Context & Objectives

- Pulmonary toxicity of the ultrafine particles (UFP) has been demonstrated by experimental and 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 alkali digestion (sodium hypochlorite previously heated to 40 °C)
  - Orbital agitation for 1h
  - Addition of 5mL iso-propanol
- 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, SIA'MX)

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

<table>
<thead>
<tr>
<th>Patients</th>
<th>Lung</th>
<th>Black spot</th>
<th>Pleura</th>
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<tbody>
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</tbody>
</table>

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 (147±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)

Concentration

- Mean concentration:
  - Lung: 9.2 ±7.8 x10⁹ p/g
  - Blackspot: 16.2±26x10⁹ p/g
  - Pleura: 1.1±0.6 x10⁹ p/g

Mean concentration

- Black spot > lung > pleura

Figure 4: Composition of metallic particles

- > 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

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.