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Relevance of asbestos bodies in sputum

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Relevance of asbestos bodies in sputum. H. Teschler, A.B. Thompson, R. Dollenkamp, N. Konietzko, U. Costabel. ©ERS Journals Ltd 1996.

ABSTRACT: The presence of asbestos bodies (ABs) in sputum specimens of individuals with occupational asbestos exposure has been well-documented. The aim of this study was to determine their clinical relevance in comparison to the concentration of AB in bronchoalveolar lavage (BAL) and lung tissue.

Subjects were included following a well-documented exposure of asbestos history (n=93) or BAL fluid analysis positive for ABs (n=42). The subjects with a well documented history of AB exposure were divided into three groups: heavy (Group 1, n=29); moderate (Group 2, n=31); or occasional exposure (Group 3, n=33). BAL fluid was available from all subjects, and lung tissue from 21 subjects. To assess the variability, 10 sputum positive subjects collected subsequent sputum on days 2, 7, 14, 30 and 90. ABs were determined by light microscopy after membrane filtration of specimen digests.

The mean sputum AB content was highest in Group 1 (2.4±5.5 AB·mL^{-1}), lower in Group 2 (0.2±0.3 AB·mL^{-1}) and lowest in Group 3 (0.1±0.1 AB·mL^{-1}) suggesting a correlation with cumulative exposure. However, many negative sputum samples were noted, when BAL specimens were positive. The AB content of sputum and BAL specimens did not correlate. ABs were found in sputum of all subjects with a tissue content of >1,000 AB·cm^{-3}, but in none with contents of <1,000 AB·cm^{-3}. Substantial variability of ABs was found in the five sequentially collected sputa of 10 initially positive patients (coefficient of variation 28–93%), but only two false negatives were found in these 50 samples.

Thus, sputum analysis for asbestos bodies is an insensitive method for assessing the lung asbestos burden, much less sensitive than bronchoalveolar lavage fluid analysis. However, a sputum sample positive for asbestos bodies is suggestive of a high lung asbestos burden.

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Asbestos exposure is recognized to occur in most industrialized countries as a result of occupational or environmental sources [1, 2]. Asbestos bodies (ABs) are a hallmark of asbestos exposure in the human lung [3], and an important component of the histological diagnosis of asbestos-induced lung diseases [4, 5]. When lung digestion techniques are utilized, small numbers of ABs are found in the lungs of more than 90% of the general population [3, 5], but much higher quantities are found in lungs of occupationally asbestos-exposed subjects [3–6]. The vast majority of ABs isolated from the human lungs are formed on long amphiboles [7]. Thus, they appear to be a valuable indicator of past exposure to amphibole asbestos.

ABs are sufficiently large to be detectable by light microscopy. In histological sections, ABs may be demonstrated either embedded within the lung interstitium, or within the bronchoalveolar spaces [5, 8]. ABs deposited in the airspace compartment are accessible to bronchoalveolar lavage (BAL) [9–11], and may be found in sputum specimens from occupationally-exposed subjects [12–17].

If asbestos-related disease is suspected, diagnosis requires quantitative information on past asbestos exposure, which can often be obtained through the occupational history. However, some patients present with only limited information on past asbestos exposure. The lung content of ABs is proportional to cumulative exposure [3, 5], but quantitation of lung ABs requires surgical lung biopsy. In order to avoid the necessity of a surgical procedure, attention has turned from quantification of ABs in lung tissue [3, 6], if to BAL [9–11, 18] or sputum specimens [12–17, 19] as the means for establishing the exposure history.

BAL is less invasive than open lung biopsy, but only enables collection of intra-alveolar ABs. Several careful investigations, however, have demonstrated a reasonably good correlation of AB concentrations in BAL fluid with the degree of asbestos exposure and the AB burden of the lung [20–22]. Discrepant results in primarily chrysotile-exposed asbestos workers indicate that the type of asbestos exposure (amphibole *versus* chrysotile) might markedly alter this relationship [9]. Collection of sputum is simpler, less invasive, and less expensive than BAL. The presence of ABs in sputum is felt to be a highly specific marker of asbestos exposure, and indicative of a considerable asbestos load within the lung [12–14]. However,

sputum AB quantification appears to be an insensitive measure of lung asbestos burden. Moreover, the correlation between BAL and sputum AB content is not well-established.

