Bronchoalveolar Lavage in the Normal Volunteer Subject*

2. Safety and Results of Repeated BAL, and Use in the Assessment of Intrasubject Variability

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To investigate the safety of repeated bronchoalveolar lavage (BAL) and the variability of commonly measured parameters from BAL to BAL in the same subject, we performed a total of 59 BALs in 16 normal volunteer subjects. The BAL was performed with 120 ml (three aliquots of 40 ml) of room temperature, normal saline in a lingular subsegment. Four subjects had five BAL, three had four BAL, and nine had three BAL performed at minimal intervals of six weeks. The BAL analysis included percentage of lavageate returned, cell number, and percentage of alveolar macrophages, lymphocytes, neutrophils and eosinophils. Relatively similar percentages of lavageate were returned on each lavage. There was considerable variability in the cell numbers obtained both within and between subjects, although some subjects had consistently high, low, or normal cell numbers returned from each lavage. Cell differential was the most consistent parameter on repeated BAL analyses, but isolated "abnormal" elevations in the percentage of one or another cell type were occasionally noted. These were unrelated to either the number or relative sequence of the BALs. Pulmonary function tests performed both before and after the repeated BAL showed no significant change and participants noted no subjective deterioration in pulmonary function. This study supports the safety of repeated BALs in the normal subject, but the variability in cell numbers obtained and isolated, "abnormal" elevations of inflammatory cells occasionally noted in this normal population indicate that BAL parameters in patients need to be interpreted with extreme caution.

The promise of serial BAL as a safe and relatively noninvasive technique with which to follow the progression of interstitial lung disease or its response to therapy has yet to be realized. While there is evidence to suggest that BAL may serve this purpose in some clinical settings, there is also evidence which suggests limited, if any, clinical utility of BAL for the serial assessment of fibrotic interstitial disorders. Serial assessment assumes that disease activity is accurately reflected in the cells of inflammation measured by BAL and requires comparative analysis of BAL specimens before and after treatment. Even if the first, albeit controversial, assumption is true, there are several recognized pitfalls in BAL analyses which make it difficult to confirm the utility of BAL in assessment of disease activity. There is often a significantly different lavage analysis in BAL specimens taken from different areas of the lung in the same patient, leading to the potential of a pseudoimprovement or deterioration on repeat BAL analysis that may relate to area sampled rather than any real change in the status of the disease itself. Furthermore, the concept of serial sampling of lung cells by BAL presumes that the process of BAL itself does not influence analysis of subsequent lavages. There is no real basis for this presumption in man. Evidence from animal studies suggests that the process of BAL may lead to neutrophil influx. Technical aspects of repeated BAL such as volume and type of lavage fluid used, frequency and interval between lavages and which pulmonary subsegments should be lavaged are not standardized. Different BAL protocols used in different laboratories impede meaningful comparison of results.

It is also possible that repeated BAL may contribute to a detriment in lung function as conventionally measured by pulmonary function tests. Previous investigations by others have shown that there is short-term but reversible impairment in pulmonary function and gas exchange following a single large lavage in normal subjects or a single smaller lavage in patients. Although there are anecdotal and sometimes implicit evidence from series evaluating patients to indicate the safety of serial lavage, the true effects of repeated BAL in these studies may be masked by disease progression or remission. To our knowledge, serial BAL has not been studied systematically in normal subjects.

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In the current study, we performed three to five BALs (a total of 59 lavages) in the same area of the lung in each of 16 normal subjects. The questions we specifically sought to answer were as follow: (1) whether there was variability of commonly used BAL parameters from BAL to BAL in the same subject (intrasubject variability); (2) whether any such changes were related to the process of BAL itself; and (3) whether repeated BAL resulted in subjective or objective deterioration of pulmonary function.

Materials and Methods

Subjects

Sixteen normal volunteers, seven men and nine women aged 20 to 32 (average 25.4 years), were solicited for the study by word of mouth and posted notices and were reimbursed for their participation. Rigid criteria for inclusion in the study included the following: (1) lack of history of pulmonary disease; (2) nonsmokers; (3) normal posterior-anterior and lateral chest roentgenograms (within the previous year); (4) normal, complete pulmonary function tests; (5) normal heart and lung physical examination; (6) taking no medication; and (7) no history of viral or other illness for several weeks prior to the study. Performance of BAL in volunteer subjects was approved by the Committee for the Use of Human Subjects at Memorial Hospital of Rhode Island and informed consent was obtained from all subjects prior to study.

