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EDITORIAL

Report of ERS Task Force: guidelines for measurement of acellular components and standardization of BAL

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The investigatory technique of bronchoalveolar lavage (BAL) has become one of the most valuable research tools for studying inflammatory mechanisms in a wide range of diseases that affect the lungs and airways in humans, and several thousand peer review papers are published each year. In addition, cytological and microbiological testing of BAL samples are of established value for assisting in clinical diagnosis and management of many lung diseases, and these procedures are available routinely in most modern specialist respiratory centres. Yet despite its undoubted value, the interpretation of BAL findings is still hindered because the procedure cannot be precisely standardized. In particular, there is still no satisfactory method of determining the dilution factor during lavage. This prevents accurate quantification of all components in BAL fluids and causes especial difficulty in interpreting the results of measurements of soluble acellular components.

A number of Task Force reports have been published that have provided guidelines for the clinical application of BAL, and on technical aspects mainly related to evaluation of cells and other cytological features [1–4]. However, no guidelines are currently available for evaluation of acellular components, nor are any firm recommendations available for standardization of BAL procedure. The purpose of this editorial is to inform that this omission is now addressed by a new report of a European Respiratory Society (ERS) Task Force which is currently being published in the *European Respiratory Review* [5]. This Report provides a comprehensive review of the current status of techniques for measurement of acellular components in human BAL samples, and gives guidelines and recommendations to define standard procedures for the general conduct of BAL. It updates previous guidelines and gives recommendations on how to comply with the increasing demands for more effective quality control.

The Task Force was established by the BAL Scientific Group of the ERS in September 1995. The authors were privileged to be the task force co-ordinators and editors of the report, to which 49 authors and 21 invited reviewers from 15 countries contributed. It contains a series of detailed critical reviews which give recommendations according to the consensus view on 17 topics. These include three sections concerned with the general problems of BAL standardization [6–8] and one section dealing with the special problems relating to children [9]; while the remaining 13 sections provide detailed information on specific considerations that apply to the measurement of different categories of specific components including pulmonary surfactant components [10], immunoglobulins [11], proteases and antiproteases [12], angiotensin-converting enzyme [13], antioxidants, oxidants and oxidation products [14], lipid mediators [15], cytokines [16], soluble adhesion molecules [17], markers of fibrosis [18], granulocyte derived markers [19], tumour markers [20], markers of cell death [21], and other acellular components [22]. Those beginning work on lavage will find information about problems and pitfalls and how best to avoid them, while those experienced in the field will find comprehensive critical reviews to assist with selecting optimal approaches. All are encouraged to agree to the recommendations for better standardization procedures, in order to facilitate multicentre studies and the clearer comparison of findings between different workers to aid clinical interpretation.

Early BAL studies were focused mainly on defining the predominant types of inflammatory cells associated with different lung diseases. Despite concerns about the lack of standardization, it soon became apparent that the findings from independent groups of workers were remarkably similar, provided that results were expressed as differential BAL cell counts. This is because differential proportions of cells, unlike concentrations per millilitre, are unaffected by variable BAL dilution. The situation is more difficult for acellular components, because quantitative measurements per millilitre are the main approach to expression of results. Despite the inaccuracies in quantification, the Task Force report shows that a great deal of valuable information has been obtained about numerous acellular components in BAL samples and that reproducibility can be improved by a more informed approach to the methods used for expression of results and by improvements in assay standardization. The current frequent variability of approaches used by different workers may explain why the clinical utility of measurements of acellular components in BAL remains poorly defined compared to that of cellular components. This may change if future studies are conducted according to the guidelines proposed in the Task Force report to improve the reproducibility and reliability of quantitative measurements. These are summarized in table 1.

