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

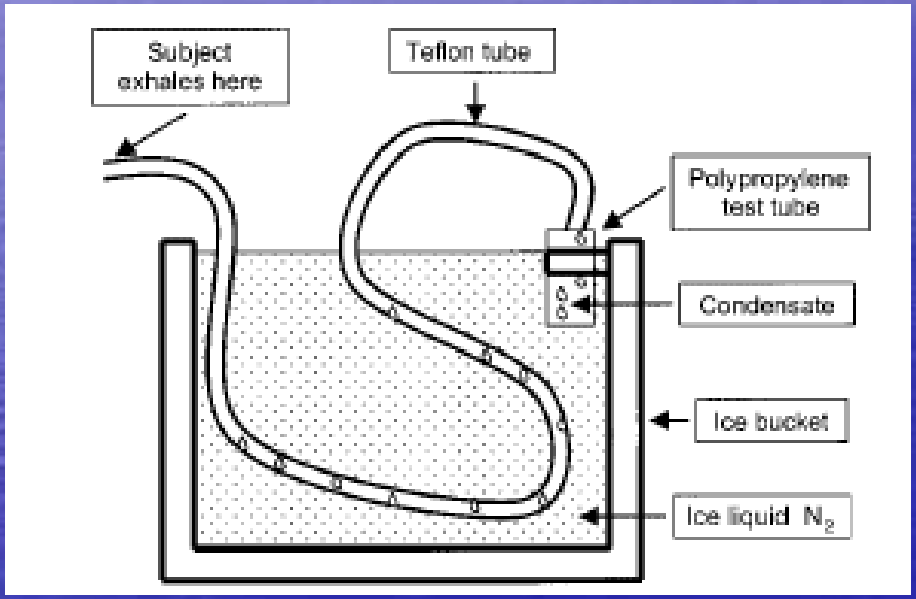
Collection of EBC

- 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. H_2O_2 is from airways (flow dependent)

EBC

- 0.1-4 particles/cm^3
- Mean diameter < 0.3µm
- 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

Condition	Compound
Cigarette smokers	H_2O_2, 8-isoprostane
COPD	H_2O_2, 8-isoprostane, serotonin, cytokines (IL-1, sIL-2R, TNF-α)
Asthma	H_2O_2, 8-isoprostane, nitrotyrosine thiobarbituric acid-reactive products, leukotrienes, pH
Chronic bronchitis	Leukotrienes
Bronchiectasis	H_2O_2
Cystic fibrosis	H_2O_2, nitrite, 8-isoprostane, IL-8
ALI/ARDS	H_2O_2, 8-isoprostane, PGE_2

Smoking

- H_2O_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

F_ENO

- 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 sm relaxation in physiological situations
- Originates in airway epithelium

F_ENO

- 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_ENO 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_ENO 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

Antczak A, Nowak D, Shariati B, Krol M, Piasecka G, Kurmanowska Z. Increased hydrogen peroxide and thiobarbituric acid-reactive products in expired breath condensate of asthmatic patients. *Eur Respir J* 1997;10:1235–1241.

- Significant reduction with treatment with ICS which remained stable for 2 weeks after discontinuation

Antczak A, Kurmanowska Z, Kasielski M, Nowak D. Inhaled glucocorticosteroids decrease hydrogen MEDICI peroxide level in expired air condensate in asthmatic patients. *Respir Med* 2000;94:416–421.

H_2O_2 levels in children

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

Dohlman AW, Black HR, Royall JA. Expired breath hydrogen peroxide is a marker of acute airway inflammation in pediatric patients with asthma. *Am Rev Respir Dis* 1993;148:955–960.

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

Hanazawa T, Kharitonov SA, Barnes PJ. Increased nitrotyrosine in exhaled breath condensate of patients with asthma. *Am J Respir Crit Care Med* 2000;162:1273–1276.

Isoprostanes

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

Montuschi P, Corradi M, Ciabattoni G, Nightingale J, Kharitonov SA, Barnes PJ. Increased 8-isoprostane, a marker of oxidative stress, in exhaled condensate of asthma patients. *Am J Respir Crit Care Med* 1999;160:216–220.

