Association between Neutrophil Concentration in Bronchoalveolar Lavage Fluid and Recent Losses in Diffusing Capacity in Men Formerly Exposed to Asbestos*

Mark R. Cullen, M.D.; and William W. Merrill, M.D., F.C.C.P.

It has been observed widely that some individuals exposed to asbestos will experience continued losses of lung function after asbestos exposure ceases. Unfortunately, there are few data on factors that determine clinical course, limiting the clinician's ability to determine prognosis in an individual case and restricting the possibility for testing or targeting any potential intervention to alter the course among the millions at risk. In an attempt to address this question, we studied a volunteer population of 50 such men from among a stable, heterogeneous population of asbestos-exposed workers who had been continuously followed in our occupational medicine clinics for up to 12 years (mean, 6.3 years); most had some clinical or roentgenographic sign of asbestos effect, pleural or parenchymal. Each subject was reexamined clinically, functionally, and roentgenographically. Asbestos and tobacco exposure histories were carefully reviewed with the subjects and quantified based on these reports and available data regarding the various work environments from which they came. Subsequently, each underwent a bronchoalveolar lavage to assess cellularity and levels of various proteins. The levels of risk factors, clinical findings, and biologic parameters from lavage were examined for their relationship to serial changes in lung function during the period over which they had been previously followed. Results of the study demonstrate that serial changes in lung function were not closely related to level or length of prior exposure, smoking behavior, chest roentgenographic findings, or lung volumes. Progressive loss of diffusing capacity for carbon monoxide (Dco) was significantly associated with two factors: level of neutrophil concentration in lavage fluid (0.043 ± 0.016 ml/min/mm Hg/yr drop for each 0.1×10^4 neutrophils per milliliter, $p = 0.02$) and the level of Dco itself (0.17 ± 0.07 ml/min/mm Hg/yr drop for each 10 percent decrease in percent Dco predicted, $p = 0.01$). The relationship with neutrophil concentration was statistically independent of the association with Dco itself and stronger; it persisted when loss of Dco was adjusted for baseline value. Lung volume changes were not associated with any predictor variables, alone or in combination. We conclude that the presence of neutrophils in bronchoalveolar lavage fluid is associated with recent disease progression that may have implications in studies of the mechanisms of asbestos-associated disease and in clinical treatment of patients at risk. *(Chest 1992; 102:682-87)*

TNC = total nucleated cells

Intense occupational exposures to asbestos have been largely abated in most developed countries for over a decade, and the occurrence of new cases of asbestosis in workers exposed to permissible levels appears low. However, clinical issues regarding asbestos-related lung disease remain relevant for the millions of living men and women, some still younger than 40 years, with heavy exposure prior to modern controls. Of particular concern is the lack of data characterizing the natural history of disease in such individuals. Even less is known about factors that may modify the risk for developing progressive fibrosis.

Certain published human observations and experimental studies provide a starting point for investigation of these questions. First, it has been clearly recognized that at least some individuals with heavy occupational exposures to asbestos will develop fibrosis that continues to progress after exposure ceases.[1-3] Animal models, based on use of specific fiber types and sizes, have provided an experimental model for this progression.[4,5] On the other hand, many heavily exposed individuals, with or without overt asbestosis when first observed, appear to have little functional or roentgenographic change over time.[6]

Cross-sectional investigations looking at bronchoalveolar lavage (BAL) specimens from patients with asbestosis have provided important clues to the pathogenesis of progressive fibrosis.[7-15] Multiple observers have confirmed the general relationship between degree of fibrosis and parenchymal alveolitis as measured by polymorphonuclear leukocytes in lavage fluid.[12-15] However, the relationship of these findings to the patients' clinical courses has not been established. The present study was developed as an effort to delineate markers of clinical progression in our own clinic

*From the Occupational Medicine Program and Section of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Yale University School of Medicine, New Haven, Conn.
These studies were supported in part by NIH grant 1 RO1 OHO 2114, the Yale Adult Clinical Research Center (NIH grant M01-RR00125), and the research service of the West Haven, Conn, Veterans Administration Medical Center.
Manuscript received September 23, 1991; revision accepted January 7, 1992
Reprint requests: Dr. Cullen, Occupational Medicine Program, Welch Building, 333 Cedar Street, New Haven 06510

population of workers with asbestos-related parenchymal and pleural disorders.

