Strengths and limitations of bronchoscopy (& related measures)
- Role of the BAL

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Learning objectives

- Airway inflammation in asthma management
- Usefulness of induced sputum
- Strengths and weaknesses of bronchial endoscopic techniques to assess airway diseases and evaluate airway pathophysiology
Endoscopic sampling for airway inflammatory diseases

Synopsis

- Endoscopic techniques for assessment of airway features
- Indications and usefulness of these techniques
- Weaknesses of this type of sampling
- Conclusions
Flexible bronchoscopy

- Initially for assessment of endobronchial tumors/lesions/foreign bodies, etc...

- Development of BAL (+TBBx) for the assessment of interstitial lung diseases
  - Sarcoidosis
  - Allergic Alveolitis
  - Others...

- Standardization for assessment of airway inflammatory disorders (e.g. Asthma...)
  - BAL
  - Bronchial biopsies
Airway inflammation and remodeling in asthma

- Acute phase:
  - Allergen
  - IgE
  - Leukotrienes
  - Histamine

- Chronic phase:
  - Macrophage
  - Leukocyte recruitment
  - TNF-α
  - Goblet cell
  - Epithelial cell
  - IL-4, IL-5, IL-13
  - Eosinophil

- Remodeling:
  - Smooth muscle hyperplasia and hypertrophy
  - Mucus gland hyperplasia
  - Airway remodeling
  - Collagen deposition
  - Neutrophil
  - Cytokines, Chemokines, Prostanoids
  - Fibroblast activation
Bronchial brushings

- Bronchial epithelial cell sampling (e.g. for cultures)
Bronchoalveolar lavage

- Cell differential
- Mediator & cytokines measurements
Bronchial biopsies

Tissue sampling

- Light microscopy,
- Electron microscopy
- Immunohistochemical stains, fluorescence, in-situ hybridization, or PCR for mRNA expression for cytokines and adhesion molecules
- Cell function
- Cell cultures
- Genes analyses

Inflammation and structural changes
Endoscopic techniques: limitations

- Should be done in a research setting mostly, by experienced endoscopists, with all human and material resources needed.
- Cannot usually be performed if airway obstruction is too severe or the patient unstable.
- Potential side-effects of the technique or medication used.
  - Safety and good tolerance of these procedures in asthma patients of mild, moderate and severe disease have been well documented.
BAL: advantages

- Samples obtained by BAL include luminal cells (predominantly macrophages) and soluble factors coming from a relatively large airway surface area, including the peripheral airways.

- With sequential lavage, it is possible to observe the results of allergen and placebo challenge in the same individual over time.

- BAL includes both mediators and cells released into the fluid phase.

Adapted from Jeffery et al. 2003
Recovery of biological samples in the form of cellular and soluble materials from medium and small airways, and alveolar spaces.

BAL has aided in the identification of many mediators currently thought to have a significant role in the pathophysiology of asthma (e.g. IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, GM-CSF, TNF-a, ICAM-1)

Murugan et al. 2009
**BAL: limitations**

- No spatial, structural, or histologic information
- Cellular signals may be affected by the method of collection
- The source of the cell sample is imprecise
- The volume of sample recovered may relate to disease severity - dilution factors
- The procedure itself or handling and processing of samples may introduce artifacts (e.g., lidocaine and bronchodilators may affect cell activity)
- The removal, washing, centrifuging, and resuspension of mucus can activate cells and confound the cell and solute information.
- BAL process may lead to transient fever within 24 hours after the lavage, occasionally associated with lung infiltrates

Adapted from Jeffery et al. 2003
BAL in asthma

- Increased numbers of eosinophils is suggestive of asthma although neutrophils can be increased, particularly in severe asthma.

- Various cytokines can be measured e.g. IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, GM-CSF, TNF-a, ICAM-1)

- No consistent correlation with asthma severity.
The predominant cell type in BAL of individuals with COPD is the alveolar macrophage.

