Asbestos bodies in bronchoalveolar lavage reflect lung asbestos body concentration

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ABSTRACT: Asbestos body (AB) countings on both bronchoalveolar lavage (BAL) fluids and digested lung tissue samples were performed in one hundred consecutive subjects submitted to a thoracotomy procedure, mostly for lung carcinoma. A good correlation (r = 0.73) was found between the two groups of values for the total group of subjects. When restrictive selection criteria were taken into account, such as lavage homolateral to the analysed lung, performed by the same trained physician, this correlation improved (r = 0.82). Absence of AB's or low AB counts (< 1 AB/ml) in BAL corresponded in about 70% of cases to concentrations of less than 1,000 AB/gm and in 90% of cases to concentrations less than 10,000 AB/gm. In subjects with BAL containing more than 1 AB/ml, the lung tissues of 85% contained more than 1,000 AB/gm and the tissues of 44% contained more than 10,000 AB/gm. Above 10 AB/ml BAL, all lung tissues contained more than 10,000 AB/gm. Since lung tissue is not readily available in patients undergoing assessment of their asbestos exposure, BAL fluid analysis seems to be a useful tool to evaluate lung AB concentrations. This technique cannot be performed, however, in patients with severe lung impairment which does not allow sufficient recovery of BAL fluid.

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Asbestos body counting in digests of lung tissue permits a rough estimation of asbestos exposure [1, 2]. It mainly reflects retention of long amphiboles [1-4] especially amosite and crocidolite, which are considered most important in the development of asbestosis, pleural plaques and mesothelioma [1, 2, 5]. Nevertheless, the presence of AB's in lung tissue or sections is only a marker of exposure and not of disease [1]. Since surgical or postmortem lung samples are not available in all the patients for whom asbestos retention has to be determined, we routinely count AB's in bronchoalveolar lavage fluid (BALF) [6, 7].

The aim of this study was to verify whether AB concentration in BALF provides a valuable estimate of AB concentration in the lung and if this procedure can be considered as an in vivo sampling tool of asbestos lung burden. The only data actually available concern a group of forty surgical and autopsy cases [8]. We performed BAL in one hundred patients who also underwent lung surgery and counted ABs in both BALF and digested lung tissue samples.

Subjects
One hundred patients (93 men and 7 women; mean age 61.4 ± 8.2 yrs) were included in this study. Ninety five patients underwent lung resection through thoracotomy and the remaining five underwent peripheral lung biopsy through thoracoscopy. Ninety three of them were part of 114 consecutive patients admitted for lung surgery in the Chest Department, who agreed to undergo bronchoalveolar lavage with overt consent in the peri-operative period. In 21 out of the 114 cases BAL was refused or was impossible to perform (due to death, post-operative infections or technical reasons such as operation schedules).

Exposure
All patients were questioned in detail by a pulmonary physician. Three categories of asbestos exposure were considered [6]: 22 patients had a definite exposure to asbestos, 15 had a probable or suspected exposure and 63 had no known exposure. The 22 following subjects were considered as definitely exposed: three insulators, one asbestos-cement worker, five heating mechanics, three thermal assays workers, one stove building worker, two car mechanics, two dental technicians, one ceiling worker, one iron foundry worker (insulated cab), one roadworks foreman, one chemical industry worker, one brewery worker (asbestos filters). There was thus no case specifically exposed to chrysotile fibres in mining, milling or brake lining factories.

The 15 following subjects were considered as probably exposed: two welders, one electrician, two
construction workers, three iron foundry workers, a roofer, three refractory material workers, two garage mechanics, one carpenter.

Amongst the 63 subjects who had not to their knowledge been exposed, 30 had been ‘blue collar’ and 33 ‘white collar’ workers, for the major part of their lives [6].

Disease

Patients were operated on by thoracotomy for lung carcinoma (84), mesothelioma (2), carcinoid tumour (1), metastasis (2), bronchiectasis (1), hamartoma (1), myoblastoma (1), round atelectasis with pleural thickening (1), bullectomy (1) and lung biopsy (1). Patients undergoing thoracoscopy had mesothelioma (3), pleural plaques (1), chronic pleural effusion (1).

Methods

Samples

1. Bronchoalveolar lavage. All patients underwent bronchoalveolar lavage shortly before (n = 52) (1-18 days; mean 4.5 days), or shortly after (n = 48) (6-24 days; mean 10.2 days) surgery.

