

## BRONCHOALVEOLAR LAVAGE - OBTAINING BIOLOGIC SPECIMENS FROM THE RESPIRATORY TRACT SURFACE

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### GLIMPSE IN THE PAST

Dating to fifth century BCE in Greece and the Hippocratic Movement of Physicians that created the beginnings of Western Medicine, a combination of bodily fluids and discharges, termed humors, described how the human body functioned. Among the four humors, phlegm was one and was connected to old age and winter (1). Consider that respiratory secretions in the elderly might reflect the cumulative effects of environmental exposures, ranging from occupational, chronic infection, smoke inhalation and mucus producing syndromes; whereas, winter connotes indoor person to person exposure of acute infections and pneumonitis. Stimulating cough to obtain expectorated sputum and grossly analyzing it has been a mainstay of clinical evaluation. Perhaps inspection of the interior of the nose and characterization of nasal secretions were included as well. Physical diagnosis of the chest with palpation, percussion (2) and succussion (commotion) and later indirect auscultation with the stethoscope (3), refined clinicopathologic associations. This all created more accurate diagnoses and methods to monitor lung disease.

Since there have been appliances available to insert into the lungs and airways, i.e., the rigid bron-

choscope (C. Jackson, 1904), balloon-anchored rubber catheters, such as double lumen tubes (E. Carlens, 1949) and metras catheters (H. Métras, 1953), and the flexible bronchofiberscope (S. Ikeda, 1967), described in detail (4,5), lavage of the airways has been done to mechanically clear secretions, and also to obtain washings for retrieval of cells and proteins from normals and from patients with lung diseases. After the advent of fiberoptic bronchoscopy (5), that is well tolerated in humans and acceptable for experimental use in normals and controls, BAL sampling of the lower airways and alveoli has become a common tool of research to obtain cells and proteinacious materials and to describe the milieu of the airways in animals (6) and humans (7).

The popularity of BAL, coupled with fiberoptic bronchoscopy, as a sampling method to obtain airway biological specimens increased quickly (reviewed 5), and led to the first BAL Conference held in Lille, France in 1976 (8). Dr. Ronald Crystal, Dr. AR Kalica, and I organized the second BAL Conference in Columbia, Maryland in 1984 (9). Over the next almost 25 years, there have been many more BAL conferences, always interesting and helpful, and held in wonderful locations, such as Vienna, Umea, Corfu, Kraków, and Coimbra to list a few.

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### 11<sup>th</sup> BAL INTERNATIONAL CONFERENCE: WHAT WAS PRESENTED ABOUT SAMPLING THE AIRWAYS?

During the joint BAL and WASOG meeting, there was considerable cross over between the use of sampling methods to obtain biological specimens

from the lung (and blood), and the analysis and interpretation of lung-derived biomarkers in many related interstitial lung diseases (ILD). Thus, the respective BAL and WASOG presentations complemented each other and interdigitated well. Among the formal presentations at plenary sessions, 18 were related to BAL sampling. Among almost 100 posters, 25 were research studies involved with BAL. The posters were all presented by their authors during several organized “poster rounds”; three were given as oral poster presentations in plenary sessions. This analysis will emphasize the oral BAL studies.

In organizing a summary of noteworthy contributions from BAL studies, these have been grouped into 6 categories:

1. Traditional use of BAL Findings to Evaluate Disease(s) – “high hopes still.”

Unquestionably, the study of cells and proteinaceous substances in lung washings have provided insight into the pathogenesis and host responses involved in inflammation, fibrosis, acute injury, and infection. BAL analysis has not been as helpful or reliable for diagnosis or a clinical monitoring modality, as in comparison with a CBC in acute microbial infection, for lung diseases such as Idiopathic Pulmonary Fibrosis (IPF) and Sarcoidosis (5). Yet, BAL fluid components are in close proximity to diseased tissue and the first approximation to in situ occurring events. Thus, “hope still” persists for BAL analysis to be a definitive diagnostic test.