The percentage of CD8+ T lymphocytes is significantly higher, and that of CD4+ T-cells significantly lower, in COPD (and healthy smokers) compared with healthy non-smokers.

Neutrophils and eosinophils have generally been shown to be increased in COPD BAL.

Mast cell numbers have also been reported to be increased.

ECP, myeloperoxidase, and IL-8 are frequently increased in patients with COPD and in healthy smokers.
The clinical utility of BAL fluid biomarkers is questioned by the lack of standardization from laboratory to laboratory in reporting the levels of various markers, making comparisons between laboratories difficult.

As disease state changes, and volume recovery changes, interpretation of serial evaluation in the same individual is troublesome.

Murugan et al. 2009
Endobronchial biopsies: - a useful research tool

- Directly samples the resident cells
- Spatial relationships of structural components maintained
- Assesses the status of the airway mucosa
- Individual structural components can be identified
- Structures can be removed and studied in isolation using laser capture dissection
- Inflammatory cell subtypes can be identified by immunostaining
- Biomarkers of epithelial damage can be studied

Adapted from Jeffery et al. 2003
Endobronchial biopsies: - a useful research tool

- Immunologic cascades can be studied using immunohistochemistry, ISH, and microarray analysis (for assessment of gene expression)

- Tissue explants and cells derived from biopsies can also be preserved in culture

- Resident cells, such as airway smooth muscle myocytes, have successfully been dissected from endobronchial biopsies from subjects with asthma, allowing measures of the proliferative responses of these cells

Adapted from Jeffery et al. 2003
Airway morphology

- Epithelium
- Basement membrane
- Smooth muscle
- Vessels
Bronchial biopsies of smoking asthmatic patients

Squamous cell metaplasia
Inflammatory cells in Bronchial biopsies
Relationship between airways responsiveness to methacholine and the number of (A) CD3 +, (B) CD4 +, (C) CD8+, and (D) CD45RO + in the bronchial lamina propria

Sont et al. Thorax 1996
Biomarkers in severe asthma

Bronchial biopsy sections from patients with severe asthma showing MBP1 eosinophils (a), CD681 macrophages (b), neutrophil elastase-positive neutrophils (c) and CD31 T cells (d)

Macedo et al. 2009
Swimmer Asthmatic

Collagen 3

Airway remodeling in swimmers

Graph showing collagen layer thickness in control, swimmers, and asthmatics.
Biomarkers in *bronchial biopsies (asthma)*

- Epithelial shedding, goblet cell hyperplasia, thickened lamina reticularis
- Submucosal eosinophilic and lymphocytic infiltration
- Increased granulation of mast cells and high affinity IgE receptor bearing cells
- Increased expression of IL-4, IL-5, endothelin, eotaxin and ICAM-1 enhanced IL-8 and IFN-γ expression, also IL-5 and eotaxin expression and reduced IL-4 expression of severe asthma compared with moderate disease
Biomarkers in *bronchial biopsies (COPD)*

- Increase in macrophages, CD8\(^+\) T-cells + eosinophils
- Increased expression of CCL5 and CXCL7 in the bronchial mucosa
- The number of IL-17A\(^+\), IL-22\(^+\), IL-22\(^+\) and IL-23\(^+\) immunoreactive cells is increased in the bronchial epithelium
- The number of cells expressing YKL-40, a chitin-binding protein that is elevated in patients with various inflammatory conditions
- Altered surfactant protein (SP)-A expression in human lung tissue samples obtained from patients with COPD
Genetic variation in immune signaling genes differentially expressed in asthmatic lung tissues Tremblay et al. 2008

LD pattern for SFRP1. A, SFRP1 LD pattern in the SLSJ familial sample. B, SFRP1 LD pattern in the CAPPS cohort. C, SFRP1 LD pattern in the SAGE cohort. D, SFRP1 LD pattern in the Busselton cohort. The location of each tested TagSNP along the chromosome is indicated in the upper part of the figure. The number in each diamond indicates the magnitude of LD (D9) as a percentage between respective pairs of SNPs. The strength of LD is depicted by progression of color.
Bronchial biopsies for cell cultures

