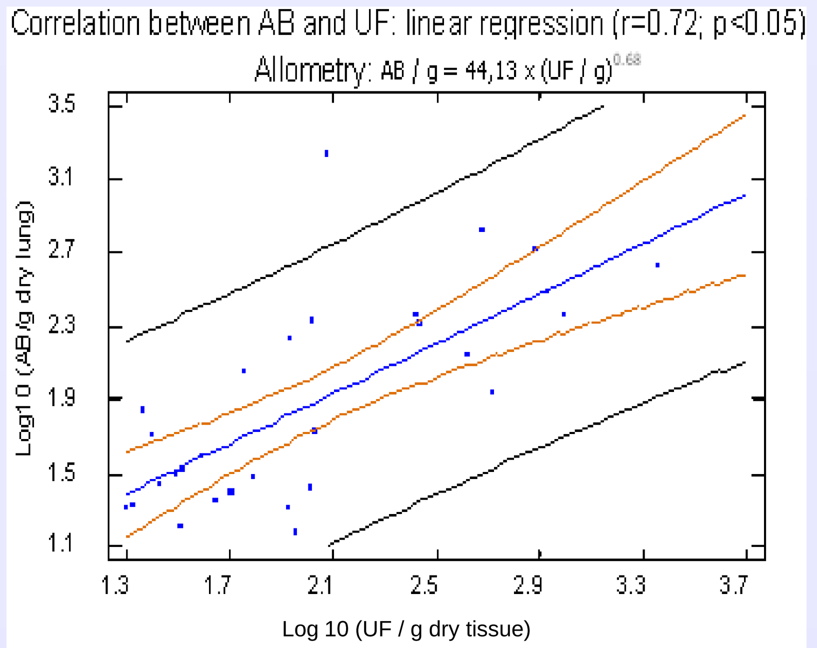

Figure 4

RESULTS

2) Dusty level : the geometric mean of our control population is 137.10^6 particles /g dry lung (range : 14.10^6-1253.10^6)

Table 3 summarize the repartition of particles by size (Feret's diameter)

- Age is statistically correllated with dusty level : r = 0,7, p < 0,05
- We have not found a positive crrrelation (linear regression test) between smohing and dusty level : r = 0,36, p > 0,05

Feret's diameter (µm)	Min (10^6)	Max (10^6)	Médian (10^6)	GM (10^6)	GSD
< 0,56	2,07	121	18,5	19,7	2,447
0,56 - 2,32	9,88	881	101	108	2,607
2,32 - 4,08	1,32	175	5,45	7,34	3,643
4,08 - 5,84	0,31	51,5	0,97	1,27	3,923
5,84 - 7,61	0,14	19,7	0,34	0,47	3,459
> 7,61	0,11	14,5	0,32	0,46	3,488

Table 3

DISCUSSION

1/ AB and UF : The average number of asbestos bodies in our general autopsy population is 67,9AB/gr dry lung (Table 2). No patient presents AB level upon 1000 (figure 3), the higher value is 648 AB/g dry lung. Our results are in accordance with literature. Churg [3] find that the average number of AB was 42 AB/g wet lung (~ 420/g dry lung) for patients (n=25) who had no occupational asbestos exposure. Dodson et al.[4] find an average of AB fewer than 20 AB/g wet lung (~ 200/g dry lung). Monso et al [5] observe an AB average of 52.35 AB/g dry lung for general population of urban industrial area and 5.37 AB/g dry lung for general population of rural area. We can note a positive correlation between (r = 0.72, p<0,05) AB and UF (Figure 4). For relationship of coated and uncoated fibers comparison of results is difficult because of differences in techniques. Studies usually used electronic microscopy to measure UF content and include short fibers (<5µ). We show that UF count by optical microscopy can give information about exposure subject. Our results are in agreement with the ones of Morgan and Holmes [6] who showed that the length distributions of UF and AB were dissimilar and who concluded that only fibers greater than 20 mm in length should be measured. Our result obtained with fibers longer than 15 mm, imply that a high UF level is an indication for asbestos exposure and more investigation (patient interview, electronic analyses) have to be proceed to identify fibers.

2/ Dusty level

The original use of numerical analysis allows us to obtain mineral particle concentration. The geometric mean number of particles 137.10^6 particles /g dry lung (range : 14.10^6–1253.10^6) is in the same order of magnitude than Churg and Wiggs [7] ($470x10^6$, range 180-$1090x10^6$) and Stettler et al [6] ($480x10^6$, range 110-$1610x10^6$) although methodology differences. Indeed dusty level estimation by electronic microscopy studies include particles under 0.5 µm. Furthermore the mean age of our control population is 48.9 year old and our subjects come from forensic institute and not from Hospitals. The greater number of particles in our study is in the size betwenn 0,56 µ and 2,32 µm. We don't observe correlation between cigarette smoking and particle concentration as Stettler et al [8]. Churg and Wiggs found correlation only for upper lobe but not for lower lobe[7]. A correlation was observed between patient age and dusty level as Stettler et al [8].

CONCLUSION

The average number of asbestos bodies (geometric mean : 67.9 AB/gr dry lung ; median : 53 ; range 15-648) of our control population of subjects from Lyon (France) urban area, who were suicide or accident victims, is in accordance with literature datas. A positive correlation (r=0,82) exist between AB and UF (>15mm). A high UF level is an indication for a possible asbestos exposure. Supplementary analyse by electronic microscopy would allow fibers identification. This study shows that numerical analysis from optical microscopy, easy and low-cost method, is useful to evaluate a dusty level. Studies on pathological and occupational cases are envisaged.

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