In all but eight cases, BALs were performed by the same trained physician. Through bronchofibroscopy, the middle lobe or the lingula was washed with three aliquots of 50 ml normal saline solution. An average of 26 ml (25.9 ± 9) of the recovered fluid from the second and third samples [9] was used for AB counting. When it was possible, BAL was performed in the same lung from which the lobe(s) was(were) resected. This was not possible in about half of the cases, mainly due to neoplastic stenosis or compression of large bronchi or, in the case of post-operative BALs, after pneumonectomy.

2. Lung Tissue. Available lung tissue consisted of a complete lung (13), two lobes (8), one lobe (6-63 upper lobes, 19 lower lobes, 4 middle lobes), a lung segment (1) or peripheral lung tissue (7) for the thoracotomy patients.

All the lung tissue samples were fixed in 10% formalin filtered on 0.45 μm porosity membrane filters. In 74 cases samples were obtained from lungs inflated by intrabronchial perfusion of formalin at a standard pressure of 25 cmH₂O for further histological studies. In the remaining 26 cases samples were obtained from uninflated fixed lung tissue. When possible, two parenchymal samples of about 2 cm³ (about 5-10 gm wet weight) were excised from each available lobe or segment: one from a proximal area and one from a subpleural (distal) area. This was not possible in fourteen cases, where only one sample per lobe could be obtained. This was the case in the twelve patients with peripheral lung tissue obtained by thoracotomy or thoracoscopy and in two patients with very large tumours. The five samples obtained by thoracoscopy were very small (less than 0.5 gm wet weight).

Processing of samples

In order to avoid contamination, all fluids used were filtered on 0.45 μm pore size cellulose esters (Millipore®) or on 0.4 μm porosity polycarbonate (Nuclepore®) membrane filters.

The BAL fluids (10-40 ml) were then centrifuged in 50 ml disposable polyethylene tubes at 1,800 G for 20 min. The sediment obtained was allowed to react for 1 h with 20 ml sodium hypochlorite at room temperature. After a second centrifugation, elimination of sodium hypochloride and resuspension in water by handshaking and a short time in ultrasound (30 s), the particles were finally collected on 0.45 μm porosity Millipore® filters.

Tissues obtained by thoracoscopy (small samples) were weighed (wet weight between 0.04 and 0.2 gm) and allowed to react with bleach until complete dissolution. For further calculations the dry weight of these samples was estimated on the basis of a ratio dry/wet weight of lung tissue of 10% [1].

For tissue samples obtained from whole lungs or lobes, the blocks were firstly put on an absorbent paper for 2 min to remove excess formalin [3]. They were then minced on a clean surface with a scalpel. Bronchioles, mucus and blood coagulated in vessels were as far as possible disregarded in order to obtain true parenchymal tissue. One part of this sample was weighed and set to dry in an oven at 60°C for 24 h to obtain the dry/wet weight ratio. After weighing, the remaining portion was attacked by sodium hypochloride overnight at 60°C. Wet weight of these fractions ranged grossly between 1 and 2 gm. If dissolution was not complete fresh hypochloride was added. The sample was then centrifuged and rinsed (see above).

In order to avoid a too heavy loading of the filters by coal particles, 30 ml of a mixture 1/1 carbon tetrachloride and ethanol was added to the sediment resuspended in 15 ml of water. Two phases segregated, carbon tetrachloride (density = 1.58) at the bottom, ethanol and water at the top. After centrifugation of this mixture, the water-ethanol phase with coal particles trapped at the interface was carefully discarded by aspiration; the sides of the tube were cleaned with a cotton tip, the ‘heavy’ particles (density > 1.58) were resuspended in 20 ml of carbon tetrachloride and finally collected on 0.45 μ Millipore® filters.

The filters were clarified and fixed on glass slides by fusion in acetone vapours.

Asbestos body quantification

ABs were counted on a known portion (roughly one third) of the filter by light microscopy at a 400 x magnification under phase contrast.

All BAL's and lung tissues with concentrations of under 500 AB/gm were counted by two trained observers. The results represent the mean of the two calculated values. Results are expressed as numbers of AB/ml of BALF and AB/gm of dry lung tissue.

The practical detection limits currently achieved by
this technique are found to be between 0.05 and 0.1 AB/ml of BALF and between 20 and 40 AB/gm of dry lung, depending on the amount of tissue used and on its dry/wet weight ratio. For tissue, this limit can be raised down to the analytical sensitivity which lies between 5 and 10 AB/gm by counting the whole filter. When several samples were available for the same lung the mean of the values was considered.

