Exhaled Biomarkers
Asthma & COPD

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DM Seminar
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Introduction

• Diagnosis and course of COPD/Asthma
  - Clinical information
  - Pulmonary function tests
  - Arterial blood gases
  - Chest X-rays

• No direct measure of lung inflammation is routinely used
What are biomarkers?

- Biomarkers are objectively measured and evaluated indicators of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention.
What can we measure?

- Exhaled gases eg NO
- Exhaled breath condensate markers
- Exhaled breath temperatures
Why is this so exciting?

- Many diseases are characterized by chronic inflammation and oxidative stress.
- Asthma, COPD, bronchiectasis, cystic fibrosis and ILD are examples.
- Inflammation is not directly measured by any routine investigation done at present.
- Measuring biomarkers may make this possible.
Asthma

- **Bronchial biopsies are the “gold standard”**
  - invasive
  - cannot be routine
  - cannot be repeated often
  - children and those with severe disease

- **BAL**
  - Invasive
  - Infection
  - Impair gas exchange

- **Symptoms are a poor indicator**
  - Perception
  - Masking by SABA/LABA
• **Histamine/methacholine challenges**
  • Confounded by bronchodilator use

• **Sputum induction**
  • Unpleasant
  • Inflammation lasts 24 h
  • Cannot be repeated in less than 24h
Exhaled Breath Condensate

- Epithelial lining fluid contains 200 volatile substances and various nonvolatile substances
- Initial focus on volatile substances particularly NO
- Studies are now focusing on nonvolatile substances e.g. proteins, lipids, oxidants and nucleotides
Possibilities

- Determining host inflammatory responses to injury in the lung
- Possible single noninvasive sampling method for point-of-care real-time analysis
Collection of EBC

- Exhaled breath is saturated with water vapor which can be condensed with cooling
- Aerosol particles from the lower tract are also present
- Source: alveoli vs. airway e.g. $\text{H}_2\text{O}_2$ is from airways (flow dependent)
EBC

- 0.1-4 particles/cm$^3$
- Mean diameter $< 0.3\mu m$
- Number depends on
  - Velocity
  - Surface tension
  - Turbulent flow
A Collection Apparatus
Problems

- Glass/polystyrene/polypropylene
- Ice/Dry ice/ liquid nitrogen
- Nose clips open nasopharyngeal velum
- 5-10 min to get 1-3 ml of EBC
- Contamination
  - Exhaled air (two way non-rebreathing valve)
  - Saliva (trap, mouth rinsing, salivary amylase)
EBC: Inflammatory mediators detected

<table>
<thead>
<tr>
<th>Condition</th>
<th>Compound</th>
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</thead>
<tbody>
<tr>
<td>Cigarette smokers</td>
<td>$\text{H}_2\text{O}_2$, 8-isoprostane</td>
</tr>
<tr>
<td>COPD</td>
<td>$\text{H}_2\text{O}_2$, 8-isoprostane, serotonin, cytokines (IL-1, sIL-2R, TNF-\alpha)</td>
</tr>
<tr>
<td>Asthma</td>
<td>$\text{H}_2\text{O}_2$, 8-isoprostane, nitrotyrosine, thiobarbituric acid-reactive products, leukotrienes, pH</td>
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<tr>
<td>Chronic bronchitis</td>
<td>Leukotrienes</td>
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<tr>
<td>Bronchiectasis</td>
<td>$\text{H}_2\text{O}_2$</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>$\text{H}_2\text{O}_2$, nitrite, 8-isoprostane, IL-8</td>
</tr>
<tr>
<td>ALI/ARDS</td>
<td>$\text{H}_2\text{O}_2$, 8-isoprostane, PGE$_2$</td>
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Smoking

- \( \text{H}_2\text{O}_2 \) a measure of oxidant activity
- Levels in smokers 5x higher
- Male smokers > Female smokers
- Levels in EBC are lower than in alveoli as there is removal by the anti-oxidant system
- Higher levels may indicate risk of developing smoking-related disease
ASTHMA
• NO levels are increased in bronchial asthma (Alving et al 93)
• Pro-inflammatory mediator with immunomodulatory effects. Predisposes to the development of AHR in pathological situations
• A weak mediator of smooth muscle relaxation in physiological situations
• Originates in airway epithelium
FE(NO)

• May rise in a large number of conditions but is most marked in allergic airway disease
• Portable inexpensive meters can measure it easily
• More relevant direct measure of inflammation which complements PFT
Rationale for $F_{E\text{NO}}$ measurement

- High degree of correlation with eosinophilic airway inflammation
- Eosinophilic inflammation responds to steroids
- Raised levels predict steroid responsiveness in pts with non-specific symptoms
- ICS treatment results in a fall in levels in a dose dependent manner
Asthma vs non-asthma