Materials and Methods

Study Population

Volunteer subjects were recruited from among over 1,000 patients of the Yale Occupational Medicine Program (New Haven, Conn) previously diagnosed as having either pulmonary asbestosis or asbestos-related nonmalignant pleural disease using ILO roentgenographic criteria. These patients had been first referred to the clinic up to 12 years earlier from a wide range of exposure backgrounds, including asbestos product manufacturing, ship-building, insulation, and other general construction trades and industrial maintenance jobs. Recruitment efforts focused initially on patients with evidence of parenchymal lung involvement to ensure adequate numbers of such subjects. Patients were excluded only if they had acute infections. The broader clinic population and the general criteria used for diagnostic classification of the recruits have been described previously.[16]

Study Protocol

The volunteers were admitted to the Yale Adult Clinical Research Center, whereupon informed written consent was obtained. During the first day, subjects underwent a physical examination and general medical history to ascertain that no untreated condition, especially cardiovascular disease, was active that would preclude safe inclusion in the protocol. Subsequently, each subject was interviewed by a clinic physician or industrial hygienist to detail the work history, with particular attention to asbestos exposure. Detailed smoking and dietary histories were also obtained. Each then had a repeated chest roentgenogram and resting lung function studies, including spirometry, static lung volumes, and diffusing capacity for carbon monoxide using the same clinical laboratory where previous studies had been obtained (Collins DSII Plus system, Collins, Braintree, Mass).

A subset of subjects (n = 18) who were willing to come to the research center 24 h earlier for injection of radionuclide also underwent quantitative gallium 67 scanning. Results were reported as a gallium index relating lung uptake to uptake in other body regions by computer analysis of the scan image.[17]

On the following morning, patients underwent bronchoscopy and BAL by the protocol that we have described previously.[18] In brief, the upper airways were anesthetized with a topical agent and a fiberoptic scope was passed through the mouth. Airways were inspected carefully for endobronchial lesions. If none were found, three to five mucosal biopsies were performed until three pieces of visible mucosal tissue had been obtained from random subsegmental branch points in the right lower lobe. The bronchoscope was then wedged into the lingula. Lavage was performed by five alternate instillations/aspirations of 50-ml aliquots of isotonic saline solution at room temperature. The entire protocol had been reviewed and approved by the Yale Human Investigation Committee prior to initiation of the study.

Exposure Analysis

Since virtually none of the subjects had worked in an environment from which adequate environmental sampling for asbestos had taken place during the periods of exposure, average and cumulative doses were calculated using the relative scale proposed by Nicholson et al.[19] Asbestos insulators and workers from highly dusty manufacturing jobs were given a score of 1 "insulator-year" for each year of work. Other tradesmen and those in less dusty trades were given estimated values ranging from 0.1 to 0.5 "insulator year equivalents." Cumulative dose was estimated by summing the values over the entire span worked prior to institution of asbestos substitutes or major controls which had generally occurred five to ten years prior to the study; no subject had been significantly exposed after the date of his first clinic visit.

Clinical Assessment

It had been our experience that chest roentgenogram changes tend to be minimal over follow-up periods even as long as a decade. Therefore, technical differences tend to dominate over real change. Hence, we elected to evaluate only the most recent roentgenogram. This film was read blindly by the first author, a NIOSH-certified B-reader, using the 1980 ILO standard films for profusion. Pleural changes were graded based on shadows of grade A2 or greater as none, unilateral, bilateral, or diffuse (involving either or both sides).