The main objectives of this study, therefore, were, 1) to compare the AB concentration in sputum and BAL fluid with each other and with the degree of occupational asbestos exposure; 2) to compare AB counts in sputum with the concentrations in the lung tissue from a subset of subjects who underwent lung biopsy; and 3) to evaluate the variability of AB counts in sequential sputum specimens from individuals with documented occupational exposure to asbestos.

Methods

Study population

The study population consisted of 135 subjects (115 males and 20 females; mean age 61±7 yrs) with a wide range of occupational exposures to mixed asbestos dust, in whom BAL was performed for suspicion of asbestos-related disease and who were able to produce sputum. Patients with a febrile respiratory infection were excluded. During their stay in hospital, medical and occupational histories were obtainable from 93 of the subjects. According to these exposure and job data, these 93 subjects were classified into three groups (table 1): Group 1 consisted of subjects who had worked in high exposure settings (>6 months, 8 h·day^{-1}) as asbestos textile workers, insulators or welders; Group 2 included subjects who had moderate occupational asbestos exposure (>6 months, <4 h·day^{-1}) as welders, mechanical-repairers, sheet-metal workers, electricians or foundry workers; and Group 3 consisted of subjects who had occasional asbestos exposure.

The variability of sputum AB counts was determined in 10 subjects (9 males and 1 female; mean age 57±8 yrs). These subjects were all cigarette smokers and met the American Thoracic Society (ATS) criteria for chronic bronchitis [23], with daily productive cough. The subjects were selected from individuals with sputum positive for ABs. Sputum was collected on days 1, 2, 7, 14, 30 and 90, and processed as described.

Table 1. – Demographic and exposure data for the three study groups

	Group 1	Group 2	Group 3
Pts n	33	31	29
Sex F/M	8/25	4/27	5/24
Age yrs*	59±6	61±7	61±8
Smoking habits			
Never n	9	7	8
Former n	18	17	16
Current n	6	7	5
Asbestos exposure*			
Duration yrs	10±6	7±5	–
Time since last exposure yrs	13±8	16±8	–

Pts: patients; M: male; F: female; Group 1: subjects who had worked in a high exposure setting (>6 months, 8 h·day^{-1}); Group 2: subjects who had moderate occupational asbestos exposure (>6 months, <4 h·day^{-1}); Group 3: subjects who had occasional asbestos exposure. *: mean±SD.

All subjects gave informed consent to BAL and, if clinically indicated, to lung biopsy or lung resection. Open lung biopsy (n=9) was performed for diagnosis of interstitial lung disease, and lung resection (n=12) for the surgical treatment of lung carcinoma. Written consent was obtained according to institutional guidelines.

Bronchoalveolar lavage

Bronchoscopy and BAL were carried out according to previously described procedures [24]. The BAL was routinely performed in a segment of the right middle lobe. However, in 21 patients undergoing open lung biopsy (n=15) or lobectomy (n=6) the lobe chosen for BAL was determined by the site of the intended surgical biopsy or lung resection. A total of 100 mL of 0.9% saline was instilled in five 20 mL aliquots, and gentle suction was applied after each instillation. The recovered fluid was pooled prior to analysis and the volume measured. For AB counting, an aliquot of 10 mL was taken from the native BAL fluid. The remaining fluid was filtered through surgical gauze. Total cell counts were determined using a Neubauer counting chamber. In addition, a trypan blue exclusion test for cell viability was performed.