Only ferruginous bodies developing on a fine, straight, transparent central core with regular or regularly segmented, yellow to brown ferroprotein coating were considered as asbestos bodies, since it was shown by analytical electron microscopy [9, 10] and microprobe [11] analysis that these are almost always built on asbestos fibres.

Ferruginous bodies with broad yellow cores (e.g. 'talc bodies') [12] or brown or black cores (e.g. 'coal bodies') [13] were not considered.

Since differences existed between the counting techniques used in this study (two readers, 400× magnification) and in the routine technique used before [6] (one reader, 250× magnification), we tried to compare the results obtained by the two methods as follows.

Twenty low-positive BAL's (< 1 AB/ml) found in the present series were selected. They were then put in the routine slides and read by one of the two counters, unaware of the test and unaware of the fact that he had read the slides previously (greater than six months interval).

**Results**

AB's were detected in 68 of the 100 BAL fluids and in 99 of the 100 lung samples. Their concentration varied from 0–3,500/ml in BALF and from 0–1,663,000/gm in lung tissue. The results are thus expressed on a log-log scale (fig 1).

For representation and calculation of the regression lines, o's were assimilated by convention to the detection threshold values (5 AB/gm in lung tissue; 0.05 AB/ml in BAL fluid).

1) **Bronchoalveolar lavage:** ABs were found in 16/33 (48.5%) of the white collar workers, with a (log) mean value of 0.14 AB/ml; 4/33 (12%) of BALF samples contained more than 1 AB/ml and no sample more than 5 AB/ml.

BAL were positive in 22/30 (73.3%) of blue collar workers with a (log) mean value of 0.26 AB/ml; 4/33 (12%) of BALF samples contained more than 1 AB/ml and no sample more than 5 AB/ml.

Among the people with definite or probable exposure, AB's were found in 30/37 (81%) of cases, with a (log) mean value of 1.6 AB/ml; 59.5% of the samples contained more than 1 AB/ml and 35% more than 5 AB/ml.

In the test of counting routinely (250×) of the twenty low-positive BAL samples (< 1 AB/ml) found with this technique (400×), only eleven were counted as positive (55%).

**Fig. 1.** Relationship between asbestos body counts in lung tissue and bronchoalveolar lavage. All patients are represented; open circles indicate patients with pleural benign or malignant disease, showing in some cases low AB burdens in lung and BAL. Large circles indicate patients who underwent biopsy through thoracoscopy.

2) **Lung tissue** (tables 1 and 2). Results are given in table 1 according to asbestos exposure. For lung tissue, sample analysis showed no systematic difference between AB counts in proximal and distal samples (paired t-test; t=0.59; ns). We therefore included in the results the group of patients which had only one lung sample for the calculations. Mean log of values for distal tissues was 3.00±86 and for proximal tissue 3.02±91.

3) **Relationship between BAL and tissue AB countings.** The relationship between AB concentration in the two studied samples is illustrated in figure 1. A good correlation was found between the two series of values (r=0.73; log Y (AB/gm dry lung)=0.684 log X (AB/ml BAL)+3.34) for the complete group (100 patients). The 95% confidence interval (in log) comprises between ±1.21 and 1.28 (fig. 1).

This correlation increased slightly (r=0.82; log Y=0.744 log X+3.35) when 57 patients were eliminated according to 'technical' selection criteria: the five patients with thoracoscopic biopsies (very small lung tissue samples likely to lead to variations in the evaluation of dry weight), and 52 patients who...
Table 1. - Relationship between AB in lung tissue and exposure (percentage of total in brackets)

<table>
<thead>
<tr>
<th>Exposure in AB/gm dry lung</th>
<th>0</th>
<th>&lt;1,000</th>
<th>1,000-10,000</th>
<th>&gt;10,000</th>
<th>Mean log</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>White collar workers n=33</td>
<td>1/33 (3)</td>
<td>25/33 (75.8)</td>
<td>7/33 (21.2)</td>
<td>0</td>
<td>415</td>
<td>0-7,550</td>
</tr>
<tr>
<td>Blue collar workers n=30</td>
<td>0</td>
<td>19/30 (63.3)</td>
<td>10/30 (33.3)</td>
<td>1/30 (3.3)</td>
<td>642</td>
<td>8-28,070</td>
</tr>
<tr>
<td>Exposed n=37</td>
<td>0</td>
<td>7/37 (18.9)</td>
<td>17/37 (46)</td>
<td>13/37 (35.9)</td>
<td>5,136</td>
<td>66-1,663,000</td>
</tr>
</tbody>
</table>