Functional status was defined as of the date of the study. Measured values for total lung capacity (TLC) (by helium dilution), forced vital capacity, forced expiratory volume in 1 s (FEV_1), and diffusion capacity for carbon monoxide (Dco) were compared with the predicted values based on age and height. The Gaensler and Wright[20] equation was used for predicted Dco, while the Morris et al[21] equation was used for FVC. Predicted total lung capacity was derived by adding the residual volume using the equation of Goldman and Becklake.[22] Values were scored as percent predicted for each of these major parameters.

Longitudinal changes in lung function were obtained by review of all studies that had been performed acceptably in the clinical laboratory using the equipment described above; studies in which technician comments suggested unacceptable technique or tracings were excluded. For each acceptable study, the absolute values and date of the study were recorded. A least-squares fit regression line was fitted and the slope used as the estimate of longitudinal change for that parameter. Where there were only two acceptable points, the line connecting these was used, but subsequent analysis was restricted to those with at least three acceptable points, in view of the variability introduced when only two are available.

Analysis of Lavage Fluid

Processing of lavage fluid was as follows: Recovered fluid was filtered through a single layer of sterile surgical gauze to remove gross mucous particles and centrifuged to pellet cellular elements. Cells and fluid from aliquot 1 were analyzed separately from the remaining fluid, which was pooled for subsequent analysis.[13,18,23] Total nucleated cells (TNC) were quantified by hemocytometer. Fifty to 100,000 TNC were pelleted onto glass slides by cytocentrifugation and stained by modified Wright's Giemsa (Dif Quick, Harleco). Differential cell counts were performed by counting the percentage from 500 stained cells. Total protein was assessed by Coomassie blue using serum albumin as standard and proteins (keratin, IgA, free secretory component, IgG) were quantified by enzyme-linked immunosorbent assay as described previously in detail.[13,24-27] Cell counts and protein assays from the pooled specimen were used for all analyses. Results of BAL were expressed as proposed by the BAL Cooperative Group Steering Committee.[28] Cellular contents were expressed as both differential cell count and concentration in pooled lavage aliquots; proteins were expressed as concentration in the pooled fluid.

Data Analysis

Results of all evaluations and analyses were coded on a microcomputer (Macintosh II) and analyzed using a data exploration package (Data Desk Professional, Odesta, Northbrook, Ill). The distribution of all relevant variables was first evaluated for normality and appropriate transformations were made where necessary. Correlations among paired variables were explored using Pearson correlation for continuous variables and Spearman ranks for categorical data such as roentgenogram scores. Subsequent statistical comparisons were made using Student's t test and analysis of variance for continuous variables and χ^2 analysis for proportions. Simple and multiple linear regression analyses were used to evaluate associa-

tions among continuous variables of interest.

Results

Characteristics of the Study Population

Fifty men, ranging in age from 37 to 74 years (mean = 60.2 ± 8.6 [SD]) volunteered for the study. They had worked, starting on average at almost 25 years of age (range, 16 to 45 years), for a mean duration of 26 years (range, 5 to 42 years) at ten different work sites or trades. Using the exposure estimates described above, 26 percent (n = 13) had average annual exposure ratings of 1.0 insulator-years; most were, in fact, asbestos insulators. Another 60 percent (n = 30) were rated at 0.5, mostly those making submarines or involved in the sheetmetal and "pipe" trades—plumbing, steam-fitting, *etc*. Only seven (14 percent) had a rating of 0.2 or less, including bricklayers, carpenters, and some factory workers. Cumulative exposure ranged from 2.5 to 36 insulator-years, with a mean of 14.8 insulator-years.

Smoking behavior reported was slightly better than typically described for manual laborers and skilled tradesmen, possibly reflecting educational activities directed at these workers. Eight (16 percent) of the subjects had never smoked regularly, while 13 (26 percent) continued to smoke. Among the 29 (58 percent) who had quit, last smoking occurred 1 to 40 years prior to the study, with a mean of 13 years; smoking behavior was not correlated with asbestos exposure.

