ULTRAFINE PARTICLES IN HUMAN PLEURA

RINALDO M [1-3], LACOURT A[2,3], ANDUJAR P[4-6], MARTINON L[7], JANSON X[7], PAIRON JC[4-7], CANAL RAFFIN M[2,3,8], SELLIER E[9], DUMORTIER P[10,11], BROCHARD P[2,3,7,8]

[1]Institut Universitaire Romand en Santé au travail, Epalinges-Lausanne Suisse, [2]Université de Bordeaux, France, [3]INSERM, U897 Bordeaux France, [4]INSERM, U955, Créteil, France; [5]Université Paris-Est Créteil, France, [6]Centre Hospitalier Intercommunal, Créteil, France, [7]Laboratoire d'étude des particules inhalées de la Ville de Paris, France, [8]CHU de Bordeaux, France, [9]Cremem-Placamat UMS 3626 87, Pessac France [10]Hôpital Erasme, Bruxelles , Belgique, [11]Université libre de Bruxelles, Belgique

email : *mickael.rinaldo@chuv.ch

Context & Objectives

- Pulmonary toxicity of the ultrafine particles (UFP) has been demonstrated by experimental et epidemiologic studies (asthma, COPD, cancer)
- Mineral particles such as coal dust or asbestos fibers can concentrate in macroscopic structures of the parietal pleura known as black spots
- Objectives : To characterize, quantify and compare particles (especially nanosized particles) found into lung parenchyma, normal and anthracotic (black spots) pleura of 10 patients, using transmission electron microscopy (TEM)

Material & Methods

Sample preparation

- Samples were kept in a filtered 10% formalin solution
- Preparation:
  - Wet alkalin digestion (sodium hypochlorite previously heated to 40 °C)
  - Orbital agitation during 1h
  - Addition of 5ml isopropanol
- Microfiltration with pre-carbonated 37 mm polycarbonate filter with a pore size of 0.2 µm (Nuclepore™, Whatman).
- Transfer on a TEM copper-indexed 200-mesh grid
  - Carbon-coating : particles are trapped between the two layers of carbon
  - Dissolution of the filter with chloroform

Electron microscopy analyses

- 1st stage : TEM Analysis (TECHNAI 12, FEI) : 50 microphotographs were taken for each sample using the same framework of preregistered coordinates and analyzed with Image J 1.43u software (NIH, USA). Each particle was contoured to calculate its surface and diameter (ferret diameter) and classified according to its morphology
- 2nd stage : Chemical composition analysis with a TEM (JEOL 2010 LaB6) equipped with an energy-dispersive X-Ray analysis (EDX) system (IDFix 12.2.1, SAM'X)

Statistical analyses

- Comparisons of particulate retention based on the Friedman test
- Comparisons of the composition of particles based on the Wilcoxon test
- Statistical analyses were performed with R.2.15.3 software.

Results

Particles concentration

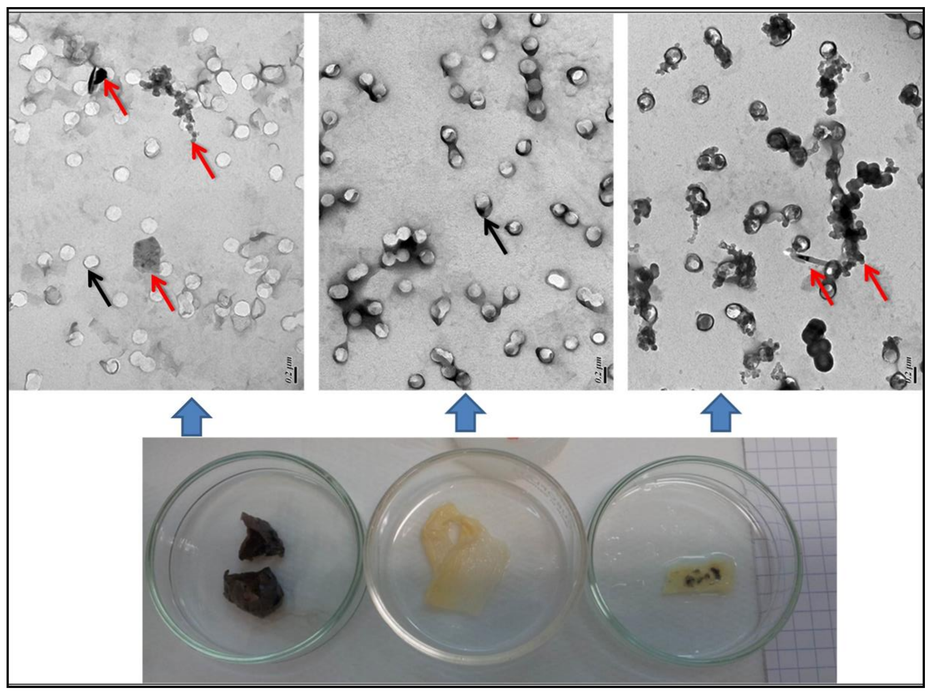


Figure 1: Macroscopic aspect of lung, normal pleura and anthracotic pleura samples and examples of associated micrographs from the same patient (magnitude x30.000). Red arrows show examples of particles; black arrows show a hole in the filter.