Leukotrienes

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

Becher G, Winsel K, Beck E, Steremann E. Leukotriene B4 in breathing condensate of patients with bronchopulmonary diseases and of normal patients. *Appl Cardiopulmon Pathophysiol* 1995;5:215–219.

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

Palaiologou A, Loukides S, Papatheodorou G, Panagou P, Xronas G, Kalogeropoulos N. pH in expired breath condensate of patients with asthma. *Eur Respir J* 2000;16:40s.

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)

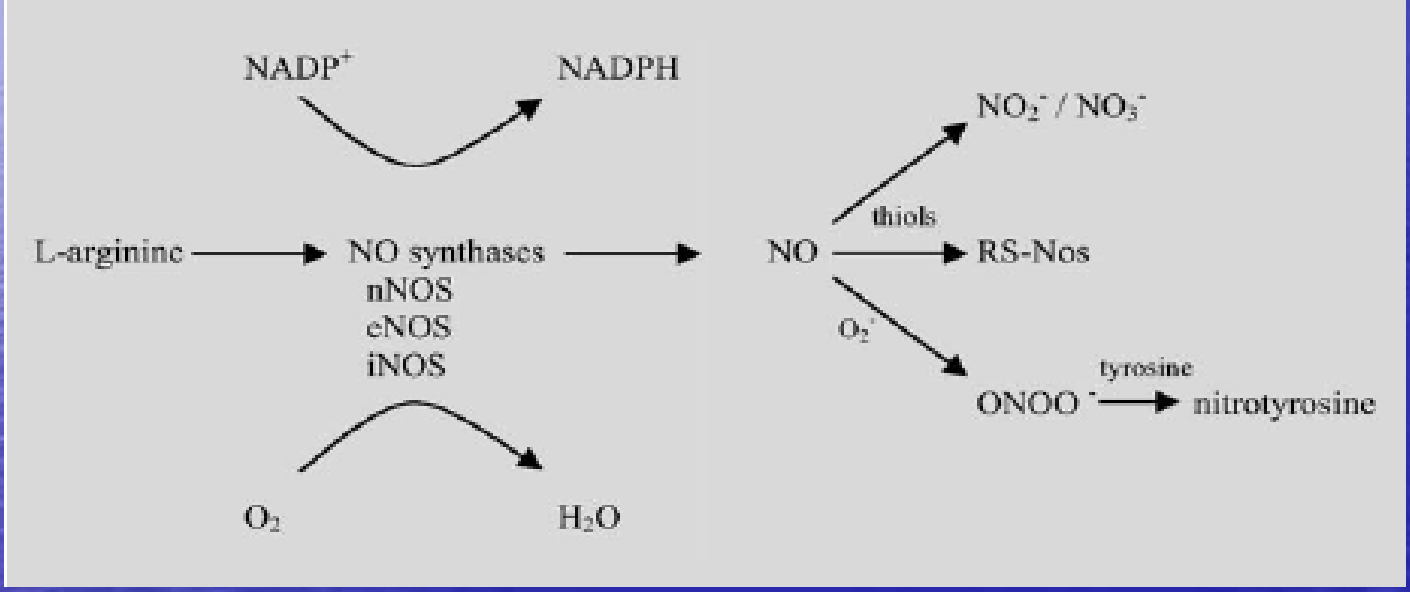