Roentgenographic changes of the lung parenchyma tended to be relatively mild in the group as a whole while over three fourths of the group had bilateral pleural plaques or diffuse pleural thickening. Summaries of the distribution of roentgenogram findings using the ILO 1980 standards for profusion are shown in Table 1.

Table 1—*Roentgenographic Findings in the Study Subjects (n = 50)*

ILO Profusion	No. of Subjects	Percent
Parenchymal grade		
0/0	1	2
0/1	21	42
1/0	12	24
1/1	10	20
1/2	2	4
2/1	2	4
2/2	1	2
3/2	1	2
	50	100
Pleural changes		
None	9	18
Unilateral plaques	3	6
Bilateral plaques	30	60
Diffuse pleural thickening	8	16
	50	100

All but one subject, who had had a laryngectomy, completed resting lung function testing. Among the 49, the percent predicted value for total lung capacity (TLC) was normally distributed with a mean of 77.3 percent (range, 48.4 to 114 percent) and a standard deviation of 14.0. Forced vital capacity (FVC) was similarly distributed with a mean of 74.3 percent of predicted (range, 45.7 to 103.6) and standard deviation of 14.4. The ratio of forced vital capacity in 1 s (FEV_1) to FVC ranged from 57 percent to 93 percent. Thirteen subjects (26.5 percent) had gross evidence of obstruction as indicated by $FEV_1/FVC < 70$ percent. The percent predicted for diffusing capacity for carbon monoxide (Dco), also normally distributed, ranged from 27 to 141.5 percent with a mean of 78.3 and standard deviation of 21.4.

Longitudinal Data

Among the 49 subjects capable of lung function testing, only two acceptable sets were available on 17. Among this group, which had been generally followed up for less than three years, the variance for major parameters of lung function was very large; therefore, subsequent analysis of this major outcome variable was restricted to the 32 subjects with at least three acceptable full sets of studies. Among this group, half had more than three assessable tests, ranging up to a maximum of ten. Average length of follow-up (interval between the first test and the last) was 63 months with a range of 29 to 139 months.

Estimating rate of change for each function (dTLC, dFVC, dDco) as the slope of the least-squares fit line for the parameter plotted against time, each of the variables had a normal distribution. Expressed as annual *losses*, these rates can be summarized as dTLC = 53.4 ml/yr ± 130.0 (SD), dFVC = 74.2 ml/yr ± 97.1, dDco = 0.52 ml/min/mm Hg/yr ± 0.88.

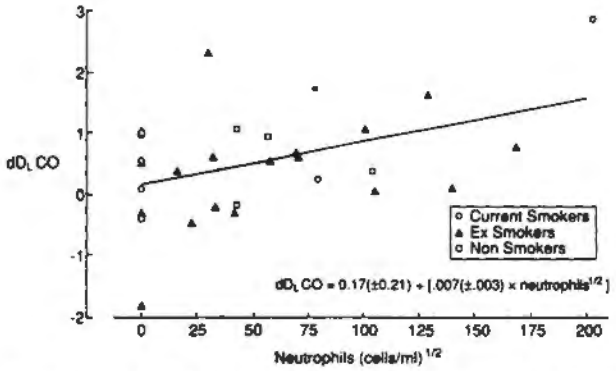

FIGURE 1. Scatter plot of losses in diffusing capacity (dDco) in ml/min/mm Hg/yr as a function of square root of neutrophil concentration in BAL fluid. Pearson correlation coefficient for combined data is $r = 0.47$, $p < 0.01$. The full regression equation, using standard deviation as the index of dispersion for the estimated parameters, is provided, along with the regression line derived from the estimates.