Bronchoscopy and BAL

Bronchoscopy was performed and the specimen processed as previously described in detail with one individual (M.J.J.) performing most of the differential analyses. The BAL was performed using three aliquots of 40 ml sterile, room temperature saline solution and processed as previously described. Not all BAL parameters were available for all subjects. On one occasion for each of five subjects, cell differentials were inadvertently not recorded (subject 4, 2nd BAL; subject 5, 5th BAL; subject 7, 4th BAL; subject 13, 2nd BAL; subject 15, 1st BAL). Other parameters such as cell number and percentage of return for these occasions were available and have been included in the results. All bronchoscopic examinations were performed by, or under the supervision of one of us (D.B.E.) using a bronchoscope with an external diameter of 4.8 mm, between 7:30 and 9:30 am. All BALs were performed in a lingual subsegment. Although an attempt was made to choose the same lingual segment in each subject for each BAL, this could not be assured in some taller subjects in which the bronchoscope was wedged in a subsegment several divisions from the main lingual bifurcation. All subjects requested consideration for repeat BAL.

Figure 1. Scattergram of percentage of lavageate returned from repeated BAL (120 ml) in each of 16 normal subjects (see Methods).

Figure 2. Scattergram of: A, the total cell numbers and B, cell number per milliliter of fluid returned from repeated BAL (120 ml) in each of 16 normal subjects.
without prompting, following their first BAL and were scheduled for repeat BAL at their convenience but no sooner than six weeks from the preceding BAL. This was an arbitrarily chosen time interval but one that has clinical relevance to serial BAL protocols in patients with interstitial lung disease. Schedule conflicts resulted in greater intervals between BAL in many of the subjects. The average interval between BAL was 4.7 months with a range from six weeks to 12 months. Some 50 percent of the repeated BAL were performed within 12 weeks and 75 percent within six months. Four subjects underwent five BALs; three subjects, four BAL; and nine subjects, three BALs. One subject (No. 1) was lavaged (five occasions) precisely at six-week intervals in the same subsegment.

**Pulmonary Function Testing**

All subjects had complete pulmonary function testing (PFTs) which included spirometry, lung volumes, and a single breath carbon monoxide diffusion capacity (DLco) prior to their first BAL. Repeat PFTs were performed using the same equipment, usually no sooner than within four weeks of the final BAL. One subject had PFTs performed within two weeks of the last BAL because she was leaving the community, and two others were not retested because they relocated without notice. The average interval between final BAL and repeat PFTs was ten weeks with a range of two weeks to 52 weeks; ten of the remaining 14 who had final PFTs were retested within six weeks of the last BAL. Percentage change between the first and second PFT was calculated by the following formula:

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\% \text{ change} = \frac{\text{value from the second PFT} - \text{value from the first PFT}}{\text{value from the first PFT}} \times 100
\]

For example, if the vital capacity from the first PFT was 4,680 ml and that from the second PFT was 4,580 ml, the calculated percentage change would be 4,580 - 4,680 / 4,680 \times 100 = -2.1 percent, the negative sign indicating a decrease relative to the previous PFTs.

**RESULTS**

**Percentage of Return and Cell Number**

The percentage of return and cell number for each BAL for each subject are shown in Figures 1 and 2, respectively. The collective percentage lavageate returned (mean ± SD) from the repeated BALs of all subjects from all lavages (64.8 ± 9.8 percent) generally fell within the normal range for our laboratory (63.4 ± 10.8 percent), derived from the first BAL of 78 normal subjects. Although there were no technical problems in the performance of BAL in any subject, there were a few subjects who had markedly different returns from BAL to BAL (see subjects 2,3, and 7). The differences in percentage returned did not seem to significantly influence the number of cells obtained by the respective BALs. As a group, the number of cells recovered 8.9 ± 4.7 \times 10^6 (mean ± SD) from the repeated BALs was somewhat higher (but not statistically significant) than the value (7.3 ± 3.9 \times 10^6) obtained from the first time lavage of 78 normal volunteer subjects. There was considerable variation in the cell numbers obtained from the different BALs from subject to subject and even within the same subject (Fig 2A). Expressing the results as cells recovered per milliliter of lavageate (Fig 2B) did not significantly influence the variability. However, some trends were discernible. The cell number recovered from one subject (No. 4) was consistently higher than our usual recovery, while the cell number recovered from five other subjects (No. 7, 10, 14, 15, 16) were consistently below our mean. Most of the subjects had more variability in the number of cells recovered on each lavage, but tended to be fairly evenly distributed around the mean.