Dr. V. Poletti (Italy) discussed BAL findings in acute lung injury as a surrogate for a lung tissue biopsy. Dr. D. Israel-Biet (France), evaluating patients after lung transplantation for graft rejection and onset of the bronchiolitis obliterans syndrome, looked for markers of these conditions and suggested that the persistence of polymorphonuclear neutrophils might be a signal. For environmental exposure, Dr. S. Constantopoulos (Greece) assessed the role of BAL in non-occupational asbestos exposure, Dr. F. Kokkris presented about chrysotile exposure, and D. F. Evyapan did so for metal induced lung disease. Dr. M. Drent (Netherlands) updated the usefulness of a computer data bank of BAL components for analysis in making a diagnosis of interstitial lung disease.

2. More Evolving Technology.

New analytical modalities applied to BAL components continue to reveal more biomarkers and mechanisms defining cellular activities. With the “omic” approach to biologic materials, intimating capture that will provide a comprehensive analysis of output, a total profile of proteins or genes in BAL fluid and cells from a normal or a patient specimen can be viewed. Dr. P. Rottoli (Italy) a pioneer in proteomics, continues to use this tool, “omics” to search for biomarkers in ILD. Similarly, gene arrays from cells, as describe by Dr. N. Kaminski (USA) sampled from lung tissue, BAL, and blood described cellular activity in ILD and for many other diseases.

3. Timing of BAL Fluid Analysis

As BAL can be well tolerated by normals (controls) and patients, the samplings can be repeated. This may have value in monitoring changes in BAL components during early development and aging, and during persistent illness. It was revealing to hear Dr. D. Phelps (USA) describe age-related changes in BAL components with a rat model. Similarly, Dr. N. Kaminski (USA) discussed serial analyses on blood cells, but which could apply to BAL cells. Longitudinal evaluation to monitor airway BAL changes is to be encouraged in patients with illness, and in volunteer controls to observe changes that occur with normal aging, and to provide BAL data in older control subjects that would better approximate or contrast with these found in older patients. Controls for research often need to be better age-approximated with patients, particularly in metabolic comparisons of drug activities when pharmacomics are desirable.

4. Sources of Special Cells from BAL.

Lung lavage retrieves important detachable types of cells and other biological specimens for in vitro study, especially alveolar macrophages (10) and lymphocytes. So a method to isolate dendritic cells (DC’s) from BAL fluids was of particular note (11). Dr. T. Berge and colleagues (Netherlands) and Dr. B. Lambrecht (Belgium) have a method to obtain dendritic cells; they estimate that about 0.1% of normal lavage cells are DC’s. The presence of DC’s among alveolar cells, which

are mostly macrophages, raises two interesting possibilities. First the finding of spontaneous lymphocyte rosettes in BAL fluids from patients with pulmonary sarcoidosis, described by Yeager and colleagues (12) and considered to be a distinguishing immunologic feature of the disease, "a LE cell prep equivalent" as in lupus erythematosus. The macrophage and surface attached T cells may in fact represent DC-T helper cell interactions involved in an antigen-innate immune TH<sub>1</sub> process that is a pathogenic mechanism in sarcoidosis (13). Second, the in vitro study of alveolar macrophages as phagocytes and effector cells in initiating inflammation and alveolitis has been considerable (10). But, Dr. C. Saltini's (Italy) use of them to reproduce the kinetics of tuberculosis infection, seemingly replicates an "in vivo" approach to creating a Mycobacterium exposure to AM's with subsequent infection of 40% of these cells and then describes their production of inflammatory cytokines. This seems the way tuberculosis may begin in a susceptible person.

#### 5. Perturbations in Animals or Humans that Affect BAL Components.

Inhalation of cigarette smoke, exposure to various environmental, often occupation-related, toxins, and aspiration of refluxed gastric secretions, all induce special changes in the composition of BAL components. Another exposure illustration with ozone by Dr. J. Floros (USA) causes oxidative stress in the airways that effects surfactant protein A. The result was illustrated with a proteomics analysis that used the term "discovery proteomics." Thus, an example of a stress for the lungs, new technology employed, and new terminology to describe what was caused are linked.

#### 6. Use of BAL in Children.

As this procedure is finding more applications in young patients, performed shortly after birth in some with congenital diseases such as cystic fibrosis, and in children with asthma or interstitial lung diseases, the safety and consequences of the lavage procedure require continued surveillance.