The 10 mL of native BAL fluid were mixed with 100 mL distilled water and then allowed to incubate for at least 1 h at room temperature to lyse cells. The mixture was vacuum filtered through a 0.45 μm membrane filter (Millipore Corp., Bedford, MA, USA) after gentle agitation. The membrane was air-dried at room temperature and mounted onto a glass slide coated with Histokitt® (Karl Hecht GmbH, Sondheim, Germany). The membrane was subsequently covered with a second layer of Histokitt® and overlaid with a coverslip. After 24 h, the Histokitt® had cleared the filter, allowing unobstructed visualization of the optically transparent AB core. ABs were counted by examining the entire area of the membrane under the light microscope at 400 fold magnification. Only yellow-brown bodies that fulfilled the morphological criteria given by CHURG and co-workers [5, 7] were considered to be ABs. The detection limit was 0.1 AB·mL^{-1} BAL fluid, since 10 mL of BAL fluid were filtered and 1 AB·filter^{-1} was the minimum AB count. The concentrations were reported as AB·mL^{-1} BAL fluid.

Sputum

Patients were asked to expectorate spontaneous, early morning sputum into a large plastic cup. Each sample was surveyed for the presence of a typical sol and gel phase, which was used to indicate that the material represented sputum of lung origin instead of spit. No microscopic examination of macrophages or epithelial cells was performed. On the first occasion, only 58% of the patients produced a satisfactory specimen. The remaining patients provided an appropriate morning specimen

during a sampling period of 14 days. The volume was measured and the sputum sample then digested by 5.25% sodium hypochloride solution. The digestate was then filtered onto a 1.2 μm membrane filter. The entire membrane was prepared for light microscopy analysis as described above. The results were given as AB·mL^{-1} sputum.

Lung tissue

From each patient 1 cm^3 wet, formalin-fixed lung tissue was digested in 5.25% sodium hypochloride solution. After digestion was completed, the suspension was treated in an ultrasonic bath for 30 s and the residue filtered onto a 1.2 μm membrane filter. The membrane was processed and ABs were counted as described above. The results were reported as AB·cm^{-3} wet lung tissue. A comparison between AB counts in wet and dry lung tissue was possible in six patients, and led to a conversion factor of 9.2 (AB·g^{-1} dry lung tissue=9.2×AB·cm^{-3} wet lung tissue).

Statistical methods

Data were expressed as mean±SD. Group comparisons were made using the nonparametric Mann-Whitney U-test. The chi-squared test was utilized for comparing proportions in contingency tables. Spearman's correlation coefficient (r) was employed to evaluate the relationship between AB concentrations in sputum, BAL, and lung tissue. To measure agreement between sputum and BAL AB counts, a plot of the difference between the results of both techniques against the mean was applied [25]. The variability over time of AB·mL^{-1} sputum was expressed by the coefficient of variation, and counts for the sequential time periods were compared by analysis of variance (ANOVA). Statistical calculations were performed with the CSS Statistica package (StatSoft Inc., Tulsa, OK, USA) on a personal computer. Significance was defined as a p-value less than 0.05.

Results

Demographic data

Demographic data for the three exposure groups are listed in table 1. No significant difference was found with regard to mean age, sex distribution or smoking habits.

Relationship between AB counts, smoking habits and exposure history

Sputum Ab counts (n·mL^{-1}) within each group were significantly higher in smokers (Group 1: 2.9±4.7 *vs* 1.9±4.1, p<0.01; Group 2: 0.26±0.21 *vs* 0.16±0.24, p<0.05; Group 3: 0.12±0.10 *vs* 0.08±0.11, p<0.05). A significant correlation was found between levels of ABs in sputum and time since last exposure (Group 1: r=-0.34, p<0.01; Group 2: r=-0.42, p<0.001) and duration of exposure (Group 1: r=0.40, p<0.001; Group 2: r=0.31, p<0.001). Reliable exposure data for Group 3 were not available.

Table 2. – Asbestos body counts in sputum and bronchoalveolar lavage (BAL) among sputum producing patients in the three study groups

Asbestos bodies	Group 1 n=33	Group 2 n=31	Group 3 n=29
Sputum n·mL^{-1}	2.4±5.5 (0.0–28.4)	0.2±0.3 (0.0–1.3)	0.1±0.1 (0.0–0.5)
BAL n·mL^{-1}	66±106 (3.2–452)	2.5±1.9 (0.1–6.1)	0.2±0.3 (0.0–1.2)

Data are presented as mean±SD, and range in parenthesis. Comparison between groups: p<0.01, Group 2 *vs* Group 3; p<0.05, all other group comparisons. For description of groups see legend to table 1.