- Helps to discriminate asthma from non-asthma
- Viral illnesses can give false positive results (wait 6 wks)
- More sensitive than spirometry and therefore will pick up disease where lung fn is still normal
Non-specific respiratory symptoms

- Role in assessing undiagnosed respiratory symptoms
- Eosinophilic bronchitis, cough variant asthma, post-viral hyperresponsiveness, Post-nasal drip, GE reflux, VCD, COPD
- A rise in FENO predicts steroid responsiveness
Pre-school children

- Diagnosing asthma from non-asthma in wheezy infants using $F_E NO$ either offline or online.
- Evidence for reliability as a screening tool is mixed.
- May allow better targeting of anti-inflammatory therapy.
Influence of atopy

- Levels are raised in atopic individuals even in the absence of symptoms suggesting low levels of airway inflammation.
- Complements skin testing and correlates well with IgE levels.
- No evidence to treat asymptomatic individuals.
Management of chronic asthma

- Predicting exacerbations
- Predicting outcomes of ICS withdrawal
- Adjustment of ICS dose
$H_2O_2$ and TBAR

- Increased levels in asthma
- High correlation between the two
- Increase in levels associated with a drop in $FEV_1$

• Significant reduction with treatment with ICS which remained stable for 2 weeks after discontinuation
H$_2$O$_2$ levels in children

- Correlate well with symptoms
- Decrease with ICS treatment
- May be a good measure for monitoring improvement with treatment

Nitrotyrosine

- A stable end product of peroxynitrite
- Mild (steroid naïve)
- Moderate (on ICS)
- Severe (on oral CS)
- Increased levels were found in the first group

Isoprostanes

- Compounds formed by non-enzymatic peroxidation of membrane phospholipids during oxidative stress
- Levels are elevated in all asthma with higher levels in more severe disease
- Correlation with PFT however is not good

Leukotrienes

- Airway smooth muscle contraction, microvascular leakage, mucus hypersecretion
- Increased levels of LTB4 in asthma which increase with severity
- No correlation with FEV1

pH

- Acute asthma associated with pH decline of two-log
- Normalised with corticosteroid therapy
- Suggested that serial measures can help titrate therapy
- Hampered by poor reproducibility
Future Prospects for EBC in asthma

- Some markers persist despite ICS
- Leukotriene pathway is not suppressed by steroids
- Persistent elevation of leukotrienes may be used to initiate therapy with specific inhibitors
- Lack of correlation with FEV$_1$ does not preclude the use of these markers
- If rise in markers precedes physiological changes greater utility is likely
Exhaled biomarkers

COPD
Inflammation in COPD

- Chronic inflammation throughout the airways, parenchyma and pulmonary vasculature
- Macrophages, T-lymphocytes (CD8+) and neutrophils
- Tissue eosinophils (unlike asthma not degranulated)
Biomarkers in exhaled air

- Exhaled NO: most used and standardised
- Exhaled CO
- Exhaled Ethane
Exhaled Nitric Oxide

• A gas which regulates vascular and bronchial tone
• Alveolar macrophages synthesize it after stimulation by endotoxin and cytokines; part of host defence
• Converted to peroxynitrite: a potent epithelial toxin
• Promotes proliferation of T lymphocytes
Synthesis of NO

NADP⁺ → NADPH

L-arginine → NO synthases
  nNOS
cNOS
iNOS

NO → RS-Nos

NO₂⁻ / NO₃⁻

thiols

O₂

H₂O

ONOO⁻ → nitrotyrosine
NO levels

- Healthy subjects: 3-7 ppb
- Lower in smokers
- No difference between healthy individuals and stable COPD/ lower in those still smoking.
- Increased levels in unstable disease owing to neutrophilic inflammation
- Increased levels in subsets with an asthmatic component to disease.
• Levels decrease with treatment with ICS (probably the effect on some eosinopholic inflammation also present)

• Levels correlate with sputum eosinophil levels.