**Cell Differential Analysis**

As shown in Figure 3, differential counts for the individual subjects were generally similar from lavage to lavage. A few exceptions were noted. Subjects 1, 5, 11, and 16 had isolated increases in percentage of lymphocytes that were not related to the order of the lavage, clinical symptoms, or cell recovery. Four subjects (No. 4, 6, 8, 12) had isolated abnormal (increased) percentage of neutrophils recovered. There was no logical explanation for the first three, but the 4th (No. 12) developed a respiratory, flu-like syndrome three days after BAL and in retrospect, felt that she was prodromal for a viral illness at the time of BAL. One subject (No. 4) had a persistently higher than normal neutrophil count on all BALs but was asymptomatic and had normal PFTs and chest roentgenogram.
As we have shown in the companion article to this study, in a larger group of normal subjects undergoing lavage, the cell number ranged more widely than any other BAL parameter that we measured. In one subject, who underwent BAL five times (subject 1), one “normal” \((8.5 \times 10^6)\), two higher than normal \((15.8 \text{ and } 16.0 \times 10^6)\), and two lower than normal \((5.6 \text{ and } 6.0 \times 10^6)\) cell returns were noted despite comparable percentage return of the lavageate. In contrast, some subjects gave consistently similar cell returns whether high, low, or normal. There was generally more consistency between the number of cells returned within an individual subject than between subjects. Although the lingula was the site of all BAL in all subjects, it is possible that different subsegments were lavaged on repeated BAL. This may have contributed to some of the variability of cell number since it may be argued that repeated BAL of a single segment may lead to either an increase or a decrease in cell number on subsequent BALs. This would lead to an “artificial” variability if results were compared to those of a subsegment which had not previously been lavaged. Although we cannot totally discount this possibility, there was no relationship between the cell number obtained and the order or the number of times BAL was performed. This also was true in subject 1 in whom BAL was performed in the same segment five times at precise six-week intervals.

In contrast to the cell number, cell differential was much more consistent from BAL to BAL and almost always within established norms for our laboratory. There were notable exceptions to this consistency. Four subjects had mildly abnormally elevated lymphocyte counts on a single occasion, and four had elevated neutrophil counts. Other than inherent variability, there was no other explanation for the abnormal results in most of these subjects. However, one of the subjects subsequently developed a respiratory flu-like syndrome that may have accounted for the elevated neutrophil count. These isolated abnormalities serve to point out another danger of BAL analysis. Seven (43 percent) of these 16 subjects would have been labeled as abnormal on the basis of a single BAL. In three of these seven subjects, the abnormal result occurred on the first BAL, thus precluding previous BAL as the cause of the abnormality. This possibility cannot be excluded in the others. We also cannot exclude the possibility that observer variation contributed to different results in these subjects. However, nearly identical repeat evaluation by the same observer, and confirmation by another observer (D.B.E) make this possibility less likely.

Pulmonary function tests performed before and after the repeated BALs showed no significant change. None of the volunteers reported any subjective sense of BAL-related morbidity. These current findings

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**Percentage of Change of Pulmonary Function Test Parameters**

All subjects had normal pulmonary function tests both prior and subsequent to repeated BALs. As shown in Figure 4, the mean change in either vital capacity (VC), total lung capacity (TLC), % FEV1, or Dco was less than 1.5 percent.

**Complications**

There were no significant untoward effects of BAL in any of the subjects. Two subjects each reported one episode of low grade temperature \(<37.7\text{°C}\) in the postbronchoscopic period. Another developed a respiratory viral syndrome (myalgias, rhinorrhea, cough) three days following her third BAL, accompanied by fever \(38.6\text{°C}\), which resolved within a week without specific therapy. None of the subjects felt that they had suffered any deterioration in their respiratory status as a result of the BALs. Although not quantified in the current study, there was little question that successive BAL procedures were better tolerated and progressed more smoothly than the initial BAL. This probably could be attributed to less anticipatory anxiety on the part of the subject on second and subsequent BALs.

**Discussion**

The data from the current study indicate that serial BAL in the same subject, in the same general area performed by the same experienced operator, using the same protocol, usually yields very similar results. However, even under these controlled circumstances, an occasional atypical result was recorded that would have labeled some of these normal subjects as “abnormal” had they been observed in the course of an isolated analysis of BAL.
agree with previous studies supporting the safety of BAL in normal subjects, but extend those findings to include the safety of repeated BAL. However, we would like to emphasize that all of the subjects in the current study were carefully screened to confirm their health, and that "small" (120 ml) lavages were performed. It is possible that repeated BAL with a larger aliquot of fluid, as is commonly used by many investigators, may not be as well tolerated. Single lobar BAL has been performed in a limited number of normal subjects with a large (1,000 ml) amount of fluid with transient but reversible (within 24 hours) decrement in gas exchange and PFT parameters. A number of smaller lavages performed in multiple sites rather than in a single subsegment is apparently less well tolerated and accompanied by more side effects. All BAL in the current study were performed by an experienced operator, in a carefully monitored setting with resuscitation equipment immediately available. Although BAL is a very safe procedure for both patients and normal subjects, it should never be approached in a casual manner.

There are practical clinical and research implications from the current study. Given the variability of cell numbers obtained in normal subjects under rigorously controlled conditions, it is difficult to envision any clinical utility in this parameter from lavages performed in patients with interstitial lung disease. There should also be appropriate caution exercised in the interpretation of a single abnormal BAL analysis, since even the cells of normal subjects may exhibit isolated and apparently inconsequential abnormalities. The safety of repeated BAL in normal volunteer subjects, at least in the protocol as reported in the current study, supports the use of repeated lavage in research protocols where the study of alveolar lining cells from the same individual over a period of time may be desirable. Although extrapolation to clinical practice may not be possible (no patients were studied), this study validates the use and safety of serial lavage as a research tool.

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