AB counts both in sputum and BAL were compared among the subject groups (table 2). The number of ABs both in sputum and BAL was related to exposure history, and was statistically significantly higher in Group 1 compared to Groups 2 and 3 (p<0.01); and also in Group 2 compared to Group 3 (p<0.05). However, all groups included subjects who had no ABs found in their sputum. In contrast, all members of Groups 1 and 2 had ABs in their BAL fluid. As a result, 33% of subjects in Group 1, 68% of subjects in Group 2, and 45% of subjects in Group 3 had BAL AB, but not sputum AB. No subjects without ABs in BAL fluid had AB in sputum (table 3).

Comparison of BAL fluid and sputum AB content

In the study population of 135 patients, the sputum content of ABs ranged 0–28.4 (0.6±2.9) AB·cm^{-3}. The BAL content of AB ranged 0–451.7 (16.9±58.9) AB·cm^{-3}. Figure 1a shows the percentage of negative sputum specimens and figure 1b the AB concentrations of the subjects grouped by BAL AB concentrations. The ranges of AB for each group were: 0 (n=30); 0.1–0.4 (n=33); 0.5–0.9 (n=13); 1.0–4.9 (n=26); 5.0–49.9 (n=23); and >50 (n=10) AB·mL^{-1} BAL fluid.

As shown in figure 1a, a large percentage of sputum samples from subjects with ABs in their BAL fluid contained no ABs. This was true even for some subjects with a high number of ABs in their BAL fluid. As can be seen in figure 1b, sputum analysis considerably

Table 3. – Number of sputum producing patients with positive and/or negative sputum and bronchoalveolar lavage (BAL) samples among the three study groups

	Group 1 n=33	Group 2 n=31	Group 3 n=29
+ve Sputum	22 (67)	7 (23)	5 (17)
+ve BAL	33 (100)	28 (90)	18 (62)
-ve Sputum +ve BAL	11 (33)	21 (68)	13 (45)
+ve Sputum -ve BAL	0 (0)	0 (0)	0 (0)

Values in parenthesis are percentages. For description of groups see legend to table 1.