Applications of BAL have diversified into many fields of respiratory medicine, and it must therefore be emphasized that the conventional BAL technique was not

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Table 1. – Causes of variability in bronchoalveolar lavage (BAL) and recommendations on standardization

Source of Variability	Recommendation
Disease process itself	Specify underlying disease
Smoking	Specify whether non- ex- or current smoker
Drug treatment	Specify whether on anti-inflammatory or other drugs
Associated diseases	Specify any relevant associated diseases *e.g.* asthma
Dwell time to aspirate fluid	Keep to minimum, and specify dwell time if prolonged
Suction pressure during aspiration	Keep to minimum (3·33–13·3 kPa (25–100 mmHg))
Physician doing lavage procedure	Specify
Contamination from airways	Standardly determine epithelial cell counts
The handling of lavage fluid (*e.g.* filtered, unfiltered, dithiothreitol, concentrated, other)	State which technique
Volume of instilled fluid	Use ≥100 mL in adults and report volume standardly instilled (200–240 mL is recommended)
Number of aliquots instilled	Specify and standardize (four is recommended)
Position of patient	Specify
Area which is lavaged (one or more lobes, right middle lobe, right lower lobe, other?)	Specify
Variability of return of lavage fluid	Report volume and percentage of fluid recovered Establish criteria for minimum percentage recovery
Reporting measurements of acellular components in BAL	Standardly report values per millilitre of BAL fluid recovered (as well as any other special approaches).
Sample handling and storage	Refer to recommendations in specific sections of the Task Force report
Assay procedures and assay controls	Refer to recommendations in specific sections of the Task Force report

designed to investigate airways diseases but rather to investigate diseases that predominantly affect the alveolar structures. Approaches to BAL standardization therefore aim to preferentially sample material from the alveoli and reduce contamination from other sites (*e.g.* the airways). Alternative modifications of the lavage procedure are available to preferentially sample components from the bronchi by bronchial lavage", but the recommendations in the Task Force report apply only to conventional "bronchoalveolar" lavage.

The report considers in detail the four main problems that prevent accurate quantification of components in alveolar epithelial lining fluid (ELF) using BAL: 1) the problem of the unknown amount of dilution during lavage; 2) the problem of contamination of the ELF sample with material from the bronchi; 3) the problem of inadequate sampling due to incomplete mixing; and 4) the problem that lung permeability varies allowing loss of introduced lavage fluid into the tissues and increased leakage of soluble components from the blood capillaries and tissues into the ELF. It provides a critical review of the approaches that have been used to address these problems and advises on which are recommended. It also shows that great care must be taken to identify the source of the components in BAL samples for correct interpretation. In addition, it shows that a previously only seldom discussed reason for variability in BAL measurements between different workers is due to a lack of standardization in the assays themselves rather to variation in BAL procedure.

Regarding the problem of the unknown BAL dilution, the Task Force concludes that there is still no method to accurately determine the dilution factor. It therefore recommends that to reduce variability, workers should employ a standard introduction volume of lavage fluid of at least 100 mL in adults (240 mL is recommended as the most common), a standard number of input aliquots (four is recommended as the most common), and a standard site (the middle lobe of the right lung is recommended as the most common). However, it must be accepted that these standards will not be achievable in all subjects, owing to clinical constraints such as patient noncompliance, uncontrolled coughing, airways obstruction, severe lung injury, and the patient being a child.

Although introduction volumes can be standardized, the volume of fluid recovered cannot be controlled due to differences in lung collapse and drainage. The amount of lung fluid retrieved is also dependent upon dwell time, permeability, and other factors. Thus concentrations per millilitre of components in BAL fluid cannot be accurately extrapolated to the *in vivo* levels in ELF. To overcome this problem of unknown dilution, measurements of two or more components can be expressed as proportions relative to each other using the same principal as for differential counting of BAL cells. For example, individual proteins can be expressed as a proportion of the total protein, and individual phospholipids as a proportion of the total phospholipid. However, the Task Force emphasizes that it is also essential to report the quantitative measurements per millilitre of BAL fluid. These indicate whether changes in relative proportions are due to an abnormal increase or to a decrease in one or more components, and facilitate comparison of results from different workers. It is also recommended that data on lavage fluid input and recovery volumes and percentage recoveries should be included in reports to confirm that quantitative differences are not merely a reflection of these variables.