AB: Asbestos bodies

Table 2. - Comparison between AB countings in BAL fluid and in lung tissue (percentage in brackets)

<table>
<thead>
<tr>
<th>AB/ml BAL Fluid</th>
<th>n</th>
<th>&lt;1,000</th>
<th>1,000-10,000</th>
<th>&gt;10,000</th>
<th>Mean log</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>32</td>
<td>29/32 (90.6)</td>
<td>3/32 (9.3)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>0.05-0.99</td>
<td>36</td>
<td>18/36 (50)</td>
<td>18/36 (50)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>&gt;1</td>
<td>32</td>
<td>13/32 (40.6)</td>
<td>14/32 (43.7)</td>
<td>14/32 (43.7)</td>
<td>8/14 (57.1)</td>
<td></td>
</tr>
<tr>
<td>&gt;5</td>
<td>14</td>
<td>6/14 (42.8)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>&gt;10</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

AB: Asbestos bodies; BAL: bronchoalveolar lavage

had BAL contralaterally to the lung which was actually analysed. The same physician performed all 43 BAL's remaining.

Again considering the one hundred subjects, a better correlation between BAL and lung tissue analyses was found (r = 0.83) if we considered only those with definite or probable exposure (n = 37).

Discussion

To our knowledge, this is the first systematic study in virtually consecutive patients for whom simultaneous BAL and surgical lung tissue samples were obtained and analysed by standardized methods. Our study concerned a group of patients from whom a vast majority had lung carcinomas and who were not selected for asbestos exposure. The results demonstrate that the number of ABs recovered by BALF is correlated with the number of total lung ABs.

1) ABs in BAL.

The values of AB countings in BAL fluid are essentially consistent with our previous experience [6], being generally less than 1 AB/ml for subjects without known exposure. However, a major difference was the greater number of low-positive BALs in the study group (48.5% for white collar workers and 73.3% for blue collar workers as compared to 25 and 52% respectively in our previous study).

We believe that this can be explained by methodological differences. Firstly, all BALs of control subjects (white and blue collar) were performed by the same trained physician, working in the same conditions (material, assistance). We reported previously that low positive results can be influenced by BAL technique [6]. Secondly, higher magnification was used for counting under light microscope and this considerably enhanced the proportion of low positive results (see results). All the slides were counted separately by two trained observers, considering as the final value the means of both countings. This also improves the detection of positive cases under 1 AB/ml [6]. Finally, this control series comprised fewer women (7.5%) than the previous one (17%), and women can generally be considered to be less exposed to occupational risks [1].

These differences were essentially found for the low-positive results (<1 AB/ml) and were much less apparent for the higher ranges of results among control subjects.

In the asbestos-exposed group, the (log) mean number of AB in BAL was 1.6 AB/ml, a relatively low result compared to that which can be found in asbestos workers [6]. However, it must be reemphasized that our patients were consecutive surgical patients not selected for heavy asbestos exposure and that we included in the 'exposed' group patients with definite (even intermittent), probable or suspected exposures.
2) AB's in lung tissue

The value of 1000 AB/gm of dry weight is generally considered as the threshold of asbestos exposure [1,3,9,14-16]. Concentrations under 1,000/gm are classically found in women and white collar workers [1], representing "background environmental exposure". Values higher than 1,000/gm, up to 10,000/gm are commonly found in blue collar workers, reflecting "additional occupational or para-occupational contaminations" [1]. Lungs usually contain more than 10,000 AB/gm when asbestos exposure is definite, up to several millions [1,17].

The values and the ranges of AB's we report in lung tissue in the different exposure groups (table 1) are largely similar to previous data from literature [1-3].

Interestingly, the AB countings in lung samples obtained by thoracotomy were consistent with the results for the whole group (fig. 1). This technique therefore seems to be valuable for mineralogical analysis as well as for histological diagnosis.

3) Relationship between AB counts in both samples

The good correlation between the two groups of values was obtained despite the approximations inherent in the method. Bronchoalveolar lavage fluid was recovered from the lingula or middle lobe whereas lung tissue samples were generally taken from lower or upper lobes, in the same lung or in the other one. This is illustrated by the improvement in correlation when we selected BALs compared with homolateral lung samples.