Table 2—*Findings on Bronchoalveolar Lavage (n = 48)*

Parameter	Mean	Median	Range
Volume, ml	115.1	122	11-183
Total nucleated cells, 10^6	53.0	28.8	1.1-335.0
Pooled differential, %			
Alveolar macrophages	83.4	88.2	26-97.6
Lymphocytes	11.9	8.5	0-43
Neutrophils	2.7	1.0	0-41
Eosinophils	1.7	0.2	0-33
Concentration of cellular elements, $\times 10^4$/ml			
Alveolar macrophages	37.4	22.8	3.8-152.0
Lymphocytes	5.26	2.07	0.0-41.9
Neutrophils	0.66	0.19	0-5.9
Eosinophils	0.69	0.11	0-13.4
Pooled proteins, mg/L			
Total protein	93.8	76.1	30.9-368.7
Albumin	49.1	41.2	14.1-125.4
IgG	11.3	7.2	1.9-60.4
IgA	16.8	14.2	1.2-62.4
Free secretory component	0.56	0.47	0-3.2
Keratin	3.6	0	0-42.6

BAL Findings

Forty-eight of the subjects underwent BAL successfully; two subjects could not tolerate the procedure (one because of presyncope, the other because of intractable coughing). The results of the studies are summarized in Table 2.

BAL results were analyzed for relationships with smoking status, which has been shown previously to influence cellular and protein constituents.[20] Recovered volumes did not differ among smoking strata (108 ml ± 25.1 for nonsmokers; 114 ± 42.5 for ex-smokers; 121.7 ± 51.8 for smokers), although there was more variability among the smokers. As anticipated, total recovered cells were markedly increased by smoking. Nonsmokers averaged 27.7×10^6 cells ± 20.1, ex-smokers had a mean of $36.7 \times 10^6 \pm 42.3$, and active smokers averaged $107.8 \times 10^6 \pm 93.0$. The difference among the groups by analysis of variance is significant at $p<0.002$. As in previous reports,[28] the proportions of cellular constituents were similar among the groups for all cell types except lymphocytes, which showed a nonsignificant trend toward decrease in the smoking groups (14.7 percent ± 13.5 in nonsmokers; 11.8 percent ± 9.6 in ex-smokers; and 10.2 percent ± 8.6 in current smokers).

The relationship between concentration of neutrophils and eosinophils as a function of smoking status was further explored. As expected, current and ex-smokers had higher neutrophil concentrations than nonsmokers (mean, 0.19×10^4/ml ± 0.22 for nonsmokers; 0.71 ± 10^4/ml ± 1.3 for ex-smokers; 0.89×10^4/ml for ± 1.3 for current smokers). Concentration of neutrophils was weakly but significantly related to smoking amount (log pack years) at $p<0.05$, $R^2 = 10.3$ percent. A similar trend was seen for eosinophils, but it did not reach significance. No relationship could be found between any of the protein constituents in BAL and smoking.

Relationship of Risk Factors, Clinical Findings, and Lavage to Functional Change

The simple correlations between each of the indices of progression—dTLC, dFVC, and dDco—and the major suspect risk factors were unrevealing. While the changes in TLC (dTLC) are closely correlated with those in FVC (dFVC), there is little relationship between change in lung volumes and change in Dco (dDco).

Pearson correlation coefficients between the measures of lung function at the time of lavage, expressed as percent predicted for TLC, FVC, and Dco and percent of FVC for FEV_1 were examined. The only significant relationship is that between Dco and its rate of change, with $r = 0.406$. Simple regression demonstrates an increase in the rate of loss of Dco equal to 0.17 ± 0.07 ml/min/mm Hg/yr for each 10 percent drop in percent predicted Dco, significant at $p = 0.02$. On the other hand, there was minimal correlation between dDco and either ILO grade on chest roentgenogram (Spearman rank correlation $r = 0.19$) or quantitative gallium scan (Pearson $r = 0.29$); no correlation on either study could be seen with changes in lung volumes.

Finally, the results of the BAL were inspected. There were no significant associations between any of the proteins in the supernatant and functional decline. Neither differential cell counts nor their normal transforms correlated well with the outcome variables, so the cell concentrations were examined. For lymphocytes, coefficients for all outcomes were negative; this reached borderline significance for dTLC. Eosinophils and neutrophils showed no effect on lung volumes but are positively related to loss of Dco (dDco) and to each other.