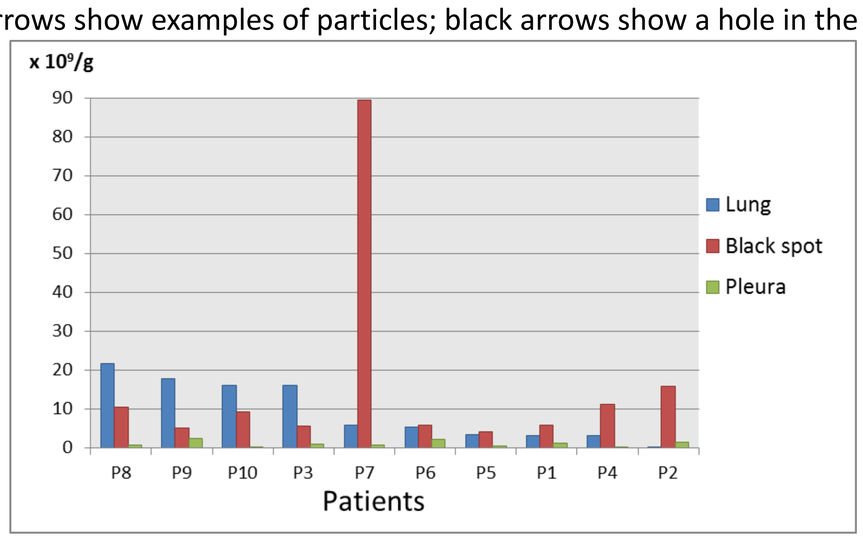
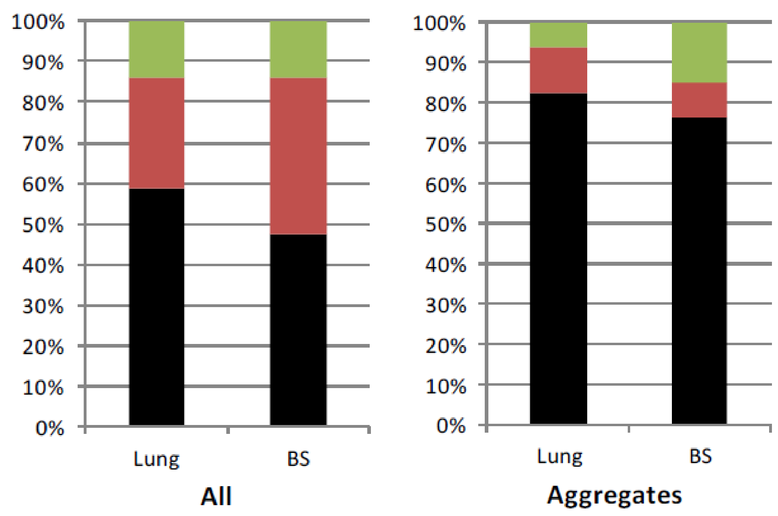


Figure 2: Concentration of particles across sample types for all patients. Patients are ranked in order of decreasing concentrations in lung samples.

Concentration
Black spot > lung > pleura

Mean concentration
- Lung : 9.2 ±7,8 x10^9 p/g
- Blackspot : 16.2±26 x10^9p/g
- Pleura : 1.1± 0.6 x10^9 p/g

Chemical composition of particles



Chemical composition is similar in lung and in black spot (BS)
≈ 50% are carbon particles (Lung: 56.6±18.1%, Black spot: 47.4±18.4%).
≈ 14% are metallic particles (14±7.5% vs 13.9±10.9%).

Figure 3: Composition of particles according to size. (Black bar: carbonaceous particles, red bar: mineral particles, green bar : metallic particles)

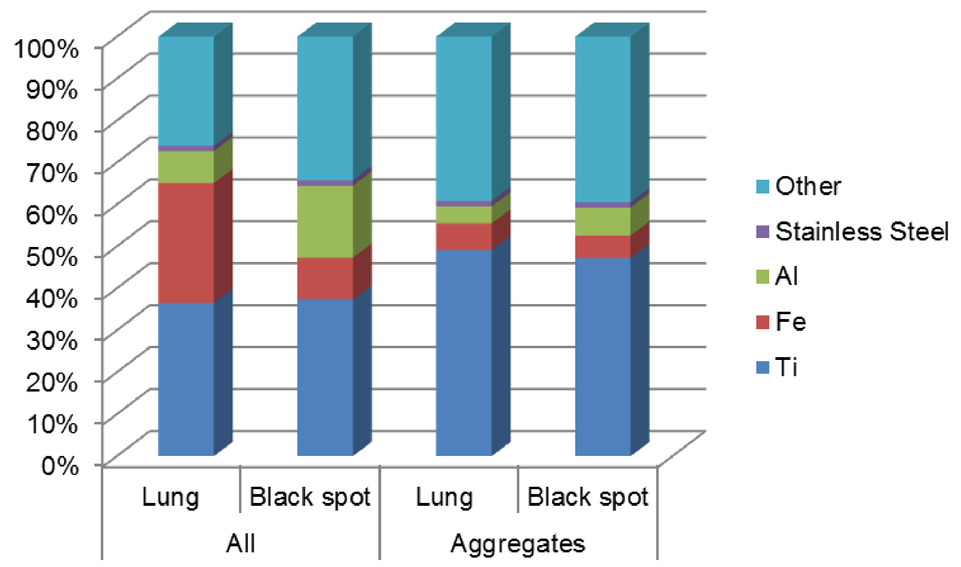


Figure 4: Composition of metallic particles

Titanium :
> 1/3 metallic particles (36.6% in the lung, 37.3% in the black spots
≈ half of metallic aggregates (49.1%, 47.3%).

Conclusion

- First demonstration of the accumulation of fine and ultrafine particles in human parietal pleura
- These particles accumulate in black spots at concentrations similar to or exceeding those in the lung
- According to present knowledge about pleural physiology, the similarities in chemical composition between the lung and the parietal pleural suggested a process of translocation through the pleural space
- Particles found in both tissues were mainly combustion-derived nanosized particles.
- Further investigations would be helpful to understand the kinetics of their translocation to the pleura and the consequences of their concentration in black spots

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