Although correlation was strong, a wide range of lung AB concentrations was found for a given BAL concentration (large confidence interval) so that it is impossible to exactly evaluate AB burden in lung by BAL fluid analysis. Moreover, since this series comprises only three patients with very high concentrations in both samples (over 100 AB/ml and over 200,000 AB/gm), we cannot extrapolate with certainty the same correlation for subjects with heavy exposures.

This large range can be due to variations in the efficacy of lavage fluid recovery and in the area of lung concerned with the lavage, but it seems probable that the ratio of alveolar ABs to parenchymal ABs can vary between individuals. This ratio, evaluated to roughly 1% through total lung lavage [15] could be influenced by many unknown parameters: AB clearance, intensity and duration of exposure, delay since exposure, fibre size and length, etc.

Zero counts as well as low positive counts (<1 AB/ml) almost always corresponded to low tissue concentration (69.2% of cases under 1,000 AB/gm and 100% of cases under 10,000 AB/gm).

When BAL is performed correctly, such results practically exclude an important lung retention of AB (>10,000 AB/gm). BAL fluids containing from 1-5 ABs/gm appeared in 84.3% of cases with values over 1,000/gm and in 43.7% with concentrations higher than 10,000. If more than 5 AB/ml were detected, lung tissues always contained more than 1,000 AB/gm (this agrees with BIGNON et al. [15]); in 60% of the cases the figure was more than 10,000 AB/gm. Subjects with BAL results above 10 AB/ml always had lung concentrations of more than 10,000 AB/gm.

This corresponds to occupational exposure and is consistent with our experience (99.5% of all controls with less than 10 AB/ml) [6].

To conclude, AB concentration in BALF is reasonably well correlated with asbestos exposure [6]; and with lung concentration of AB's. Since ferroprotein coating rarely develops on chrysotile and on small fibres, asbestos bodies are only a fraction of total asbestos burden in the lung [1,18] and thus in bronchoalveolar lavage. Nevertheless, their presence reflects that of long amphibole fibres, considered the most frequently associated with asbestosis and mesothelioma [1,2,5]. Since it is easy to perform routinely, AB counting in BAL appears to be a useful diagnostic sampling technique for the evaluation of exposure to long fibres [6], industrial or environmental [19]. This technique, however, can not be performed in patients with severe obstructive lung impairment, in whom a good fluid reaeration does not occur.

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RéSUMÉ : Les comptages de corps asbestiques dans les liquides
de lavage bronchoalvéolaire et dans des échantillons digérés de tissu
pulmonaire, ont été réalisés chez 100 sujets consécutifs soumis à une
thoracotomie, la plupart du temps pour un cancer du poumon. Une
bonne corrélation (r = 0.73) a été mise en évidence entre les deux
groupes de valeurs pour l’ensemble du groupe de sujets. Si on prend
en considération des critères plus restrictifs de sélection, par exemple
le lavage homolatéral à l’égard de l’analyse pulmonaire exécutés par
le même médecin entraîné, la corrélation s’améliore (r = 0.82).
L’absence de corps asbestiques ou des comptages faibles (< 1
corps asbestique/ml) dans le lavage broncho-alvéolaire corres-
pond, dans environ 70% des cas, à des concentrations inférieures à
1 000 corps asbestiques/g, et dans 100% des cas à des
concentrations inférieures à 10 000 corps asbestiques/g. Chez les
sujets dont le lavage broncho-alvéolaire contient plus de 1 corps
asbestique/ml, les tissus pulmonaires contiennent dans 85% des
cas plus de 1 000 corps asbestiques/g, et dans 44% des cas plus de
10 000 corps asbestiques/g. Au-dessus de 10 corps asbestosi-
ques/ml de lavage broncho-alvéolaire, tous les tissus pulmonaires
contiennent plus de 10 000 corps asbestiques/g. Il est donc que
les tissus pulmonaires ne sont pas facilement accessibles chez les
patients qui subissent une évaluation de leur exposition à l’asbeste,
l’analyse du lavage broncho-alvéolaire semble être un outil utile
pour évaluer les concentrations de corps asbestiques dans le
poumon. Toutefois, cette technique ne peut pas être pratiquée chez
tous les patients qui ont une atteinte pulmonaire sévère qui rend
impossible une récupération correcte du liquide de lavage broncho-
alvéolaire.