Fibroblasts

Epithelial cells

Reconstitution of bronchial mucosa
Endobronchial biopsies: limitations

- Samples are limited in size to between 1 - 2 mm diameter.
- They may not be representative of the entire conducting airways (mainly from proximal airways and from subcarinae peribronchial tissue (deep to the mucosa) is not included in endobronchial samples).
- The procedure itself may induce artifacts, especially due to forceps damage.
- Biopsy procedure itself creates scar tissue and can initiate cellular processes that can proceed during the interval (seconds) between sampling and fixation.
- Biopsy provides only a snapshot in time of an ongoing disease process.

Adapted Jeffery et al. 2003
Endobronchial biopsies: limitations

- Bronchial biopsies to quantify inflammation are limited by normal anatomic variability, patchy focal inflammation, sample size, and number of biopsy specimens and quantification of cellular infiltrate.

- Studies evaluating the variability and reproducibility of data obtained from bronchial biopsies of asthmatics suggest a sample size of 8–25 individuals to study individual cell types and 13–48 individuals per arm in parallel design drug studies are required to establish adequate statistical power.

Murigan et al 2009
# Morphometric Measurements

## Comparison of Commonly Used Morphometric Methods in Asthma Research

<table>
<thead>
<tr>
<th>Parameter</th>
<th>2-Dimensional: One Section per Biopsy or &gt; Two-Step Section</th>
<th>3-Dimensional: Multiple Biopsies and Multiple Sections per Biopsy</th>
<th>Semiquantitative Methods</th>
<th>Automated Methods</th>
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<tbody>
<tr>
<td><strong>Parameter</strong></td>
<td>No. per area&lt;br&gt;No. per length</td>
<td>Volume density (mm³/mm³)&lt;br&gt;Volume to surface&lt;br&gt;Uses established stereologic principles&lt;br&gt;Volume-oriented measurement&lt;br&gt;Can use technique to quantify cells and structures&lt;br&gt;Easily mastered&lt;br&gt;“Do more less well”—requires more subjects and more biopsies</td>
<td>Scoring system (1+, 2+, 3+)&lt;br&gt;Useful for pilot studies&lt;br&gt;Fast&lt;br&gt;Consistency of staining</td>
<td>No. per area&lt;br&gt;Systems may have difficulty discerning cells; differences based on color, not cell type</td>
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<tr>
<td><strong>Advantages</strong></td>
<td>Easily mastered</td>
<td></td>
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<tr>
<td><strong>Disadvantages</strong></td>
<td>May invite bias because larger cells are overestimated, smaller cells underestimated</td>
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This table compares the 2-dimensional and 3-dimensional morphometric methods used in asthma research. The 2-dimensional method involves taking one section per biopsy or using a two-step sectioning technique. Its advantages include ease of mastering the technique, but it may invite bias due to overestimation of larger cells and underestimation of smaller cells. Its disadvantages include potential for bias in cell size estimation.

The 3-dimensional method involves multiple biopsies and sections per biopsy, providing a more accurate representation of volume density and other stereologic principles. Its advantages include the ability to use advanced techniques for quantifying cells and structures, with ease of mastering this technique. However, it requires more subjects and more biopsies to be effective.

For semiquantitative methods, the scoring system ranges from 1+ to 3+, useful for pilot studies, emphasizing speed and consistency of staining. Automated methods may face challenges in accurately discerning cells, with differences based on color rather than cell type.
Bronchoscopy in the treatment of asthma: thermoplasty!
Conclusions

- Endoscopic techniques may be very helpful in the study of the pathophysiology of airway diseases.
- These techniques are invasive and not suitable for clinical assessment of asthma & COPD.
- New developments can optimize the usefulness of these sampling methods.