The relationship with neutrophilic granulocytes was further explored. Figure 1 shows the annual loss in Dco as a function of square root neutrophil concentration for each assessable study subject. Simple regression for all subjects demonstrates that this variable can explain one fifth of the total variance of dDco ($R^2 = 0.20$) with a slope equivalent to a drop of 0.043 ± 0.016 ml/min/mm Hg/yr in Dco for each additional 0.1×10^4 neutrophils per milliliter in the lavage fluid ($p = 0.01$) in the midrange of the data. The slope is significantly greater than 0 ($p<0.01$).

The possible confounding role of smoking was assessed in several ways. As noted above, smoking itself had little association with dDco. When log pack years or smoking strata were added as a variable into the regression for dDco, it had virtually no impact on the correlation coefficient for square root neutrophil

concentration, nor on its significance with the added variable, $0.01<p<0.02$. Finally, the relationship between dDco and neutrophil concentration was separately examined in each smoking group. As can be visually appreciated in Figure 1, the general relationship between neutrophil concentration and dDco is similar for each stratum. In fact, although the regression approaches significance only for current smokers ($p<0.06$) because of small numbers in each group, the regression coefficients are very similar: 0.008 in nonsmokers; 0.005 in smokers; 0.010 in current smokers; and 0.007 for the set overall.

Because the value of Dco as a percent predicted *itself* was correlated with dDco, the possible confounding effect of disease severity on the relationship between neutrophil concentration and dDco was explored. Analysis in the whole study population of the relationship between Dco at the time of study and root square neutrophil concentration showed a moderate correlation (Pearson $r=-0.34$, $p\approx0.03$), as would be expected from prior reports.[13-16] When only the subjects with assessable longitudinal data ($n=32$) are considered, this relationship weakens ($r=-0.26$) and fails to achieve significance. Thus, Dco itself and neutrophil concentration are not colinear. When both Dco and neutrophil concentration or its square root in BAL are included in a multiple regression for dDco, neutrophil concentration but not Dco remains significant ($p<0.05$). Together the two variables explain 28.4 percent of the variance in dDco.

Finally, multiple regression was used to explore the combined effects on dDco of all pertinent risk factors, clinical measurements, and BAL constituents. No variable even approached significance nor added to the proportion of dDco explained beyond neutrophil concentration in BAL and Dco itself.

Discussion

We have attempted in this study to examine factors that may be associated with longitudinal change in lung function in men formerly exposed to biologically significant doses of asbestos. The importance of establishing such information is underscored by three observations. First, millions of living workers and retirees in the United States and other developed countries have this environmental history and are increasingly coming under medical observation. Individual prognosis, appropriate intensity and timing of repeated examinations, and the possibility of interventions to modify course all hinge on the ability to predict accurately functional outcome. Second, no data broadly applicable to this large, heterogeneous group are presently available. Third, it is evident to all who observe such patients that the rate of loss of function of at least some of these individuals greatly exceeds comparable rates in the healthy population, suggesting that they are at risk for significant and increasing functional impairment. This is clearly demonstrated by the findings in our population, where the average losses of lung volumes were greater than 50 ml/yr and mean loss of Dco was over 0.5 ml/min/mm Hg/yr, each two to three times the values predicted for healthy men of comparable age.[20,21]

The lack of correlation of all clinically available indicators but Dco itself with recent changes in Dco renders the finding of the association between neutrophil concentration on BAL and progressive loss of Dco potentially important. Although this parameter explained only one fifth of the variability in Dco loss, it was more strongly associated in a full model than any clinical or other lavage parameter. Given the potential predictive value of this association, if confirmed, we must address the possibility that the study design may have distorted reality. Most notably, the study population described herein is quite small. Although up to 50 subjects were available for other components of the overall study, only the 32 with multiple acceptable lung function studies could be used for the analysis of disease progression. While it is a strength of the study that men of differing clinical stage, exposure, age, *etc*, are included in terms of generalizability, small numbers in any particular category make statistically robust associations impossible. A second consideration that potentially limits the usefulness of our observations are potential inaccuracies in classification of risks and measurement of predictor and outcome variables.