If was in part this consideration of safety with research use of investigative fiberoptic bronchoscopy, often coupled with broncho-provoca-

tion to simulate asthma and then perform lavage, that the NHLBI convened a workshop to review the scientific merit gained from this approach (14). The consensus among the workshop participants was positive that the risks compared with new scientific information obtained was worthwhile; continued use of this form of research was reaffirmed. But, there were some concerns about adequate training of personnel performing these procedures, and obtaining surrogate permission for these young, vulnerable patient subjects that should be reviewed for possible updating.

### FUTURE EXPECTATIONS: 12<sup>th</sup> BAL INTERNATIONAL CONFERENCE

In several years, the next BAL Conference will occur: What should we expect this sampling procedure to yield? Perhaps these items will be included:

#### 1. Continued New Revelations from Research on BAL Samples.

As this method of obtaining airway-alveolar space biological specimens has contributed many important insights into the normal and diseased respiratory tract, hopefully, more scientific observations will be forthcoming, and include the broad spectrum of lung diseases discussed at the 11<sup>th</sup> BAL conference and at all of them before. Although this sampling method is termed "BAL," operationally the procedure is broader and encompasses other strategies (15), such as local sampling in large versus peripheral airways, analysis of exhaled breath condensates, comparisons with blood cells and proteins, and genetic arrays of respiratory cells. As the "omic" wave of measurement technologies continues, the discovery of new substances or cellular functions with proteomics, genomic arrays, metabolomic revelations from activated cells, and applied pharmacomics to assess likely drug and therapeutic effectiveness, all will enhance the understanding of many diseases. Perhaps more research will occur to assess and compare the upper respiratory tract secretions and cells with the lower tract that might find similarities that would promote more upper airway sampling. This could facilitate less invasive longitudinal monitoring, be acceptable for children and

other younger subjects, and explore diseases that affect both portions of the respiratory tract, such as allergic rhinitis-sinusitis and hyperactive airways of asthma syndromes.

## 2. Review of Any Ethical Issues Related to Bronchoscopy Related Research.

The use of BAL in patients and controls or normals is well accepted and usually quite well tolerated in the volunteer on whom it is performed. As mentioned, we reaffirmed the contribution of bronchoscopy and related procedures done, such as airway brushing, transmucosal or trans-bronchial biopsies, and bronchoprovocation with an allergen to induce a localized airway reaction, at a NHLBI and NIAID sponsored workshop held July 25-26, 2003; the recommendations were published in October, 2005 (14). However, investigation has been expanded into patients with more advanced disease, more vulnerable patients, and involving younger subjects including infants and young children. In using bronchoscopy with BAL and other procedures as a research tool, it is always appropriate to consider and reevaluate the risk and discomfort for the research volunteer. Thus, in that spirit, the workshop report (14) produced an Appendix available online in the journal issue ([www.atsjournals.org](http://www.atsjournals.org)) was included with the workshop summary (titled: Appendix II: "Ethical Issues Related to Bronchoprovocation and Bronchoscopy Research.") Some issues considered included: informed consent, financial incentives, and additional protections for research involving children and those subjects who need surrogate permissions.

The BAL Conference, with its broadly representative and international composition of participants, might consider a formal review of some of these topics.

## 3. Training of Future Respiratory Clinical Scientists Who Use Respiratory Tract Sampling Methods.

The continued output of relevant scientific research on respiratory tract diseases is predicated on having a sufficient supply of interested, well trained, innovative investigators. How we motivate, and support this next generation is an important task for the academic research communi-

ty (16, 17). The success to fund and train these investigators is a collective effort between the supporting agencies, the professional societies and specialty groups, and the pharmaceutical industry. An assessment of "how are we doing" might be considered (18).

## 4. BAL Will Help Reveal "Secrets" Still in the Lungs.

It remains surprising what "we don't know" about cells in the respiratory tract and their functions, so a NHLBI workshop was held (July 9-10, 2007) to discuss how more information can be obtained about still unrecognized and insufficiently studied cells. The summary of this workshop and companion papers that review in detail the development of the lung, the airway, the alveolar unit, and the pulmonary vasculature (19) presents research recommendations. The use of BAL and other local sampling methods to retrieve cells and other biological specimens will be needed. Future BAL conferences will likely deal with these new findings, as the lung's cellular secrets are revealed.

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