Biomarkers in exhaled air

- 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



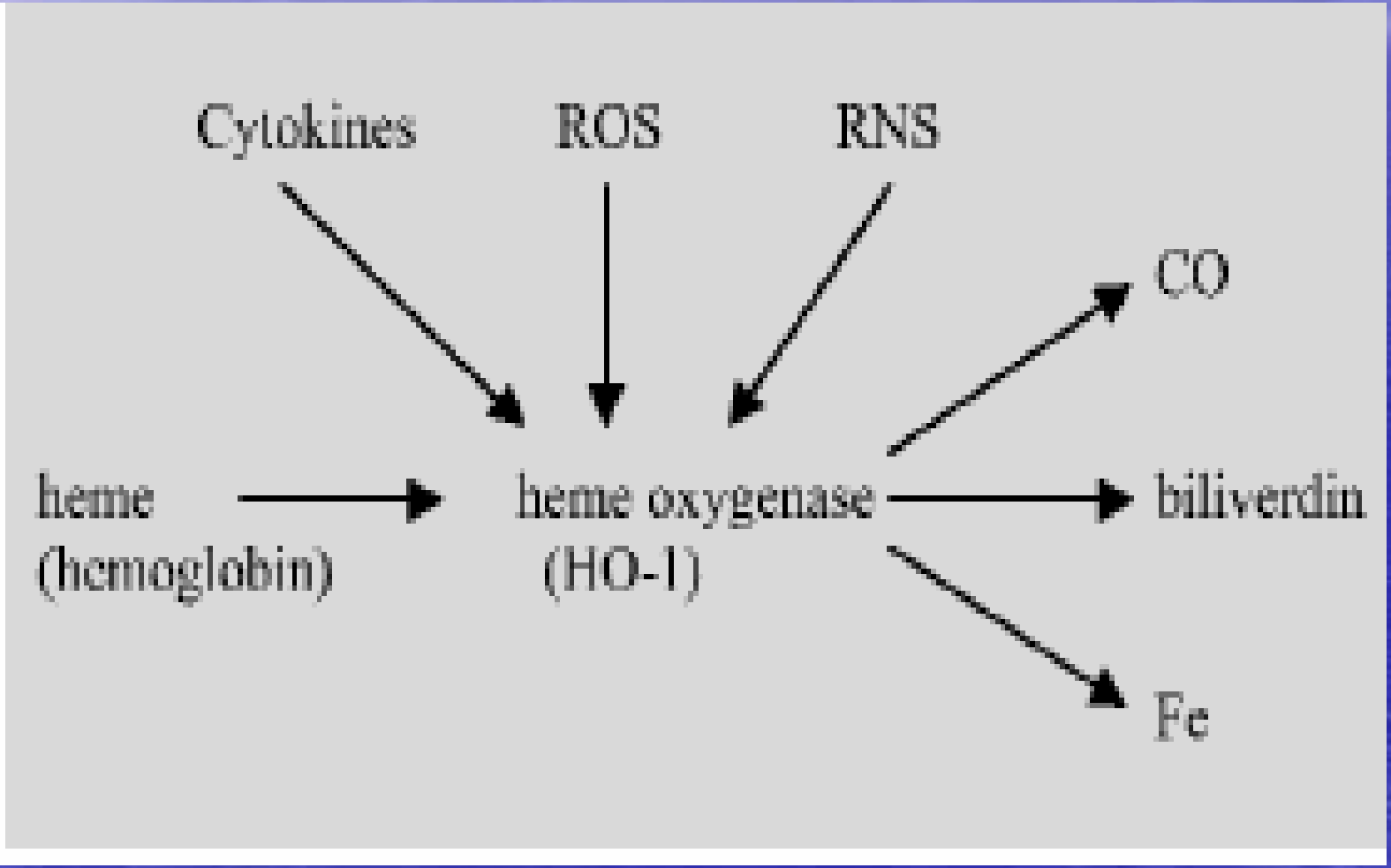
NO levels

- 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


Cytokines
ROS
RNS
CO
heme
(hemoglobin)
heme oxygenase
(HO-1)
biliverdin
Fe

CO

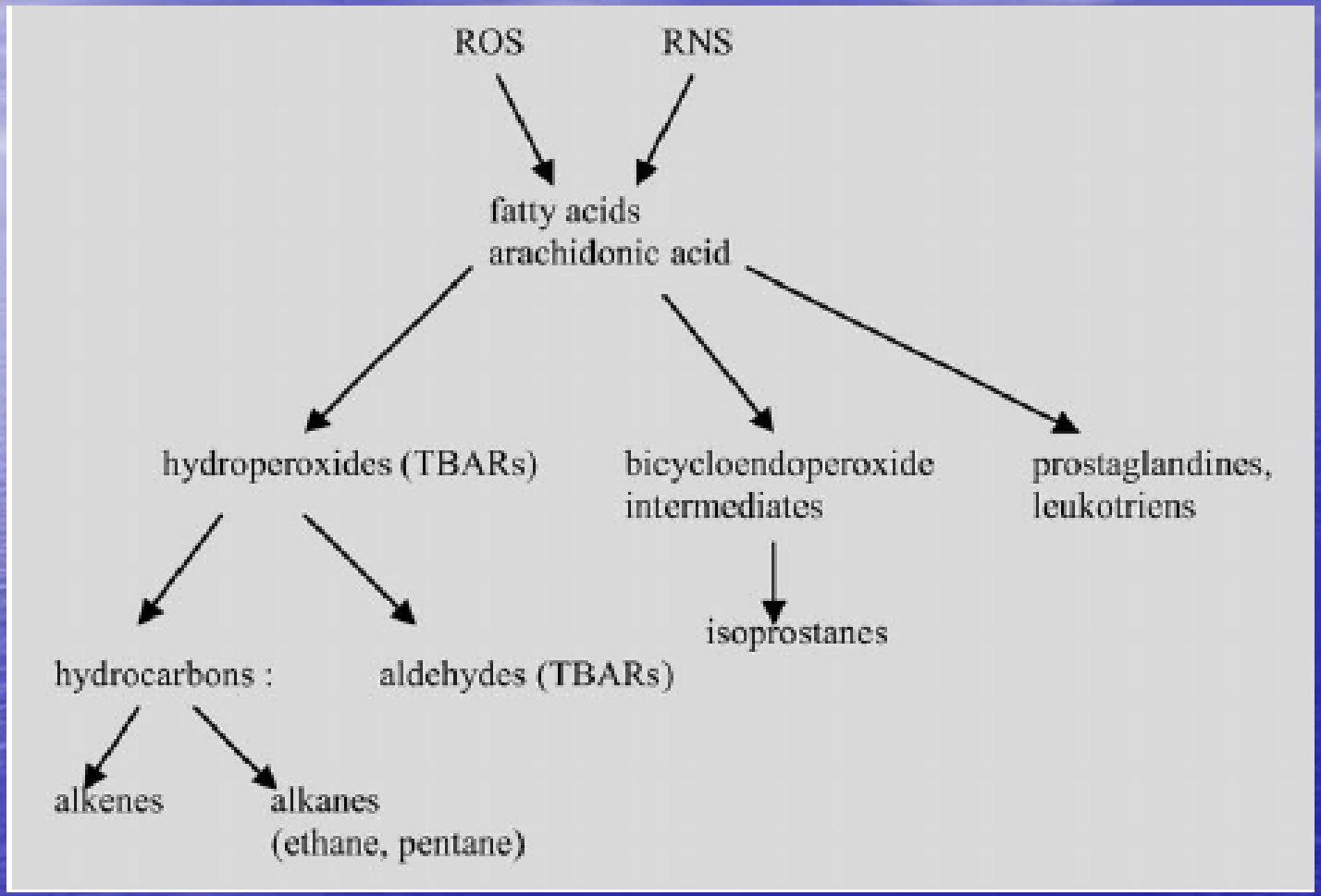
- CO is produced in alveoli, nose and paranasal sinuses
- Environmental levels affect measurements
- Higher in stable COPD
- Smoking has the greatest effect 8ppm
- URTI also raises levels
- Healthy subjects 1-8ppm

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_2O_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
RNS
fatty acids
arachidonic acid
hydroperoxides (TBARs)
bicycloendoperoxide
intermediates
prostaglandines,
leukotriens
isoprostanes
hydrocarbons :
aldehydes (TBARs)
alkenes
alkanes
(ethane, pentane)

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
- H_2O_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 0.01-0.09mmol/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

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

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