The third and perhaps most serious limitation of this study is the fundamental reversal of the observational design itself. Ideally, we would have liked to lavage the subjects and then observe them forward in time to plot their rates of functional decline. In so doing, we could have collected more frequent observations, limiting the effect of measurement error and allowing for the possibility that some subjects may have had nonlinear temporal changes in their courses, due perhaps to changes in smoking habits or the natural history of the disease itself. More importantly, by establishing the "baseline" status and biologic indices at the outset of follow-up, rather than at the conclusion, we could take into account the possibility that these parameters, too, change over time. Thus, the value of our observed observations to predict future course is limited by the possibility that both the neutrophil concentration "marker" and the outcome variable dDco have been misclassified.

Despite these limitations, several pieces of information suggest the association between neutrophil concentration and progressive loss of Dco is real. For one thing, the relationship in our data set appears fairly robust, consistent among smoking strata, and withstanding the addition back of other variables, including Dco itself, back into a full model. Further-

more, the effect of presumably random misclassification of subjects by progression due to variability in lung function testing and inadequate numbers of data points would be to blunt an association, not exaggerate it. Most important, however, are the compelling biologic reasons to believe that a casual relationship exists between granulocytosis in lavage and progression of parenchymal lung disease. A strong association between neutrophils and Dco has been shown in most studies of lavaged asbestos-exposed subjects[12-15] and is evident in our group as well. Mechanistically, it seems most plausible that granulocytes are associated with the active, ongoing disease process while Dco reflects cumulative past activity.

References

1 Barbers RG, Abraham JL. Asbestosis occurring after brief inhalational exposure: usefulness of broncheoalveolar lavage in diagnosis. Br J Ind Med 1989; 46:106-10

2 Cookson W, Deklerk N, Musk AW, Glancy JJ, Armstrong B, Hobbs M. The natural history of asbestosis in former crocidolite workers of Wittenoom Gorge. Am Rev Respir Dis 1986; 133:994-98

3 Barnes R. Progression of radiographic changes in asbestos workers and ex-workers. J Soc Occup Med 1986; 36:9-12

4 Begin R, Rola-Pleszczynski M, Masse S, Nadeau D, Drapeau G. Assessment of progression of asbestosis in the sheep model by bronchoalveolar lavage and pulmonary function tests. Thorax 1983; 38:449-57

5 Begin R, Masse S, Sebastein P, Bosse J, Rola-Pleszczynski M, Boctor M, et al. Asbestos exposure and retention as determinants of airway disease and asbestos alveolitis. Am Rev Respir Dis 1986; 134:1176-81

6 Jones RN, Diem JE, Hughes JM, Hammad YY, Glindmeyer HW, Weill H. Progression of asbestos effects: a prospective longitudinal study of chest radiographs and lung function. Br J Ind Med 1989; 46:97-105

7 Hayes AA, Bullan B, Lovegrove FT, Rose AH, Musk AW, Robinson BW. Gallium scanning and bronchoalveolar lavage in crocidolite-exposed workers. Chest 1989; 96:22-6

8 Wallace JM, Oishi JS, Barbers RG, Batra P, Aberle DR. Bronchoalveolar lavage cell and lymphocyte phenotype profiles in healthy asbestos-exposed shipyard workers. Am Rev Respir Dis 1989; 134:33-8

9 Costabel U, Bross KJ, Huck E, Guzman J, Matthys H. Lung and blood lymphocyte subsets in asbestosis and mixed dust pneumoconiosis. Chest 1987; 91:110-12

10 Gellert AR, Perry D, Langford JA, Riches PG, Rudd RM. Asbestosis: bronchoalveolar lavage fluid proteins and their relationship to pulmonary epithelial permeability. Chest 1985; 88:730-35