Regarding the problem of the unknown sampling location during lavage, the available literature shows that a smaller introduced volume will increase the relative amount of airways:alveolar material sampled, because the same bronchial surface area will be lavaged but a reduced alveolar area will be washed. The Task Force therefore

recommends the use of large introduction volumes of at least 100 mL which minimize the effect of bronchial contamination by diluting them sufficiently to have little effect on the final overall measurements unless the subject has substantive airways disease. The Task Force concludes that discarding the first 20 mL aspirate is not an accurate method to selectively remove bronchial contamination and this is not recommended. An indicator should be standardly used to assess airways contamination. The recommended most common approach is to count the numbers of ciliated bronchial epithelial cells and squamous epithelial cells present in the BAL samples. If these exceed 5% of the total BAL cells, the lavage sample may be unsatisfactory to draw reliable conclusions on alveolar composition.

The fact that the number of aliquots used to introduce lavage fluid has an influence on quantification has received little attention in previous reports on standardization. However, this becomes important for measurements of acellular components which are standardly expressed as amounts per millilitre of fluid recovered. The evidence shows that the concentrations of acellular components progressively decrease in sequential aspirates indicating an increasing dilutional effect, although not following a simple dilution model. Nevertheless, it must be concluded that to better standardize quantitative measurements it is clearly important to standardize the number of aliquots used for lavage (four is the most common).

The Task Force has also critically reviewed the information on the use of external and internal markers as reference standards to assess dilution. External markers can be added to the introduced lavage fluid and then the change in concentration in the fluid recovered can be determined as an indication of the extent of dilution. However, the Task Force report shows that none of the external makers investigated satisfies all of the essential criteria, namely that it is easily measured, safe, inert, resistant to biodegradation, not taken up by cells, of a high enough molecular weight to ensure that there is no transepithelial migration, and that it is homogeneously distributed in the lavage fluid. Research which is currently in progress, on external markers with the potential for use in the future, such as high molecular weight dextrans, is also discussed in the report.

Internal markers used as alternative reference standards must satisfy the criterion that they are present at constant levels in body fluids so that comparison of lavage with blood levels should give an accurate measure of dilution. Urea is widely used as an internal marker, but this report reviews evidence showing conclusively that this small molecule is unsuitable for use as an internal marker because it continues to pass rapidly from the blood and tissue spaces into the newly instilled lavage fluid. This demonstrates that the "dwell" time of fluid in the lungs during lavage should be minimal, but clinical constraints make this very difficult to standardize. Albumin is also widely used as an internal marker but the Task Force concludes that this also continues to diffuse into the instilled lavage fluid during the procedure. It diffuses more slowly than urea and can be of value as an internal marker in subjects who have normal lung permeability, but if lung permeability is increased its value as a reference standard is negated. It then becomes more useful as a marker of increased lung permeability.

The Task Force concludes that a pragmatic approach should be used when expressing results of acellular components as amounts per millilitre in order to facilitate comparison of data from different workers until such a time as a reliable external marker can be defined. The additional use of semiquantitative approaches such as ratios or relative proportions that are not influenced by dilution are also recommended to detect a quantitative imbalance in a mixture of components that normally occur in relatively constant proportions. This approach is already well accepted for differential counting of BAL cells to overcome the problems of dilution.

Finally, earlier guidelines on BAL standardization have not considered the extent to which contradictory findings between different workers might also be due to differences in the actual assay techniques. The Task Force report shows that assay variability is a very serious problem that applies to the measurement of many acellular components. There is an urgent need for international standard preparations that can be used as assay controls so that techniques can be optimized and results compared around the world. In addition, differences in the procedures used for handling, storage and processing of BAL samples can have a profound effect on the variability of results.

In summary, the information presented in the European Respiratory Society Task Force report points the way to improved standardization of procedures for the measurement of components in bronchoalveolar lavage. It provides a comprehensive survey of current information on the measurement of acellular components in bronchoalveolar lavage. It also identifies where there are inconsistencies which adversely affect assay results that urgently need to be addressed. The report will be of value to the bronchoalveolar lavage community as a whole if it succeeds in promoting standardization by providing these guidelines to update and extend the previous European reports. The bronchoalveolar lavage Scientific Group of the European Respiratory Society will continue to provide a forum where these guidelines and any other matters related to standardization can be annually reviewed. A workshop of the Group is organized at each annual European Respiratory Society Conference, and anybody that wishes to contribute to future discussions is very welcome.

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