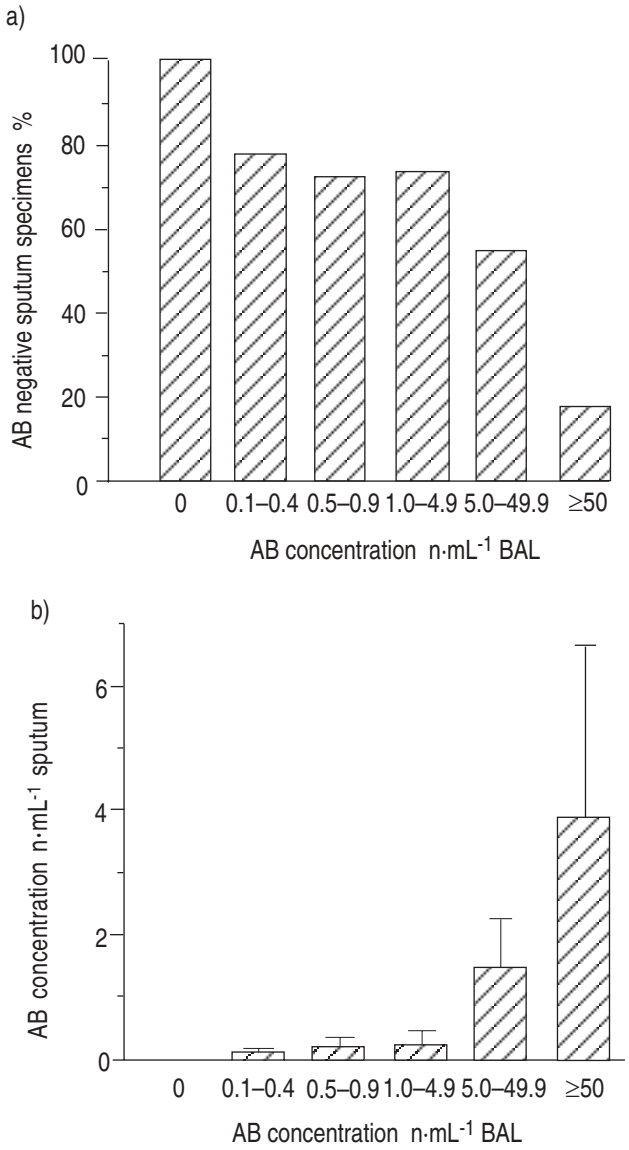

Fig. 1. – a) Percentage of asbestos body (AB) negative sputum samples and b) concentration of ABs in sputum for different ranges of AB concentrations in bronchoalveolar lavage (BAL). Data show mean±SD.

under-estimated the concentration of ABs in BAL fluid. Comparison of the AB content of BAL fluid and sputum demonstrated no significant correlation (r=0.13, p=0.34), either for the entire study population, the three exposure groups (r=0,27, 0,17, 0,19 for the groups with high, moderate and occasional exposure, respectively), or for the five subgroups with positive AB counts in the BAL fluid.

Plotting the data as the difference between the AB counts in sputum and BAL fluid *versus* the mean value for the two sampling procedures, allowed for analysis of sampling bias [25]. Plotted in this fashion (fig. 2), the data demonstrate that the recovery of ABs by sputum is systematically lower than the number recovered by BAL fluid (mean=-8.2±4.9 AB·mL^{-1}, 95% confidence limits (95% CI) -3.3 to -13.1 AB·mL^{-1}). There was a linear increase in the difference between the two sampling methods as the BAL content of AB increased, reflecting

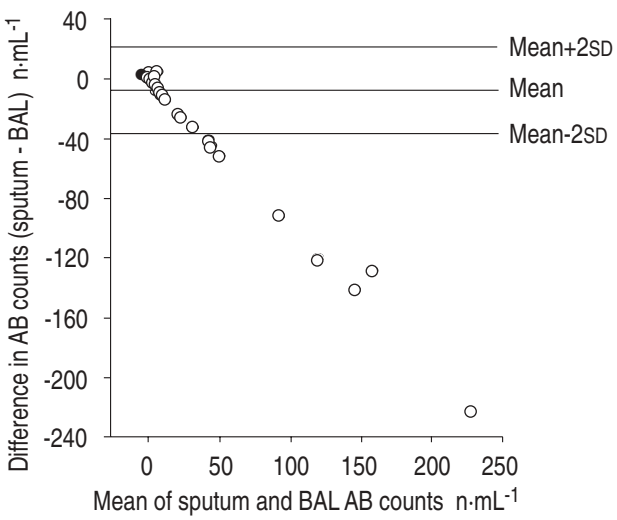

Fig. 2. – Difference between asbestos body (AB) counts in sputum and bronchoalveolar lavage (BAL) fluid *versus* the mean of the counts.

very low recovery rates by sputum, particularly in those individuals with high AB counts in BAL fluid.

Comparison of tissue and sputum AB content

Analysis of the open lung biopsy specimens revealed ABs in all samples. The number of ABs ranged 12–44,000 (mean±SD 6,811±10,796) AB·cm^{-3} wet lung tissue (fig. 3). For subjects with less than 1,000 AB·cm^{-3} of wet lung tissue, no ABs were found in the sputum samples. In contrast, all subjects with asbestos counts greater than 1,000 AB·cm^{-3} of wet lung tissue had ABs in their sputum. However, in the 11 subjects with ABs in their sputum there was no correlation with the number of ABs in the lung biopsy specimens (r=0.26, p=0.34).