• Inverse correlation with $FEV_1$ levels in stable patients
• NO is not a good marker for disease severity in COPD

• Increased levels
  • Asthmatic subset
  • Exacerbations

• Smoking reduces levels confounding the picture
Exhaled CO
CO

- CO is produced in alveoli, nose and paranasal sinuses
- Environmental levels affect measurements
- Higher in stable COPD
- Smoking has the greatest effect 8ppm
- URTI also raises levels
- Healthy subjects 1-8ppm
CO

- Also increased in asthma
- No data exist on correlation with ICS use
- Limited utility as a marker because of wide variation with environmental levels and smoking
Exhaled alkanes

- Oxidants can cause injury by lipid peroxidation
- ROS and H$_2$O$_2$ released by activated inflammatory cells can induce peroxidation of polyunsaturated membrane fatty acids
- This impairs function and inactivates receptors and enzymes, increases permeability and causes airflow limitation
ROS  
\[ \rightarrow \text{fatty acids} \]  
\[ \rightarrow \text{arachidonic acid} \]  
\[ \rightarrow \text{hydroperoxides (TBARs)} \]  
\[ \rightarrow \text{bicycloendoperoxide intermediates} \]  
\[ \rightarrow \text{prostaglandines, leukotriens} \]  
\[ \rightarrow \text{isoprostanes} \]  
\[ \rightarrow \text{hydrocarbons: \ aldehydes (TBARs)}\]  
\[ \rightarrow \text{alkenes} \]  
\[ \rightarrow \text{alkanes (ethane, pentane)} \]  
RNS
Ethane

- Easier to measure
- Analyzed by gas chromatography
- Expensive and time consuming
- Environmental contamination has to be avoided
- Age does not affect levels
- 0.88 ppb is the normal level
- Increased in smokers and those with airway obstruction. Decreased with steroid use
Biomarkers in EBC

- Biomarkers which are not gases cannot be measured directly
- Hydrogen peroxide
- Isoprostanes
- NO metabolites
- TBARS
- Salivary contamination is a problem
Exhaled Hydrogen peroxide

- Airway inflammation causes a "respiratory burst" producing ROS
- $\text{H}_2\text{O}_2$ levels reflect oxidative stress in the lung
- Measurement is based on reaction with suitable substrates leading to the release of color, light or fluorescence
- Normal levels are almost undetectable
Hydrogen peroxide

- Collection and storage is a source of error
- Exercise increases levels
- Food and beverages increase levels
- Levels vary widely with repeated measurements
- Healthy young non-smokers 0.01-0.09 mmol/l
- Increased in stable COPD/ increased further during an exacerbation
- Lower levels in current smokers
- Levels decrease with ICS/NAC
- Standardization is poor and large intraindividual variability exists.
Isoprostanes

- Reduction of bicycloendoperoxide intermediates (from arachidonic acid reacting with oxygen radicals)
- Stable in body fluids
- No diurnal variation
- Higher levels in smokers
- Higher in COPD regardless of smoking status
Isoprostanes

• Also high in healthy smokers, asthma and ILD which may confound its use for diagnosing or monitoring COPD
Nitric oxide metabolites

- NO is highly reactive and has a short life in vivo
- Stable end products include nitrite and nitrate. Peroxynitrite results from a reaction with superoxide
- Nitrotyrosine and nitrosothiols result
- All can be measured in EBC
- Increased directly after cigarette smoking/in COPD /asthma
- Steroids reverse the increase in asthma
Thiobarbituric acid reactive substances (TBARs)

- Volatile products of lipid peroxidation
- Undetectable in healthy non-smokers
- Raised in smokers with no relation to other inflammatory markers
- Raised in stable COPD with no difference with smoking status
- Also increased in asthma to a higher degree with significant correlation with H2O2 levels
• TBAR levels can differentiate stable COPD and healthy individuals and also between those with COPD and asthma

• Current smoking status does not change levels in COPD
Advantages and Limitations
Advantages

1. Simple, point-of-care intervention
2. Inclusive rather than intrusive (e.g., healthy children, mechanically ventilated neonates)
3. Domiciliary
4. Longitudinal sampling
5. Nonvolatile compounds associated with pulmonary pathophysiology
6. Amplified DNA and RNA from prokaryotic and eukaryotic cells
7. Pharmacokinetics/pharmacodynamics of drugs
8. Solute clearance
Limitations

1. Lack of standard breath-sampling method
2. Not anatomic site specific
3. Lack of evidence for the origin of the aerosol particles (bronchi versus terminal airways)
4. Concentration artifact (due to evaporation of samples)
5. Feasibility and utility of biomarkers unrelated to oxidative stress not tested
6. Little information on biomarkers of interstitial lung disease
Conclusions

• EBC has potential as a non invasive real time technique in the future
• Lack of standardisation in collection and analysis for most markers makes comparison of studies and clinical application difficult at present
• Collected fluid is not anatomic site specific
• Reference data for healthy individuals needs to be available
• Smoking status affects different markers in different ways
• Data on reproducibility and variability is scarce
• Effect of treatment on different markers needs to be determined before they can be used for follow up
Take home message!

• Biomarkers may be a useful non invasive adjunct in the diagnosis and follow-up of patients with various pulmonary inflammatory conditions at the point of care in real time

• Further work is needed to validate standardize and better define the clinical utility of this emerging instrument in pulmonary disease
Thank you