11 Rebuck AS, Brande AC. Bronchoalveolar lavage in asbestosis. Arch Intern Med 1983; 143:950-52

12 Robinson BW, James A, Ross AH, Sterrett GF, Musk AW. Bronchoalveolar lavage sampling of airway and alveolar cells. Br J Dis Chest 1988; 82:45-55

13 Hayes AA, Ross AH, Musk AW, Robinson BW. Neutrophil chemotactic factor release and neutrophil alveolitis in asbestos-exposed individuals. Chest 1988; 94:521-25

14 Xaubet A, Rodriguez-Roinsin R, Bombi JA, Marin A, Roca J, Agusti-Videl A. Correlation of bronchoalveolar lavage and clinical and functional findings in asbestosis. Am Rev Respir Dis 1986; 133:848-54

15 Gellert AR, Langford JA, Winter RJ, Uthyakumar S, Sinha G, Rudd RM. Asbestosis: assessment by bronchoalveolar lavage and measurement of pulmonary epithelial permeability. Thorax 1985; 40:508-14

16 Cullen MR, Cherniack MG. Spectrum of occupational disease in academic hospital-based referral center in Connecticut from 1979 to 1987. Arch Intern Med 1989; 149:1621-26

17 Begin R, Cantin A, Drapeaux G, Lamoreux G, Boctor M, Masse S, et al. Pulmonary uptake of gallium-67 in asbestos-exposed humans and sheep. Am Rev Respir Dis 1983; 127:623-30

18 Merrill WW, Naegel GP, Olchowski JJ, Reynolds HY. Immunoglobulin G subclass proteins in serum and lavage fluid of normal subjects: quantitation and comparison with immunoglobulins A and E. Am Rev Respir Dis 1985; 131:584-87

19 Nicholson WJ, Perkel G, Selikoff IJ. Occupational exposure to asbestos: population at risk and projected mortality 1980-2030. Am J Ind Med 1982; 2:259-311

20 Gaensler EA, Wright GW. Evaluation of respiratory impairment. Arch Environ Health 1966; 12:146-89

21 Morris JF, Koski A, Johnson LC. Spirometric standards for healthy non-smoking adults. Am Rev Respir Dis 1971; 103:57-67

22 Goldman HI, Becklake MR. Respiratory function tests: normal values at median altitudes and prediction of normal results. Am Rev Tuberc 1959; 79:457-67

23 Rennard SI, Hunninghake GW, Bitterman PB, Crystal RG. Production of fibronectin by the human alveolar macrophage: mechanism for the recruitment of fibroblasts to sites of tissue injury in interstitial lung diseases. Proc Natl Acad Sci USA 1981; 78:74147-51

24 Fick RB Jr, Olchowski J, Squier SU, Merrill WW, Reynolds HY. Immunoglobulin-G subclasses in cystic fibrosis: IgG2 response to *Pseudomonas aeruginosa* lipopolysaccharide. Am Rev Respir Dis 1986; 133:418-22

25 Merrill WW, Goodenberger D, Strober W, Matthay RA, Naegel GP, Reynolds HY. Free secretory component and other proteins in human lung lavage. Am Rev Respir Dis 1980; 122:156-61

26 Merrill WW, Naegel GP, Reynolds HY. Reaginic antibody in the lung fluid: analysis of normal human bronchoalveolar lavage fluid IgE and comparison to immunoglobulins G and A. J Lab Clin Med 1980; 96:494-500

27 Merrill WW, Barwick KW, Madri J, Strober W, Matthay RA, Olchowski J, et al. Bronchial lavage proteins as correlates of histopathologic airway changes in healthy smokers and patients with pulmonary carcinoma. Am Rev Respir Dis 1984; 130:905-09

28 The BAL Cooperative Group Steering Committee. Bronchoalveolar lavage constituents in healthy individuals, idiopathic pulmonary fibrosis and selected comparison groups. Am Rev Respir Dis 1990; 141(5 pt 2): S169-201