Variability of sputum AB counts over time

In the initial positive sputum samples, used as the criteria for enrolling subjects in this part of the investigation,

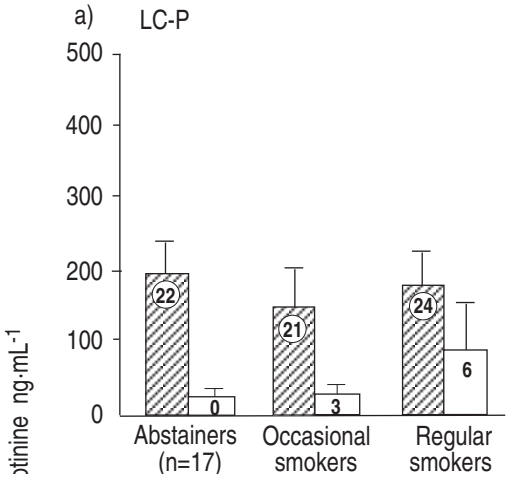

Fig. 3. – Relationship between asbestos body (AB) concentrations in sputum and lung tissue specimens of 21 asbestos-exposed subjects.

Table 4. – Variability of asbestos body counts ($n \cdot mL^{-1}$) in subsequent sputum specimens of 10 subjects

Subject No.	Day 1	Day 2	Day 7	Day 14	Day 30	Day 90	Mean count	CoV %
1	14.0	10.0	18.0	11.0	21.0	16.0	15.0	28
2	2.4	0.6	5.0	3.1	0.2	2.5	2.3	76
3	2.8	0.0	8.0	6.3	12.0	3.1	5.4	80
4	0.2	0.4	0.0	0.3	0.6	0.2	0.3	71
5	5.8	2.6	3.9	7.8	1.3	5.2	4.4	53
6	18.0	56.0	12.0	29.0	34.0	2.6	25.3	75
7	0.3	1.9	4.0	0.7	0.2	2.9	1.7	93
8	2.9	0.3	0.7	1.8	1.5	3.2	1.7	67
9	0.3	1.4	3.1	1.0	0.6	4.2	1.8	87
10	8.7	0.1	12.0	9.0	2.5	4.9	6.2	72

CoV: coefficient of variation.

the sputum content of ABs ranged 0.2–18 $AB \cdot mL^{-1}$ sputum (table 4). In the subsequent 50 samples of sputum, only two were found to contain no ABs, suggesting that sputum positive for ABs was a consistent finding. ANOVA suggested that the AB counts in the sputum samples did not differ over time (p=0.98). However, the coefficient of variation of the sequential counts ranged 28–92%, demonstrating that the quanti-fication of lung asbestos burden by sampling sputum introduces significant variability.

Discussion

The results of this study suggest that quantification of ABs in sputum, whilst related to history of occupational asbestos exposure, is less sensitive than BAL for the assessment of the lung asbestos body content. Furthermore, a significant percentage of the subjects, all of whom had asbestos exposure, had negative sputum analyses. Thus, analysis of sputum for ABs is an insensitive indicator of exposure. However, the data from the open lung biopsies would suggest that the presence of ABs in sputum indicates a relatively heavy asbestos burden in the lung of the subject under investigation.

These results are similar to previous reports of sputum analysis for ABs in occupationally-exposed subjects. In one report 35%, and in another 29%, of heavily exposed subjects were found to have sputum positive for ABs [12, 26]. According to our results, smokers are more likely to produce a satisfactory sputum sample with higher asbestos body counts than former or nonsmokers. In agreement with this, it has been demonstrated that smokers appear to have higher lung asbestos body burden than nonsmokers, probably due to a higher retention on the basis of an impaired clearance [27, 28]. The number of ABs present in the sputum correlated both with interval since last exposure and duration of exposure. This is in agreement with previous investigations [12, 27], and indicates that cumulative exposure is the significant factor in relation to the occurrence and amount of AB in sputum. In two other reports, sputum analysis yielded positive findings of ABs only if the lung content of ABs was higher than 900 $AB \cdot g^{-1}$ wet tissue or 1,000 $AB \cdot cm^{-3}$, respectively [13, 29]. This is in perfect agreement with our results and underlines that the occurrence of ABs in sputum is an exquisitely specific marker of heavy occupational exposure, mainly to amphiboles. However, ABs are generally a poor indicator of the pulmonary chrysotile asbestos burden and great variability exists in AB formation on longer amphibole fibres [3–5, 9]. Recent studies suggest that detection of uncoated asbestos fibres in digested sputum specimens by means of electron microscopy is a more sensitive way of confirming chrysotile and total asbestos exposure [15, 19].

The specificity of sputum ABs as an indicator of occupational asbestos exposure was not addressed by the investigation, as the subjects did not include a group of occupationally-unexposed individuals. This issue has been addressed in a previous publication [30]. In cytology specimens from 11,000 patients processed by a cytology laboratory, there were only five samples positive for ABs, and all of these were in retrospect found to be from subjects with significant occupational exposures.

BAL has been investigated as a means for quantifying the lung content of ABs [9–11, 18]. Previous investigations have demonstrated that there is a statistically significant correlation between BAL fluid AB content and lung AB content. It has been shown that the finding of 1 $AB \cdot mL^{-1}$ of BAL fluid roughly correlates with a tissue concentration of 1,000 $AB \cdot g^{-1}$ (or cm^{-3}) lung tissue [20–22]. In the current study, sputum AB counts were found not to correlate with BAL counts. Furthermore, many subjects with positive BAL fluid analysis had negative sputum results. These findings suggest that BAL is the superior of the two methods for assessing lung AB content.

The high coefficient of variation of the AB counts made on sequential sputum samples underscores the poor accuracy of lung AB quantification by sputum analysis. These results are similar to those reported previously in an analysis of sputa from 20 subjects, sampled over seven consecutive days [26]. This earlier report differs in the timing of the collections (over 1 week *versus* over 3 months) and in that some of the subjects had very high sputum AB counts (up to 2,458 $AB \cdot mL^{-1}$), but very similar variations were noted in sputum AB counts. Importantly, many more "false negatives" were reported. The majority of the subjects had at least one negative sputum in spite of previous positive sputa. This is in contrast to our results, in that only two of the 50 sequential sputa were negative.

The design of our study relied upon early morning collection of spontaneously expectorated sputum. It is

possible that the use of induced sputum may have yielded fewer false negatives.

Inducing sputum has been reported to significantly increase the number of positive specimens and the concentration of ABs [31, 32]. However, the improvement was far lower than would be necessary to significantly improve the correlation between BAL and sputum AB counts noted here. The subjects for the study of the variability of sequential sputum AB counts were chosen for the ability to produce sputum on a daily basis. This could potentially bias the result of the analysis as daily expectoration of sputum could conceivably alter AB clearance from the lower respiratory tract. Another potential limitation concerns the issue of whether sputum or spit was collected. This determination was based on the macroscopic appearance of the expectorated material instead of a microscopic examination of cells.

In conclusion, sputum analysis for asbestos bodies would appear to be an insensitive method for estimating lung asbestos body load. A negative sputum analysis for asbestos bodies in no way excludes the possibility of a high lung asbestos burden. However, a sputum sample positive for asbestos bodies suggests a significant lung burden of asbestos. Bronchoalveolar lavage would appear to be a superior nonsurgical method of sampling the lung for evidence of asbestos exposure.

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Notes for Author

1. Table 1. – Population numbers appear to be **17** female, 76 male = 93. However, initial study population was 135, 125 male and **10** female. Thus does the study include only the 93with medical history - where did the **extra** females come from?

2. Results – When discussing smokers vs nonsmokers you refer to tables 1 and 2. However, neither table shows the results for ABs in smokers/non smokers. Thus you do not show the values - should those be in table 2 and not just a statistical value?

3. p-values for table 2 in text do not appear to correspond to p-values in text *e.g.* table Group 2 *vs* 3 = $p<0.01$ but text states 2 *vs* 3 $p<0.05$. Please check.

4. ? Redraw figure 1a to show **negative** correlation which is the **point** of the figure? - see mock up.

5. Figure 1 - Are you happy with the zero BAL asbestosis column?

6. Abstract states group 3 sputum AB 0.01 ± 0.001 and table 2 shows 0.1 ± 0.1. Please check.